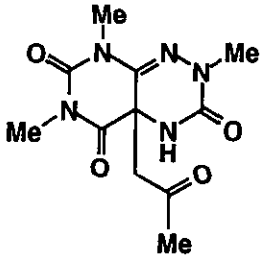
ENANTIOSELECTIVE SYNTHESIS OF PYRIZINOSTATIN, A PYROGLUTAMYL PEPTIDASE INHIBITOR

Masayuki Kitagawa and Kuniaki Tatsuta*

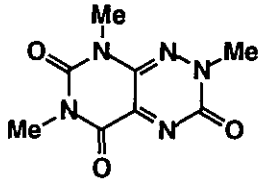
Graduate School of Science and Engineering, Waseda University, 3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169, Japan

Abstract - Enantiospecifically pure pyrizinostatin was synthesized from 2-methylfervenulone by using chiral imines of acetone.

Pyrizinostatin [(-)-**1**] isolated from fermentation broth of *Streptomyces* sp. is a strong inhibitor against pyroglutamyl peptidase.[1,2] Recently, racemic pyrizinostatin (**1**) and its analogs have been synthesized in only one step from an antibiotic, 2-methylfervenulone (**2**) in our laboratories.[3,4] As racemic pyrizinostatin (**1**) has been obtained only by dissolving 2-methylfervenulone (**2**) in acetone, natural pyrizinostatin could be similarly considered to be an artifact derived from **2** and acetone. However, natural pyrizinostatin [(-)-**1**] is endowed with the optical activity to show $[\alpha]_D^{24}$ -15.6° (*c* 1.0 , MeOH),[1,2] although the absolute configuration remained undetermined. This indicates that natural pyrizinostatin [(-)-**1**] is a biologically synthesized metabolite.



Pyrizinostatin : 1



2

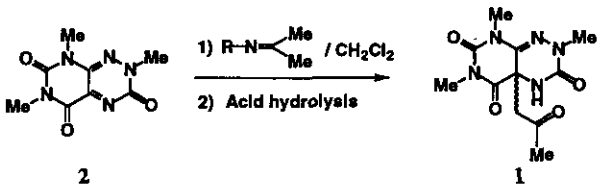
Herein, we report the enantioselective synthesis of (-)-pyrizinostatin [(-)-**1**] from a structurally plane compound (**2**) by using chiral imines derived from acetone including the hydrazone. A variety of chiral amines and a chiral hydrazine derivative (SAMP) reacted with acetone to give the corresponding imines,[5] the carbanion of which attacked to 2-methylfervenulone (**2**) to yield, after acid hydrolysis, optically active pyrizinostatin (**1**) as summarized in Table 1. In considering the completely planar structure of **2**, the good result was obtained by reaction with the imine derived from acetone and (*S*)-(+)-1-(*p*-nitrophenyl)ethylamine (Entry 4) to give a 57% enantiomeric excess of (-)-**1**, the optical purity of which was calculated on the basis of the optical rotation of natural (-)-**1**. The isolation of an enantiomerically pure sample of (-)-**1** was realized by using the fact that the solubility of the racemate in methanol was much lower than that of (-)-**1**. Namely, the enantiomerically pure pyrizinostatin [(-)-**1**] was obtained in 21.5% yield from the mother liquor after recrystallization of the aforesaid 57% ee product from methanol.

As the absolute configuration of (-)-**1** has not been determined, this enantioselective reaction has not been reasonably rationalized so far.

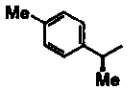
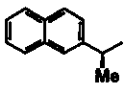
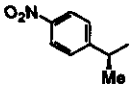
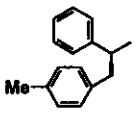
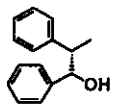
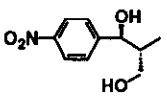
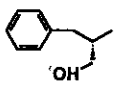
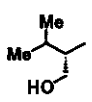
A typical synthetic procedure is the following.

A solution of (*S*)-(+)-1-(*p*-nitrophenyl)ethylamine (1.0 g) in acetone (30 ml) was refluxed with Dean-Stark trapping (MS 3A) for 1 day and then evaporated to a residue, which was dissolved in EtOAc. The solution was washed with water, dried (Na_2SO_4), and concentrated to give a syrup (about 1.2 ml) of the corresponding imine of acetone. The syrup (0.4 ml) was added dropwise to a solution of 2-methylfervenulone (**2**: 45.8 mg) in CH_2Cl_2 (2 ml) at 5°C, and the reaction mixture was allowed to stand at the same temperature for 12 days. After addition of AcOH (0.6 ml) in $CHCl_3$ (18 ml), the solution was stirred at room temperature for 1 h until the imine derivative was hydrolyzed. The reaction mixture was directly chromatographed on silica gel column (10 g) with 1% AcOH in $CHCl_3$ to elute *p*-nitrophenylethylamine and then with 1% AcOH in EtOAc. The fractions having R_f value 0.33 on tlc (EtOAc) were combined and concentrated to give a crude solid of pyrizinostatin (**1**: 42 mg, 73%): $[\alpha]_D^{23}$ -8.99° (*c* 1.54, MeOH). This solid was

Table 1.



2 1

Entry	R	Yield(%)	$[\alpha]_D^{23}$ ° (*c*, MeOH)	ee(%)*
1		70.3	-8.18 (1.81)	52
2		72.8	-7.40 (1.54)	47
3		48.7	-2.28 (1.05)	15
4		73.0	-8.99 (1.54)	57
5		57.3	-5.31 (1.47)	34
6		56.4	+0.59 (1.34)	0
7		41.9	+2.82 (1.56)	18
8		48.2	-1.05 (1.14)	7
9		44.8	+0.3 (1.32)	0
10		55.1	+5.96 (0.94)	38

*Determined by comparing the optical rotation with that of the natural product[1] and further by the nmr analysis in the presence of $Eu(hfc)_3$.

recrystallized from methanol to give crystals of racemic pyrizinostatin (**1**: 23 mg). The mother liquor was concentrated to give a residue, which was chromatographed on silica gel column (8 g) with EtOAc to yield, after recrystallization from EtOAc-hexane, enantiomerically pure pyrizinostatin [(-)-**1**: 12.4 mg. 21.5%]: mp 184-186°C; $[\alpha]_D^{23}$ -15.4° (*c* 0.84, MeOH); ^{1}H nmr (500 MHz, $CDCl_3$) δ: 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).

ACKNOWLEDGMENT

We are grateful to Nippon Kayaku Co. Ltd., Yamanouchi Pharmaceutical Co. Ltd. and Shikoku Chemicals Co. for their generous support of our program.

REFERENCES

1. T. Aoyagi, M. Hatsu, C. Imada, H. Naganawa, Y. Okami, and T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1795.
2. M. Hatsu, H. Naganawa, T. Aoyagi, and T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1961.
3. K. Tatsuta and M. Kitagawa, *J. Antibiot.*, 1994, **47**, 389.
4. K. Tatsuta and M. Kitagawa, *Heterocycles*, 1994, **38**, 1233.
5. An example of enantioselective synthesis by using chiral imines is the following: M. Pfau, G. Revial, A. Guingant, and J. d'Angelo, *J. Am. Chem. Soc.*, 1985, **107**, 273.

Received, 9th May, 1994