NEW INHIBITORS OF MELANOGENESIS, OH-3984 K1 AND K2

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURAL ELUCIDATION

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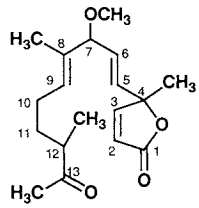
New melanin synthesis inhibitors, OH-3984 K1 and K2, were isolated from the fermentation broth of *Streptomyces* sp. OH-3984, and their structures were elucidated by spectroscopic methods and by chemical transformations. OH-3984 K1 (M.W.: 306; $C_{18}H_{26}O_4$) and K2 (M.W.: 308; $C_{18}H_{28}O_4$) have unique γ-lactone rings, both of which correspond to oxidative products derived from C_1-O_{14} cleavage of the 14-membered lactone group.

In our continuing search for novel antibiotics showing melanin synthesis inhibitory activity, two new antibiotics, OH-3984 K1 (**1**) and K2 (**2**) were isolated from the culture broth of *Streptomyces* sp. OH-3984, together with three known macrolide antibiotics, albocycline (**4**), 2,3-dihydroalbocycline (**5**) and 2,3-dihydro-11-hydroxyalbocycline (**6**) (Fig. 1)[1]. The taxonomic studies of the producing strain, the isolation procedure and the biological characteristics of **1** and **2** were reported in a previous paper[2]. This paper

Fig. 1. Structures of OH-3984 K1, K2 and albocyclines.



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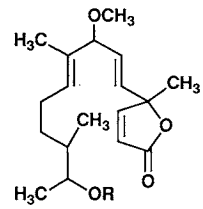
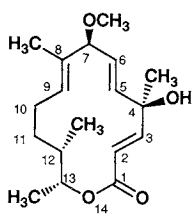


2 R=H
3 R=$COCH_3$



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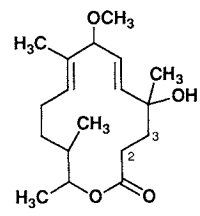

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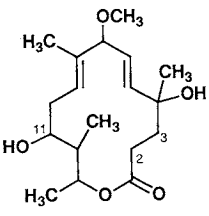

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Table 1. Physico-chemical properties of OH-3984 K1 (**1**) and K2 (**2**).

	OH-3984 K1 (**1**)	OH-3984 K2 (**2**)
Appearance	Colorless oil	Colorless oil
Optical rotation $[\alpha]_D^{24}$	$-154.9°$ (*c* 0.366, EtOH)	$-167.9°$ (*c* 0.927, EtOH)
Molecular formula	$C_{18}H_{26}O_4$	$C_{18}H_{28}O_4$
MW	306	308
UV λ_{max} (MeOH)	End absorption	End absorption
IR ν_{max} ($CHCl_3$) cm^{-1}	2975, 2925, 1740, 1700, 1440, 1350, 1090, 940	3600, 3475, 1750, 1440, 1375, 1220, 1100, 940, 820
Pos. FAB-MS (*m/z*)	307 $(M+H)^+$	309 $(M+H)^+$
HREI-MS (*m/z*)		
Obsd.	306.18229	308.19864
Calcd. for $C_{18}H_{26}O_4$	306.18236	308.19864
HR Pos. FAB-MS		
Obsd.	329.1713	331.1859
Calcd. for $C_{18}H_{26}O_4Na$	329.1729	331.1855
Color reaction		
Positive	50% $H_2SO_4+\Delta$, iodine	50% $H_2SO_4+\Delta$, iodine,
Negative	Ninhydrin reagent, DRAGENDORFF's reagent	Ninhydrin reagent, DRAGENDORFF's reagent

outlines the determination of the structure of **1** and **2**.

Results

Structures of OH-3984 K1 (**1**) and K2 (**2**)

Physico-chemical properties of **1** and **2** are summarized in Table 1. Compound **1** was isolated as a colorless oil. The molecular formula of **1** was determined as $C_{18}H_{26}O_4$ by HR-FAB mass spectrum. The IR absorptions at 1740 cm^{-1} and 1700 cm^{-1} of **1** showed the presence of an α,β-unsaturated γ-lactone ring and a carbonyl function, respectively. In the ^{13}C NMR spectrum of **1** (Table 2), the signal of C-13 was observed in the downfield region compared with that of albocycline (**4**). In the ^1H NMR spectrum of **1** (Table 3), the methyl signal at C-13 was shifted downfield in the range of 0.9 ppm compared with that of **4** and appeared as a singlet, while the methyl signal at C-13 of **4** was observed as a doublet. From these results, the carbon at C-13 of **1** was evidently a ketonic group which is attached to the methyl group at δ 2.12. The tertiary carbon signal of C-4 of **1** was observed in the downfield range compared with that of **4**. The olefinic proton signals of C-2 (δ 5.98, d, $J=5.5$ Hz) and C-3 (δ 7.36, d, $J=5.5$ Hz) were also shifted downfield in the range of 0.11 ~ 0.48 ppm compared with those of **4** (C-2, δ 5.87, d, $J=16.0$ Hz, *trans*; C-3, δ 6.88, d, $J=16.0$ Hz, *trans*). Therefore, the presence of an α, β, unsaturated γ-lactone ring was anticipated, and this was also supported by a consideration of the IR

Table 2. ^{13}C NMR chemical shifts of OH-3984 K1 (**1**), K2 (**2**) and albocyclines (**4**, **5**)[a].

C	(1)	(2)	(4)	(5)
1	172.3 (s)	172.3 (s)	166.2 (s)	173.6 (s)
2	119.7 (d)	119.5 (d)	115.4 (d)	30.4 (t)
3	159.4 (d)	159.4 (d)	154.7 (d)	36.9 (t)
4	87.6 (s)	87.6 (s)	73.2 (s)	72.2 (s)
5	128.8 (d)	128.5 (d)	136.5 (d)	137.3 (d)
6	131.4 (d)	131.5 (d)	130.8 (d)	128.7 (d)
7	86.0 (d)	85.9 (d)	84.8 (d)	87.8 (d)
8	133.8 (s)	132.8 (s)	135.9 (s)	136.3 (s)
9	128.6 (d)	129.7 (d)	129.1 (d)	127.6 (d)
10	25.3 (t)	25.2 (t)	24.7 (t)	22.6 (t)
11	32.3 (t)	32.0 (t)	34.2 (t)	31.8 (t)
12	46.6 (d)	39.5 (d)	39.0 (d)	35.4 (d)
13	212.3 (s)	71.3 (d)	75.6 (d)	72.4 (d)
7-OMe	55.7 (q)	55.5 (q)	57.0 (q)	55.5 (q)
4-Me	24.0 (q)	23.8 (q)	27.0 (q)	11.2 (q)
8-Me	11.1 (q)	10.9 (q)	14.0 (q)	30.3 (q)
12-Me	16.2 (q)	14.3 (q)	15.7 (q)	15.1 (q)
13-Me	28.1 (q)	19.3 (q)	17.8 (q)	15.0 (q)

[a] δ ppm from TMS in $CDCl_3$.
( ): Multiplicity.

Table 3. ^{1}H NMR chemical shifts of OH-3984 K1 (**1**), K2 (**2**), acetate of K2 (**3**) and albocyclines (**4**, **5**)[a].

H	(1)	(2)	(3)	(4)	(5)
2	5.98 (1H, d, 5.5)	6.00 (1H, d, 5.6)	6.00 (1H, d, 5.6)	5.87 (1H, d, 16.0)	2.42 (1H, ddd)[f]
2′	—	—	—	—	2.26 (1H, ddd)[g]
3	7.36 (1H, d, 5.5)	7.40 (1H, d, 5.6)	7.38 (1H, d, 5.6)	6.88 (1H, d, 16.0)	1.94 (1H, ddd)[h]
3′	—	—	—	—	1.82 (1H, ddd)[i]
5	5.73 (1H, d, 16.0)	5.72 (2H, d, 2.0)	5.72 (2H, d, 2.3)	5.77 (1H, d, 15.9)	5.53 (1H, dd)[j]
6	5.67 (1H, d, 16.0)	5.72 (2H, d, 2.0)	5.72 (2H, d, 2.3)	5.64 (1H, dd)[e]	5.82 (1H, dd)[k]
7	3.94 (1H, d, 3.0)	3.98 (1H, d, 2.3)	3.97 (1H, br s)	4.06 (1H, br d, 5.7)	4.05 (1H, d, 6.6)
9	5.37 (1H, t, 7.0)	5.43 (1H, t, 6.9)	5.40 (1H, t, 7.1)	5.28 (1H, br t, 6.0)	5.31 (1H, br dd)[l]
10	2.02 (2H, dd)[b]	2.06 (2H, m)	2.07 (2H, m)	2.22～2.08 (1H, m)	2.19 (1H, ddd)[m]
10′	2.02 (2H, dd)[b]	2.06 (2H, m)	2.07 (2H, m)	1.90～1.76 (1H, m)	2.02 (1H, m)
11	1.73 (1H, dddd)[c]	1.64～1.48 (2H, m)	1.73～1.40 (2H, m)	1.30～1.16 (2H, m)	1.48 (1H, ddd)[n]
11′	1.38 (1H, dddd)[d]	1.25～1.15 (1H, m)	1.26～1.18 (1H, m)	1.30～1.16 (2H, m)	1.35 (1H, m)
12	2.50 (1H, dq, 14.7)	1.64～1.48 (2H, m)	1.73～1.40 (2H, m)	1.50～1.38 (1H, m)	1.73 (1H, septet, 6.0)
13	—	3.66 (1H, quintet, 5.9)	4.82 (1H, quintet, 6.3)	4.55 (1H, dq, 6.0)	4.56 (1H, quintet, 6.0)
7-OMe	3.18 (3H, s)	3.20 (3H, s)	3.20 (3H, s)	3.29 (3H, s)	3.20 (3H, s)
4-Me	1.55 (3H, s)	1.56 (3H, s)	1.58 (3H, s)	1.54 (3H, s)	1.30 (3H, s)
8-Me	1.46 (3H, s)	1.49 (3H, s)	1.49 (3H, s)	1.64 (3H, s)	1.61 (3H, s)
12-Me	1.09 (3H, d, 6.0)	0.96 (3H, d, 6.6)	0.91 (3H, d, 6.9)	0.89 (3H, d, 6.4)	0.91 (3H, d, 7.0)
13-Me	2.12 (3H, s, 6.0)	1.13 (3H, d, 6.3)	1.15 (3H, d, 6.3)	1.22 (3H, d, 6.4)	1.11 (3H, d, 6.0)
Acetyl	—	—	2.04 (3H, s)	—	—

[a] δ ppm from TMS in $CDCl_3$. [b] J=15.0, 12.0 Hz. [c] J=14.0, 7.0, 7.0, 2.0 Hz. [d] J=14.0, 7.0, 7.0, 2.0 Hz. [e] J=15.9, 5.7 Hz. [f] J=15.6, 11.6, 4.0 Hz. [g] J=15.6, 11.0, 4.0 Hz. [h] J=14.7, 9.0, 4.0 Hz. [i] J=14.7, 11.9, 4.0 Hz. [j] J=15.8, 1.2 Hz. [k] J=15.8, 6.6 Hz. [l] J=10.0, 4.5 Hz. [m] J=14.3, 10.0, 4.0 Hz. [n] J=14.0, 10.5, 6.0 Hz.

band at 1740 cm^{-1}.[1] Final confirmation of the structure of **1** was undertaken using HMBC experiments. In the HMBC spectrum of **1**, correlation peaks between the proton signal at C-3 and the γ-lactone carbonyl signal at C-1, and the methyl signals at the C-12 and C-13 positions and the carbonyl signal at C-13 were observed. Other partial structural connections of **1** were established by H-H COSY and H-C COSY spectroscopy. These results clearly indicated that the structure of **1** was as shown in Fig. 1.

The molecular formula of **2** was assigned as $C_{18}H_{28}O_4$ based on the HR-FAB mass spectrum. The IR spectrum of **2** (Table 1) showed absorptions at 3600 cm^{-1} (OH) and an α,β-unsaturated γ-lactone ring at 1750 cm^{-1}. Acetylation of **2** with acetic anhydride in pyridine at room temperature gave the monoacetate (**3**), which showed that **2** has a hydroxy group in the molecule. In the ^{13}C NMR spectrum of **2**, the signals were very similar to those of **1**, except for the carbon signal at C-13 and its methyl signal. The latter two signals were similar to those of **4**. The proton signal at C-13 of **2** shifted upfield in the range of 0.89 ppm compared with that of **4**. Furthermore, the C-13 proton-signal of **2** was shifted downfield in the range of 1.16 ppm (δ 3.66→δ 4.82) with acetylation of **2**. Final elucidation of the structure of **2** was performed using HMBC experiments. In the HMBC spectrum of **2**, the cross peak between the proton signal at C-13 and the γ-lactone carbonyl signal at C-1 was not observed unlike in that of **4**. From these results, the structure of **2** was determined as shown in Fig. 1.

Discussion

We isolated two inhibitors of melanogenesis of B16 melanoma cells, **1** and **2**, with three albocycline group macrolides (**4**, **5** and **6**). Among these compounds, **5** was reported as a biomodification product by SLECHTA *et al.*[3], although the NMR chemical shifts were not described in detail in their paper. Compound

6 was reported as a minor component of **4** by HARADA *et al.*[1] Compounds **1** and **2** have unique γ-lactone rings, both of which correspond to oxidative products derived from the C_1-O_{14} cleavage of **4**. A similar relationship in the structure has been found between macrocyclic 12-hydroxyalbocycline (M-2) and γ-lactone-type M-8[1]. However, inhibition of melanogenesis by these microbial metabolites has not been reported previously. It is of interest to examine the mechanisms of action of the metabolites, since they do not show inhibitory activity against tyrosinase[2].

Melanin synthesis inhibitors of microbial origin have been reported by several investigators[4~10], but most of them were known to be tyrosinase inhibitors. Therefore, the present results are of interest in the search for prospective antibiotics showing melanin synthesis inhibitory activity.

Experimental

UV spectra were recorded on a Shimadzu model UV-160A spectrophotometer. IR spectra were taken with a JASCO model A-102 infrared spectrophotometer. MS were obtained with a JEOL model DX-300 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 270. Preparative HPLC was performed using a YMC-Pack D-ODS-5 (5 μm, i.d. 20 × 250 mm) column with a solvent system of MeOH-H_2O (70:30) at 220 nm.

Acknowledgments

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References

1) 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
2) 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
3) SLECHTA, L.; J. CIALDELLA & H. HOEKSEMA: Biomodification of albocycline by *Streptomyces venezuelae*. J. Antibiotics 31: 319~323, 1978
4) ARGOUDELIS, A. D.; F. REUSSER, S. A. MIZSAK & L. BACZYNSKYJ: Antibiotics produced by *Streptomyces ficellus*. II. Feldamycin and nojirimycin. J. Antibiotics 29: 1007~1014, 1976
5) ARGOUDELIS, A. D.; S. A. MIZSAK, L. BACZYNSKYJ & R. J. WNUK: The structure of feldamycin. J. Antibiotics 29: 1117~1119, 1976
6) TOMITA, K.; N. ODA, M. OHBAYASHI, H. KAMEI, T. MIYAKI & T. OKI: A new screening method for melanin biosynthesis inhibitors using *Streptomyces bikiniensis*. J. Antibiotics 43: 1601~1605, 1990
7) IMAE, K.; H. KAMACHI, H. YAMASHITA, T. OKITA, S. OKUYAMA, T. TSUNO, T. YAMASAKI, Y. SAWADA, M. OHBAYASHI, T. NAITO & T. OKI: Synthesis, stereochemistry, and biological properties of the depigmenting agents, melanostatin, feldamycin and analogs. J. Antibiotics 44: 76~85, 1991
8) ISHIHARA, Y.; M. OKA, M. TSUNAKAWA, K. TOMITA, M. HATORI, H. YAMAMOTO, H. KAMEI, T. MIYAKI, M. KONISHI & T. OKI: Melanostatin, a new melanin synthesis inhibitor. Production, isolation, chemical properties, structure and biological activity. J. Antibioties 44: 25~32, 1991.
9) TERAO, M.; K. TOMITA, T. OKI, L. TABE, M. GIANNI & E. GARATTINI: Inhibition of melanogenesis by BMY-28565, a novel compound depressing tyrosinase activity in B16 melanoma cells. Biochem. Pharmacol. 43: 183~189, 1992
10) OHYAMA, Y. & Y. MISHIMA: Melanogenesis-inhibitory effect of kojic acid and its action mechanism. Fragrance J. No. 6: 53~58, 1990