NOTES

A New Inhibitor of Melanogenesis, Albocycline K3, Produced by *Streptomyces* sp. OH-3984

SATOSHI TAKAMATSU, YONG-PIL KIM, MASAHIKO HAYASHI, KANKI KOMIYAMA, GENJI IMOKAWA† and SATOSHI ŌMURA*

The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan
†Kao Corporation, Biological Science Laboratories, 2606 Akabane, Ichikaimachi, Haga, Tochigi 321-34, Japan

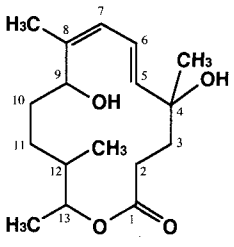
(Received for publication December 20, 1995)

In our continuing a search for melanogenesis inhibitors of microbial origin, we have reported albocyclines K1 and K2, which were produced by *Streptomyces* sp. OH-3984[1,2]. The absolute configuration of both compounds was clarified by chemical conversion[3]. A further study of the strain led to discovery of another macrocyclic compound named albocycline K3 (**1**) (Fig. 1). This paper describes the isolation, physicochemical properties, structure determination and biological characteristics of **1**.

The fermentation and isolation of **1** were carried out in the same way as reported previously[1]. A stock culture of the producing organism was inoculated into a test tube (i.d. 2×20 cm) containing 10 ml of seed medium consisting of 2% glucose, 0.5% peptone, 0.3% dry yeast, 0.5% meat extract, 0.5% NaCl and 0.3% $CaCO_3$ (pH 7.0 before sterilization). The tube was incubated at 27°C for 72 hours on a reciprocal shaker. Then, 2 ml portions of the culture were transferred to a 500-ml Erlenmeyer flask containing 100 ml of the seed medium. The flask was incubated at 27°C for 48 hours on a rotary shaker (210 rpm), and 400 ml of the resulting culture was transferred into a 50-liter fermenter containing 30 liters of the same medium as described above. The fermentation was carried out at 27°C for 96 hours of an agitation rate of 160 rpm and an aeration rate of 60 liters per minute.

The fermentation broth of *Streptomyces* sp. OH-3984 (30 liters) was extracted with EtOAc (25 liters), and the EtOAc layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a brown syrup (10.0 g). The ethyl acetate extracts were chromatographed on a silica gel (70~230 mesh; i.d. 5×36 cm) column using $CHCl_3$-acetone (8:2). Finally, isolation of the active fraction by preparative HPLC (Cosmosil 5C18-AR packed column, i.d. 20×250 mm, Nacalai Tesque) using a solvent system of MeOH-H_2O (80:20) gave **1** (33 mg) and albocycline (800 mg), respectively.

Fig. 1. Structure of **1**.



The physicochemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined as $C_{17}H_{28}O_4$ by HR positive-FAB mass analysis. The IR absorption at 1770 cm^{-1} of **1** showed the presence of a lactone group. Acetylation of **1** with acetic anhydride in pyridine at room temperature gave the diacetate of **1**, which showed that **1** has two hydroxy groups in the molecule. The ^{13}C NMR spectrum of **1** showed 17 carbon signals and the DEPT spectrum of **1** indicated the presence of four methyl, four methylene, six methine and three tertiary carbon signals. In the ^1H NMR spectrum of **1**, two tertiary methyl signals observed at δ 0.83 (3H, d, J=5.9 Hz, 12Me) and δ 1.20 (3H, d, J=6.3 Hz, 13Me) were characteristic of derivatives of the albocyclines[4]. Furthermore, the ^{13}C and ^1H NMR chemical shifts between C-1 and C-5 were similar to those of 2,3-dihydroalbocycline[2]. However, the signal of the methoxy group, a characteristic functional group of albocycline, was not observed. In the ^1H-^1H COSY spectrum of **1**, the connection from C-5 to C-13 *via* a long range coupling between H-7 (δ 6.09) and H-9 (δ 3.70) was demonstrated. Final confirmation of structure **1** was undertaken using HMBC experiments (J=8 Hz) summarized in Table 2. These results clearly indicated that the structure is the derivative of albocycline without a methoxy group as shown in the Fig. 1. Although this structure is similar to that of cineromycin B[5], a 14-membered macrocyclic compound without a methoxy group, the physicochemical and biological properties of **1** are different from those of cineromycin B. Cineromycin B was reported as a

Table 1. Physicochemical data of **1**.

Appearance	Colorless oil
$[\alpha]_D^{24}$	−10.0° (*c*. 0.4, MeOH)
Molecular formula	$C_{17}H_{28}O_4$
UV λ_{max}^{MeOH} nm	End absorption
IR ν_{max}^{KBr} cm^{-1}	3448, 2927, 1770, 1365
Pos. FAB-MS (*m/z*)	297 $(M+H)^+$
HR Pos. FAB-MS (*m/z*)	Obsd. 319.0768 ($C_{17}H_{28}O_4Na$)
	Calcd. 319.0753
Color reaction	
Positive	50% H_2SO_4 + Δ, Iodine
Negative	Dragendorff's reagent, Ehrlich's reagent + Δ
	Ninhydrin reagent

Table 2. ^{13}C NMR, ^{1}H NMR and HMBC ($J=8$ Hz) data of **1** in $CDCl_3$.

No.	^{13}C	M	^{1}H (M, J value in Hz)	HMBC ($^{1}H \rightarrow ^{13}C$)
1.	176.8	*s*		
2.	28.9	*t*	2.56 (2H, m)	C-1, C-3, C-4
3.	34.5	*t*	2.18, 2.09 (1H, m, each)	C-1, C-2, C-4, 4-Me, C-5
4.	85.6	*s*		
5.	134.1	*d*	5.69 (1H, d, J=15.2)	C-3, C-4, 4-Me, C-6, C-7
6.	124.8	*d*	6.48 (1H, dd, J=15.2, 10.9)	C-4, C-5, C-7, C-8
7.	122.6	*d*	6.09 (1H, br.d, J=10.9)	C-5, C-6, C-8, 8-Me, C-9
8.	140.9	*s*		
9.	81.8	*d*	3.70 (1H, br.d, J=10.9)	C-8, 8-Me, C-10, C-11
10.	30.9	*t*	1.66 (2H, m)	C-8, C-9, C-11, C-12
11.	32.9	*t*	1.79 (1H, m), 1.26 (1H, m)	C-9, C-10, C-12, 12-Me, C-13
12.	37.1	*d*	1.29 (1H, m)	C-11, 12-Me, C-13, 13-Me
13.	79.8	*d*	3.11 (1H, m)	C-1, C-11, C-12, 12-Me, 13-Me
4-Me	26.8	*q*	1.52 (3H, s)	C-3, C-4, C-5
8-Me	13.9	*q*	1.77 (3H, s)	C-7, C-8, C-9
12-Me	17.9	*q*	0.83 (3H, d, J=5.9)	C-11, C-12, C-13
13-Me	19.7	*q*	1.20 (3H, d, J=6.3)	C-12, C-13

de-*O*-methoxy-derivative of albocycline which showed the following properties: a neutral colorless plate, mp 149~150°C, $[\alpha]_D^{24}$ −110° (*c* 1.0, MeOH), molecular formula ($C_{17}H_{26}O_4$), molecular weight (294) and antibacterial activity, however, the structure of cineromycin B was not clearly elucidated[6].

Albocycline K3 (**1**) inhibited the melanogenesis of B16 melanoma cells at a concentration of 15.0 μg/ml without cytotoxicity using the previously described method[1]. Albocycline K3 (**1**) showed no antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi or yeast at a concentration of 1.0 mg/ml in the paper disc method reported previously[7]. Recently, the search for new melanin biosynthesis inhibitors of microbial origin has been performed using some unique screening methods, and isonitrile antibiotics, trichoviridin[8] and MR304A[9] were reported to inhibit not only melanogenesis but also mushroom tyrosinase. However, the mechanism of action of **1** is unknown since no inhibition of tyrosinase activity was observed by the method of AKIU *et al.*[10] and POMERANTZ *et al.*[11]. Therefore, it is of interest to examine the mechanism of the inhibitory effect of albocycline K3 (**1**) on melanogenesis.

Acknowledgments

This work was supported in part by funds from the Japan Keirin Association.

References

1) KOMIYAMA, K.; S. TAKAMATSU, Y. TAKAHASHI, M. SHINOSE, M. HAYASHI, H. TANAKA, Y. IWAI, S. ŌMURA & G. IMOKAWA: New inhibitors of melanogenesis, OH-3984 K1 and K2: I. Taxonomy, fermentation, isolation and biological characteristics. J. Antibiotics 46: 1520~1525, 1993
2) TAKAMATSU, S.; M.-C. RHO, M. HAYASHI, K. KOMIYAMA, H. TANAKA, S. ŌMURA & G. IMOKAWA: New inhibitors of melanogenesis, OH-3984 K1 and K2: II. Physicochemical properties and structural elucidation. J. Antibiotics 46: 1526~1529, 1993
3) SUNAZUKA, T.; R. OBATA, Z. LI, S. TAKAMATSU, K. KOMIYAMA, S. ŌMURA & A. B. SMITH, III: Relative and absolute stereochemistry of the melanogenesis inhibitors, OH-3984 K1 and K2. Partial synthesis from albocycline. Tetrahedron Letters 35: 2635~2636, 1995
4) HARADA, K.; F. NISHIDA, H. TAKAGI, M. SUZUKI & T. IWASHITA: Studies on an antibiotic, albocycline VII. Minor components of albocycline. J. Antibiotics 37: 1187~1197, 1984
5) Dictionary of Antibiotics and related substance. *Eds.*, B. W. BYCROFT, pp. 24, Chapman and Hall, 1988
6) MIYAIRI, N.; M. TAKASHIMA, K. SHIMIZU & H. SAKAI: Studies on new antibiotics, cineromycins A and B. J. Antibiotics, Ser. A 19: 56~62, 1966
7) KOMIYAMA, K.; S. TAKAMATSU, Y.-P. KIM, A. MATSUMOTO, Y. TAKAHASHI, M. HAYASHI, H. B. WOODRUFF & S. ŌMURA: Louisianins A, B, C and D: non-steroidal growth inhibitors of testosterone-responsive SC 115 cells. I. Taxonomy, fermentation, isolation and biological characteristics. J. Antibiotics 48: 1086~1089, 1995
8) HASHIMOTO, R.; S. TAKAHASHI, K. HAMANO, T. MORI & A. NAKAGAWA: New method for screening the melanin biosynthesis inhibitor by using the larval haemolymph of the silkworm, *Bombyx mori*, and the inhibitory activity of trichoviridin. Biosci. Biotech. Biochem. 58: 1725~1726, 1994
9) LEE, C. H.; H. KOSHINO, M. C. CHUNG, H. J. LEE & Y. H. KHO: MR304A, a new melanin synthesis inhibitor produced by *Trichoderma harzianum*. J. Antibiotics 48: 1168~1170, 1995
10) AKIU, S.; Y. SUZUKI, T. ASAHARA, Y. FUJINUMA & M. FUKUDA: Inhibitory effect of arbutin on melanogenesis—Biochemical study using cultured B16 melanoma cells—. Jpn. J. Dermatol. 101: 609~613, 1991
11) POMERANTZ, S. H.: Separation, purification and properties of two tyrosinases from hamster melanoma. J. Biol. Chem. 238: 2351~2357, 1963