

## NOTES

**A New Trichostatin Derivative, Trichostatin RK, from *Streptomyces* sp. RK98-A74**

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The tumor suppressor gene, *p53*, is found to be mutated or deleted in more than 50% of human tumor cells. Dysfunction of *p53* by mutation or deletion resulted in down-regulation of *p53*-inducible genes, such as CDK (cyclin dependent kinase) inhibitor *p21*<sup>WAF1</sup>. Natural products, which activate the *p21* promoter and then up-regulate its expression, can be cancer chemotherapeutic drugs.

In the course of a screening program of the *p21* promoter<sup>1)</sup>, we found that three actinomycete strains produced the compound(s) enhancing the luciferase activity of H1299/tsp53-Luc cells. All of the three strains produced trichostatin derivatives, and one strain, RK98-A74, was found to produce a new trichostatin derivative, trichostatin RK.

The producing organism, strain RK98-A74, was isolated from a soil sample collected at Okutama in Tokyo Metropolis. The strain was cultured on an ISP-2 agar plate for 14~20 days at 28°C, and then its morphological characteristics were observed. RK98-A74 grew very well, and formed abundant aerial mycelia. The mature aerial mycelia corresponded to the gray color series. The reverse side of the colony was white to light gray. Substrate and aerial mycelia of strain RK98-A74 were well-branched and not fragmented. Substrate RK98-A74 formed spiral chain of spores with 15~20 per chain on the aerial mycelium. The spores were spherical or egg-shaped,

0.5~0.6×0.5~0.8 μm in size with a smooth surface (Fig. 1). Sclerotia, sporangia, and zoospores were not observed. Analysis of the whole cell hydrolysates showed the presence of L,L-diaminopimelic acid, so the cell wall of this strain is classified as type I. Based on these and other characteristics, the strain RK98-A74 was deemed to belong to genus *Streptomyces*, and named *Streptomyces* sp. RK98-A74.

Strain RK98-A74 was cultivated for 4 days at 28°C with shaking in the producing medium (containing 1.0% glucose, 0.5% yeast extract, 0.5% malt extract, adjusted at pH 7.0). Three liters of the culture broth were extracted with the same volume of acetone, and filtered. The filtrate was concentrated *in vacuo* to give an aqueous solution, which was extracted with 3 liters of EtOAc. The organic layer was concentrated *in vacuo* to yield an oily material, which was dissolved in a small volume of CHCl<sub>3</sub> and applied to a silica gel column (Silica gel 60, Merck). Absorbed material was eluted with CHCl<sub>3</sub> and MeOH. Fractions (CHCl<sub>3</sub>:MeOH=90:10) enhancing luciferase activity were collected and concentrated *in vacuo*. The crude mixture was dissolved in a small amount of MeOH, applied on a preparative HPLC (Pegasil ODS i.d. 20×250 mm, Senshu Science Co.) and developed with 70~80% aqueous MeOH. Three fractions, which UV

Fig. 1. *Streptomyces* sp. RK98-A74.

Bar presents 2.0 μm.

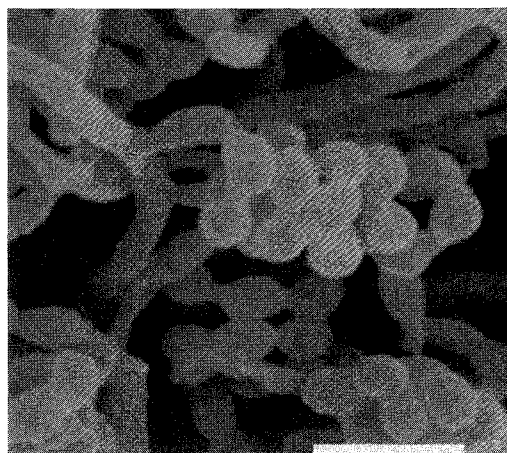
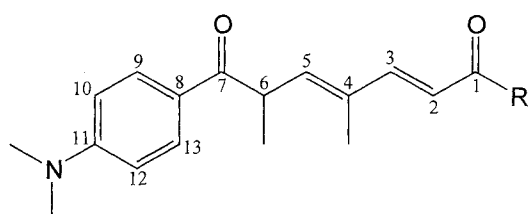


Fig. 2. Structures of trichostatins.



Trichostatin A (TSA); R = -NHOH  
 Trichostatic acid (TSacid); R = -OH  
 Trichostatin RK (TSRK); R = -NHCH<sub>3</sub>  
 FL657C; R = -NH<sub>2</sub>  
 Trichostatin C (TSC); R = -NHO-β-D-Glc  
 Monomethyl TSA; R = -NCH<sub>3</sub>OH  
 Dimethyl TSA; R = -NCH<sub>3</sub>OCH<sub>3</sub>

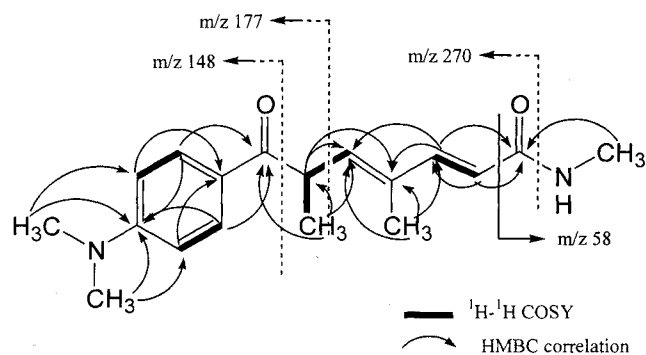
spectra were similar to each other; compounds 74-1 (1.0 mg), 74-2 (0.6 mg) and 74-3 (1.3 mg), were obtained. Based on instrumental analyses, compounds 74-1 and 74-3 were identified to be trichostatin A (TSA)<sup>2)</sup> and trichostatic acid (TSacid)<sup>2)</sup>, respectively (Fig. 2). Compound 74-2, namely trichostatin RK (TSRK), was deemed to be a new trichostatin derivative, further analysis for structure determination was carried out.

The UV spectrum of TSKK in MeOH possessed  $\lambda_{\max}$  at 265 and 350 nm, which was identical with TSA. The base peak at  $m/z$  301 ( $M+H^+$ ) and fragment ions at  $m/z$  148 ( $C_9H_{10}NO^+$ ; corresponding to *N*-dimethyl-*p*-aminobenzylacylium ion), 177 and 270 were observed in the FAB-MS spectrum of TSKK (Fig. 3), indicating that the structure of TSKK was very close to that of TSA. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of TSKK shown in Table 1 are very similar to that of TSA as described previously<sup>3)</sup>, but additional signals of a methyl carbon at 26.3 ppm and a methyl proton at 2.88 ppm were observed in the <sup>13</sup>C and <sup>1</sup>H NMR spectral data of TSKK. The structure of TSKK was deduced by <sup>1</sup>H-<sup>1</sup>H COSY, HMQC and HMBC experiments. Figure 3 shows <sup>1</sup>H-<sup>1</sup>H couplings detected by COSY, and long-range couplings observed in the HMBC experiment. The correlation from the methyl protons at 2.88 ppm to a carbon at 167.1 ppm in the HMBC spectrum was observed. Moreover, a specific fragment suggesting an aminomethyl carbonyl ion was observed at  $m/z$  58 in the FAB-MS spectrum of TSKK. These results suggest that the additional methyl group of TSKK was substituted for a hydroxyl group in the hydroxamic acid moiety of TSA. Accordingly, the structure of TSKK was determined as

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR spectral data of TSKK in CDCl<sub>3</sub>.

C-No.	$\delta_C$	$\delta_H$ (multiplicity, $J=Hz$ )
1-NCH <sub>3</sub>	26.3	2.88 (d, 5.2, 3H)
1	167.1	
2	119.1	5.78 (d, 15.5)
3	144.7	7.16 (d, 15.5)
4	132.3	
4-CH <sub>3</sub>	12.6	1.89 (s, 3H)
5	140.0	5.97 (d, 9.8)
6	40.8	4.35 (dq, 9.4, 6.8)
6-CH <sub>3</sub>	17.6	1.30 (d, 6.6, 3H)
7	198.6	
8	123.9	
9, 13	130.5	7.84 (d, 7.2, 2H)
10, 12	110.6	6.63 (d, 7.2, 2H)
11	153.3	
11-N(CH <sub>3</sub> ) <sub>2</sub>	39.9	3.05 (s, 6H)

Fig. 3. Structure of TSKK.

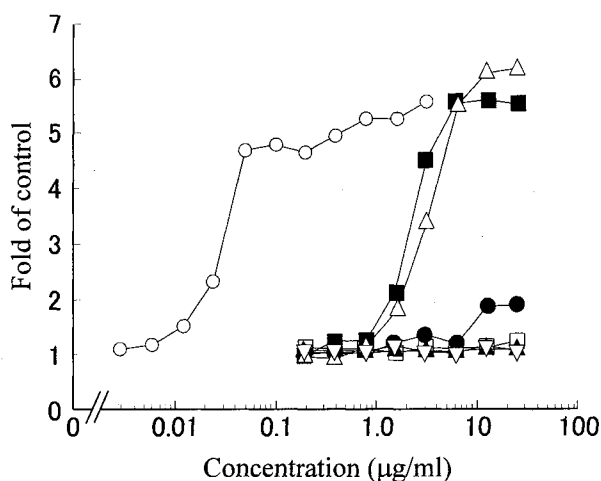


Bold bars and arrows present <sup>1</sup>H-<sup>1</sup>H COSY and HMBC correlations, respectively. Dashed lines with arrowheads indicate common fragments observed in FAB-MS spectra of trichostatin derivatives. Plain line with arrowhead shows specific fragment observed in FAB-MS spectrum of TSKK.

shown in Fig. 3.

The inducible activities of luciferase expression in H1299/tsp53-Luc cells by the trichostatin derivatives (Fig. 4) were measured the same as described in the accompanying report<sup>1)</sup>. TSA, TSacid and TSKK were

Fig. 4. Luciferase activity induced by trichostatin derivatives.



Symbols indicate the following; ○: trichostatin A, ■: trichostatic acid, ●: trichostatin RK, △: FL657C, □: trichostatin C, ▲: monomethyl trichostatin A, ▽: dimethyl trichostatin A.

isolated from the culture broth of the strain RK98-A74. Trichostatin C (TSC)<sup>2</sup> and FL657C<sup>4</sup> were isolated from another actinomycete strain RK98-A34, and monomethyl and dimethyl TSA were chemically synthesized. As shown in Fig. 4, TSA induced the luciferase activity over 4-fold at 50 ng/ml, TSacid and FL657C had the same effect at 2~3 µg/ml. However, TSRK, TSC, monomethyl and dimethyl TSA hardly induced activity. It is known that p21 promoter is activated by TSA due to its inhibitory activity against histone deacetylase (HDAC)<sup>5</sup>. A structure-activity relationship study of HDAC and TSA has revealed that the hydroxamic acid moiety of TSA is important for high affinity with the active-site cavity bearing the zinc ion in HDAC<sup>6</sup>. TSacid and FL657C reduced the inhibitory activity because of the loss of the hydroxamic acid moiety. It was reported that TSacid has no effect on cell differentiation, cell cycle<sup>7</sup> and *in vitro* HDAC activity<sup>8</sup>. However, TSacid induced the luciferase activity in H1299/tsp53-Luc cells in our assay system, which involved

its HDAC inhibition and other unknown mechanism. Induction of luciferase activity by TSRK, TSC, monomethyl and dimethyl TSA was not observed, because their bulky groups (methyl, dimethyl and glucosyl group) may obstruct binding to the active-site pocket of HDAC. The relationship between the trichostatin derivatives and luciferase activity is apparently consistent with the mode of action of trichostatin on HDAC inhibition

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