ONE-STEP SYNTHESIS OF A PYROGLUTAMYL PEPTIDASE INHIBITOR, PYRIZINOSTATIN, FROM AN ANTIBIOTIC, 2-METHYLFERVENULONE

Sir:

Pyrizinostatin (1) was discovered in 1992 as an inhibitor of pyroglutamyl peptidase from culture filtrate of the genus *Streptomyces*, which had been isolated from a marine soil. Although pyrizinostatin (1) represents a new structural class of enzyme inhibitors, its relatively low culture yields for biological study have slowed the development of this inhibitor.

Structurally, we anticipated that pyrizinostatin (1) might be biologically derived from 2-methylfervenulone (2: MSD-92), which showed broad in vitro antibiotic activity.

As 2-methylfervenulone (2) is a fluorescent compound, it is expected to be chemically reactive.

Herein, we report one-step synthesis of pyrizinostatin (1) from 2-methylfervenulone (2). The starting 2-methylfervenulone (2) was isolated from the fermentation broth of the microbial strain and also readily prepared on large scale in 4 steps from 1,3-dimethyl-4-chlorouracil according to Taylor’s elegant synthesis.

Dramatically, the desired pyrizinostatin (1) was produced by nucleophilic attack of acetone, when 2-methylfervenulone (2) was dissolved in acetone and allowed to stand at 20°C for 2 days. After evaporation of the solvent, the residue was purified by silica gel column chromatography with EtOAc, followed by recrystallization from MeOH to give crystals of pyrizinostatin (1) in 74% yield: MP 165 ~ 166°C; FAB-MS m/z 282 (M+H)+; UV λmax 282 nm (ε 4,900); 1H NMR (500 MHz, CDCl3) 2.13 (3H, s), 2.95 (1H, d, J = 16.0 Hz), 3.24 (1H, d, J = 16.0 Hz), 3.27 (3H, s), 3.31 (3H, s), 5.63 (1H, br s); 13C NMR (125 MHz, CDCl3) 28.8 (q), 30.4 (q), 30.8 (q), 37.0 (q), 49.6 (t), 54.9 (s), 138.6 (s), 149.8 (s), 151.5 (s), 166.2 (s), 202.7 (s). These physico-chemical data were identical with reported data of the natural product except for the optical rotation and MP.

The synthesized pyrizinostatin (1) inhibited the activity of pyroglutamyl peptidase at an IC50 value of 0.8 μg/ml, while the natural product showed the enzyme inhibiting activity at 0.3 μg/ml.

This synthesis naturally recalls the fact that fluorescent flavins (for example, 3) are biologically inactivated by nucleophilic attack of suicide substrates.

Since the starting material 2 has been supplied by both fermentation and chemical procedure, the described synthesis is distinctly simple and provides a preparative entry into a wide variety of designed analogs for biological studies.

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References

