Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 1273–1276

Mevashuntin, a novel metabolite produced by inhibition of the mevalonate pathway in *Streptomyces prunicolor*

Kazuo Shin-ya,[a,*] Yukiko Umeda,[a] Shuhei Chijiwa,[a] Kazuo Furihata,[b] Keiko Furihata,[a] Yoichi Hayakawa[c] and Haruo Seto[d]

[a]*Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan*
[b]*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[c]*Faculty of Pharmaceutical Sciences, Tokyo University of Science Noda, Chiba 278-8510, Japan*
[d]*Tokyo University of Agriculture, Setagaya-ku, Tokyo 113-8657, Japan*

Received 22 November 2004; revised 15 December 2004; accepted 24 December 2004

Abstract—Inhibition of the mevalonate pathway by an HMG-CoA reductase inhibitor, mevalotin, in *Streptomyces prunicolor* possessing both mevalonate and MEP pathways resulted in the production of a new metabolite mevashuntin that consisted of conjugated thiazolone and pyranonaphthoquinone moieties.

Although microorganisms belonging to the genus *Actinomycetes* are good sources for production of bioactive secondary metabolites with structural diversity, their production of isoprenoids is quite limited in number.[1] They possess the MEP pathway (formerly called the nonmevalonate pathway) in common and utilize it for production of the starter units of isoprenoids, isopentenyl diphosphate (IPP) and dimethylally diphosphate (DMAPP).[1] Some members of this group, however, were proved by labeling experiments to use the mevalonate pathway for the biosynthesis of isoprenoids such as naphterpin,[2,3] furaquinocin,[4] terpentecin,[5] napyradiomycin[6] and BE-40644.[7]

In addition to these members, our Southern hybridization experiments proved the presence of the mevalonate pathway in other strains[8] such as *Streptomyces prunicolor* 1884-SVT2 that produced benthocyanins with an isoprenoid side chain.[9–11] Our detailed genetic studies revealed that the mevalonate pathway is utilized for the production of isoprenoids as secondary metabolites.[12–14] Thus we were interested in knowing what would happen if the mevalonate pathway were inhibited in these organisms. As a model experiment, we treated *S. prunicolor* 1884-SVT2 with a mevalonate pathway inhibitor, mevalotin, that caused the appearance of a new orange pigment (silica gel TLC, R_f 0.3, $CHCl_3$–MeOH = 10:1) with concomitant complete disappearance of benthocyanins in the fermentation broth. We wish to report herein the production of a new metabolite, designated as mevashuntin (**1**).

The producing strain 1884-SVT2 was cultivated in a seed medium consisting of starch 1.0%, polypepton 1.0%, molasses 1.0% and meat extract 1.0% (pH 7.2) for 3 days at 27 °C on a rotary shaker. The seed culture was inoculated into a production medium composed of starch 2.5%, soybean meal 1.5%, dry yeast 0.2%, $CaCO_3$ 0.4% (pH 6.4 before sterilization) and cultivated on a rotary shaker (200 rpm) at 27 °C. After 24 h cultivation, 500 μg/mL of mevalotin was added to the culture medium and incubated for a further 4 days. The whole culture broth was centrifuged to give mycelial cake, which was subjected to acetone extraction. The solvent extract was concentrated in vacuo to a small volume and the residual aqueous layer was extracted twice with EtOAc. The solvent layer was dried over Na_2SO_4, and concentrated to give an oily residue. This oily material was subjected to silica gel column chromatography using $CHCl_3$–MeOH (20:1) as a solvent system. The orange fraction was concentrated under reduced pressure and rechromatographed on a silica gel column developed

Keywords: Mevalotin; Naphthoquinone; Thiazolone; Mevalonate pathway; *Streptomyces prunicolor*.
* Corresponding author. Tel.: +81 3 5841 7840; fax: +81 3 5841 8485; e-mail: kshin@iam.u-tokyo.ac.jp

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.12.134

Table 1. Physico-chemical properties of **1**

Appearance	Orange powder
Mp	166–167 °C
$[\alpha]_D^{21}$	−415.5 (*c* 0.03, $CHCl_3$)
Molecular formula	$C_{22}H_{23}NO_7S$
HRFAB-MS (*m/z*)	Found: 446.1286 $[M+H]^+$
	Calcd: 446.1273
UV λ_{max} nm (ε)	
In MeOH	434 (6500), 326 (9300), 264 (31,100), 245 (22,200)
In MeOH + NaOH	565 (6700), 420 (sh, 2900), 326 (9300), 290 (sh, 9300), 242 (33,200)
IR: *ν* (KBr) cm^{-1}	3450, 1710, 1690, 1620, 1265 cm^{-1}

with $CHCl_3$–MeOH–concd aqueous NH_4OH (50:10:1). The orange fraction was concentrated in vacuo, and the residue was further purified by column chromatography on Toyopearl HW-40F developed with MeOH to give a pure sample of mevashuntin (**1**) as an orange powder.

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was established as $C_{22}H_{23}NO_7S$ by the high-resolution FAB-MS spectrum $[(M+H)^+$, *m/z* 446.1286 (calcd: 446.1273, +1.3 mmu error)]. IR absorptions at 3450, 1710, 1690 and 1620 cm^{-1} implied the presence of hydroxyl, amide and quinone groups.

The ^{13}C and ^1H NMR spectral data are tabulated in Table 2. The structure of **1** was elucidated by the interpretation of the DQF-COSY and the constant time HMBC (CT-HMBC) spectra.[15] The sequence from an oxy-methine proton 9-H (δ_H 5.07) to geminal methyl protons 17-H (δ_H 0.85) and 18-H (δ_H 0.83) through methylene protons 14-H (δ_H 2.12, 1.91), 15-H (δ_H 1.30, 1.05) and a methine proton 16-H (δ_H 1.51) revealed the presence of a 4-methylpentyl moiety as shown in Figure 2a. Moreover, the proton spin system from a methylene proton 6-H (δ_H 2.81) to methylene protons 12-H (δ_H 2.78, 2.67) through an oxymethine proton 7-H (δ_H 3.97, δ_C 69.0) was also deduced by DQF-COSY. Homoallylic coupling between 6-H and 9-H together with the long-range couplings from 7-H to C-9 (δ_C 74.6) reveled a dihydro-pyran like substructure as shown in Figure 2a. This partial structure was also confirmed by the ^1H–^{13}C long-range couplings from 6-H and 9-H to aromatic carbons C-5a (δ_C 144.0) and C-9a (δ_C 135.0) in the HMBC spectrum. The long-range couplings between 7-H and C-5a, 14-H and C-9a corroborated the assignments of these carbons. The methylene protons 12-H was long range coupled to a carbonyl carbon C-13 (δ_C 175.0), which was elucidated to be a carboxylic group by treatment with diazomethane to produce a methyl ester derivative of **1**.[16] Thus, these results proved the presence of the substituted dihydro-pyran substructure as shown in Figure 2a.

Table 2. ^{13}C and ^1H NMR chemical shifts of **1**

No.	δ_C	δ_H
2	169.8	
3a	137.9	
4	174.1	
4a	129.3	
5	121.0	7.40 (1H, s)
5a	144.0	
6	35.7	2.81 (2H, d, *J* = 6.0 Hz)
7	69.0	3.97 (1H, quintet, *J* = 8.0 Hz)
9	74.6	5.07 (1H, br dd, *J* = 5.5, 2.0 Hz)
9a	135.0	
10	158.7	
10a	111.7	
11	182.0	
11a	125.9	
12	40.1	2.78 (1H, dd, *J* = 16.5, 7.5 Hz)
		2.67 (1H, dd, *J* = 16.5, 6.0 Hz)
13	175.0	
14	31.7	1.91 (1H, m)
		2.12 (1H, m)
15	33.7	1.30 (1H, m)
		1.05 (1H, m)
16	27.9	1.51 (1H, m, *J* = 6.0 Hz)
17	22.8	0.85 (3H, d, *J* = 6.0 Hz)
18	22.3	
19	32.2	3.77 (3H, s)
10-OH		12.33 (1H, s)

^{13}C and ^1H were observed at 125 MHz and 500 MHz, respectively.

The UV and visible spectra of **1** showed the typical absorption of a naphthoquinone with phenolic hydroxyl residue as a chromophore.[2] A phenolic hydroxyl proton 10-OH (δ_H 12.33), which is hydrogen-bonded with a quinone carbonyl, was long-range coupled to C-5a, C-9a, C-10 (δ_C 158.7) and C-10a (δ_C 111.7). An aromatic proton 5-H (δ_H 7.40) was strongly long-range coupled to *meta* carbons, C-9a and C-10a, the quinone carbonyl carbon C-4 (δ_C 174.1) and the methylene carbon C-6 at a *peri*-position. The moderate long-range couplings between 5-H and C-4a (δ_C 129.3), C-5a, C-10 and the other quinone carbonyl carbon C-11 (δ_C 182.0) were also observed. Thus, the pyranonaphthoquinone moiety of **1** was established as shown in Figure 2a.

A thiazolone moiety as the remaining unit was elucidated by CT-HMBC experiments taken under normal and specific conditions. In the CT-HMBC spectrum, methylamino protons 19-CH_3 (δ_H 3.77, δ_C 32.2) was long-range coupled to an amide carbonyl carbon C-2 (δ_C 169.8) and an aromatic carbon C-3a (δ_C 137.9). The molecular formula of **1** implied that the remaining components were assigned to a sulfur atom and an aromatic carbon C-11a (δ_C 125.9), which should be the member of naphthoquinone moiety. Thus, the remaining substructure was deduced to be an *N*-methylthiazolone moiety as shown in Figure 2b.

Lack of any correlations between naphthoquinone and thiazolone moieties left two possible combinations of these two moieties. In the CT-HMBC experiment, which enabled us to observe weak correlations by employing the longer delay time, and the methylamino proton 19-CH_3 showed long-range couplings to the quinone carbonyl carbon C-4 (Fig. 3).[17] Furthermore, a weak long-range coupling from 19-CH_3 to C-4a was also recognized in this spectrum (shown by dotted lines in Fig. 3). Thus the structure of **1** was determined as shown in Figure 1.

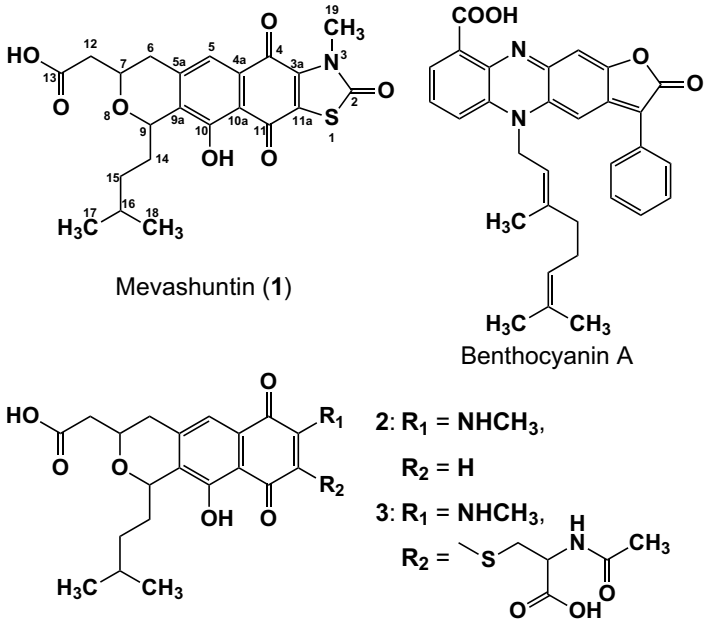

Figure 1. Structures of mevashuntin (**1**), benthocyanin A and structurally resembled naphthoquinone derivatives (**2** and **3**).

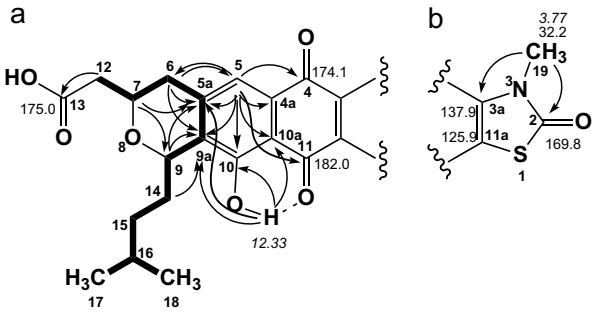

Figure 2. The NMR analyses of DQF COSY and general conditioned HMBC experiments. Bold line shows proton–proton couplings in DQF-COSY, and allows show long-range couplings in HMBC.

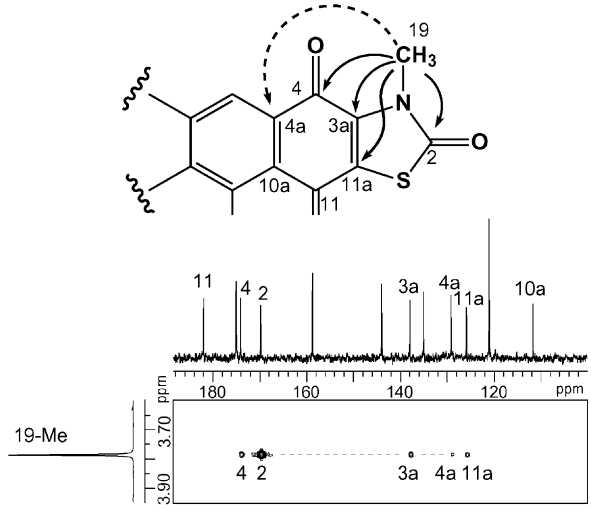

Figure 3. The correlation between naphthoquinone and thiazolone moieties revealed by special conditioned HMBC experiments. Allows us to show long-range couplings and the dotted allows show weak couplings.

In addition to **1**, treatment of *S. prunicolor* 1884-SVT2 with mevalotin also induced production of several orange or yellow pigments, which were not produced under normal culture condition. **1** is structurally related to pyranonaphthoquinones (Fig. 1) reported by Kulanthaivel et al.,[18] but it differs from them in possessing the chromophore consisting of a thiazolone fused to a naphthoquinone nucleus. It should be emphasized that this chromophore in **1** is the first example found in not only natural products but also in synthetic compounds (even thiazolone fused to benzoquinone is a novel skeleton). The pyranonaphthoquinones were described to show weak inhibitory activities against cdc25A, a family of protein phosphatases, which progresses cell cycle progression.[18] Biological activities of **1** are now under investigation.

Acknowledgements

This work was supported in part by Industrial Technology Research Grant Program in 01A04006b from New Energy and Industrial Technology Development Organization (NEDO) of Japan to K.S. and a Grant-in-Aid for Scientific Research (B), the Ministry of Education, Science, Sports and Culture to H.S. (14360067).

References and notes

1. Kuzuyama, T.; Seto, H. *Nat. Prod. Res.* **2003**, *20*, 171–183.
2. Shin-ya, K.; Imai, S.; Furihata, K.; Hayakawa, Y.; Kato, Y.; VanDuyne, G. D.; Clardy, J.; Seto, H. *J. Antibiot.* **1990**, *43*, 444–447.
3. Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **1990**, *31*, 6025–6026.
4. Funayama, S.; Ishibashi, M.; Komiyama, K.; Omura, S. *J. Org. Chem.* **1990**, *55*, 1132–1133.

5. Isshiki, K.; Tamamura, T.; Sawa, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1634–1635.
6. Shimoi, K.; Iinuma, H.; Naganawa, H.; Isshiki, K.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1987**, *40*, 1740–1745.
7. Seto, H.; Orihara, N.; Furihata, K. *Tetrahedron Lett.* **1998**, *39*, 9497–9500.
8. Kuzuyama, T.; Takahashi, S.; Dairi, T.; Seto, H. *J. Antibiot.* **2002**, *55*, 919–923.
9. Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H.; Kato, Y.; Clardy, J. *Tetrahedron Lett.* **1991**, *32*, 943–946.
10. Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Org. Chem.* **1993**, *58*, 4170–4172.
11. Shin-ya, K.; Hayakawa, Y.; Seto, H. *J. Nat. Prod.* **1993**, *56*, 1255–1258.
12. Hamano, Y.; Dairi, T.; Yamamoto, M.; Kuzuyama, T.; Itoh, N.; Seto, H. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 808–819.
13. Kawasaki, T.; Kuzuyama, T.; Furihata, K.; Itoh, N.; Seto, N.; Dairi, T. *J. Antibiot.* **2003**, *56*, 957–966.
14. Dairi, T.; Hamano, Y.; Kuzuyama, T.; Itoh, N.; Furihata, K.; Seto, H. *J. Bacteriol.* **2001**, *183*, 6085–6094.
15. Furihata, K.; Seto, H. *Tetrahedron Lett.* **1998**, *39*, 7337–7340.
16. Methyl ester of **1**. $C_{23}H_{26}NO_7S$, HR-FABMS: found 460.1456 (+2.6 mmu error) $[M+H]^+$. The NMR data for the methyl ester derivative of **1** are as follows: ^1H NMR (δ_H, $CDCl_3$ at 500 MHz): 12.34 (s, 10-OH), 7.41 (s, 5-H), 2.78 (br dd, J = 6.0, 2.0 Hz, 6-H), 3.96 (m, 7-H), 5.03 (m, 9-H), 2.73 (dd, J = 16.0, 7.0 Hz, 12-Ha), 2.60 (dd, J = 16.0, 6.0 Hz, 12-Hb), 2.12 (m, 14-Ha), 1.89 (m, 14-Hb), 1.29 (m, 15-Ha), 1.04 (m, 15-Hb), 1.50 (m, 16-H), 0.84 (d, J = 6.0 Hz, 17-H), 0.82 (d, J = 6.0 Hz, 18-H), 3.77 (s, 19-H), 3.72 (s, 13-OCH_3). ^{13}C NMR (δ_C, $CDCl_3$ at 125 MHz): 169.8 (C-2), 137.9 (C-3a), 174.2 (C-4), 129.1 (C-4a), 121.1 (C-5), 144.4 (C-5a), 35.9 (C-6), 69.3 (C-7), 74.5 (C-9), 135.4 (C-9a), 158.8 (C-10), 111.7 (C-10a), 182.0 (C-11), 125.9 (C-11a), 40.4 (C-12), 171.2 (C-13), 31.7 (C-14), 33.8 (C-15), 27.9 (C-16), 22.8 (C-17), 22.4 (C-18), 32.2 (C-19), 51.8 (13-OCH_3).
17. HMBC delay time was usually set to 60 ms. To observe weak correlations (special condition), HMBC delay time was set to 500 ms.
18. Kulanthaivel, P.; Perun, T. J., Jr.; Belvo, M. D.; Strobel, R. J.; Paul, D. C.; Williams, D. C. *J. Antibiot.* **1999**, *52*, 256–262.