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[1,2]. 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 **1**.

Structurally, we anticipated that pyrizinostatin (**1**) might be biologically derived from 2-methylfervenulone (**2**: MSD-92)[3], 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[4].

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 λ_{max}^{MeOH} 282 nm (ε 4,900); ^{1}H NMR (500 MHz, $CDCl_3$) 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), 3.34 (3H, s), 5.63 (1H , br s); ^{13}C NMR (125 MHz, $CDCl_3$) 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 IC_{50} 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[5].

Since the starting material **2** has been supplied by both fermentation[3] and chemical procedure[4], 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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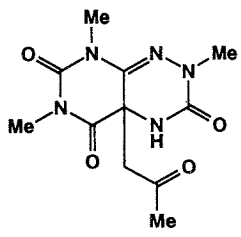

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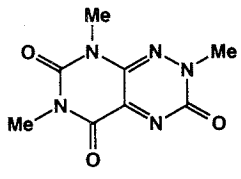

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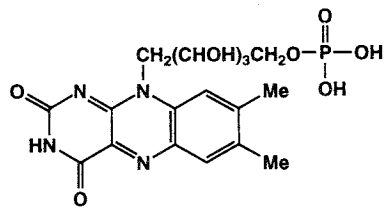

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