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Deacetylravidomycin M, a New Inhibitor of IL-4 Signal Transduction, Produced by *Streptomyces* sp. WK-6326

II. Structure Elucidation

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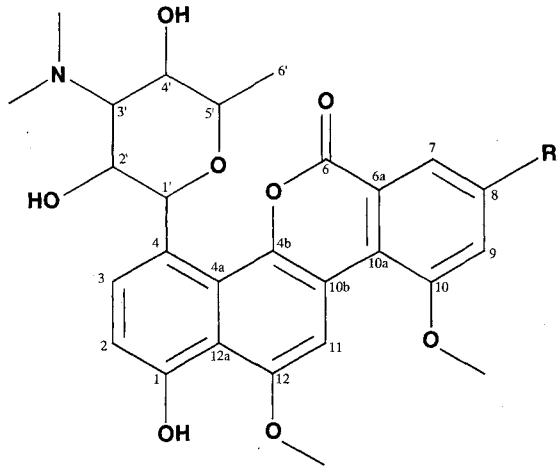
The structure of deacetylravidomycin M, an inhibitor of interleukin-4 signal transduction, was elucidated to be 6*H*-benzo[d]naphtho[1,2-b]pyran-6-one, 4-[3,6-dideoxy-3-(dimethylamino)-α-altropyranosyl]-1-hydroxy-10,12-dimethoxy-8-methyl- by spectroscopic studies including NMR measurements.

A novel ravidomycin analog named deacetylravidomycin M (Fig. 1) was isolated from the culture broth of *Streptomyces* sp. WK-6326 as an inhibitor of interleukin (IL)-4 signal transduction. A known analog deacetylravidomycin[1~3] (Fig. 1) was also isolated from the culture broth. The ravidomycin family[4~7] with a common structure of the *C*-glycoside-linked benzonaphthopyranone ring has been reported as antitumor and antimicrobial antibiotics[1~3].

As described in the preceding paper[8], deacetylravidomycin M inhibited IL-4-induced CD23 expression in U937 cells, whereas deacetylravidomycin showed no inhibitory activity. In this paper, we report the structure elucidation of deacetylravidomycin M.

Fig. 1. Structures of deacetylravidomycin M and deacetylravidomycin.



	R
Deacetylravidomycin M	$-C^{13}H_3$
Deacetylravidomycin	$-C^{13}H{=}C^{14}H_2$

Methods

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Optical rotations were obtained with a JASCO DIP-370 digital polarimeter. EI-MS spectra were recorded on a JEOL JMS-D 100 mass

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Table 1. Physico-chemical properties of deacetylravidomycin M and deacetylravidomycin.

	Deacetylravidomycin M	Deacetylravidomycin
Appearance	Pale yellow powder	Pale yellow powder
Molecular formula	$C_{28}H_{31}NO_8$	$C_{29}H_{31}NO_8$
Molecular weight	509	521
FAB-MS (*m/z*)		
Positive	510 $[M+H]^+$	522 $[M+H]^+$
	532 $[M+Na]^+$	544 $[M+Na]^+$
Negative	508 $[M-H]^-$	520 $[M-H]^-$
HRFAB-MS (*m/z*) (positive)		
	$C_{28}H_{32}NO_8$	$C_{29}H_{32}NO_8$
Calcd:	510.2128	522.2128
Found:	510.2126	522.2128
UV $\lambda_{max}^{C_2H_5OH}$ nm (ε)	244 (35,100)	248 (32,300)
	275 (20,600)	287 (35,100)
	322 (9,600)	391 (13,100)
	382 (8,900)	
IR ν_{max}^{KBr} (cm^{-1})	3351, 3324, 1716,	3548, 3425, 1712,
	1371, 1247, 1133,	1629, 1465, 1376,
$[\alpha]_D^{22}$ (*c* 0.3, $CHCl_3$:CH_3OH, 1:1)	−22°	−15°
Solubility		
Soluble:	CH_3OH, $CHCl_3$,	CH_3OH, $CHCl_3$,
	CH_3CN, Acetone,	CH_3CN, Acetone,
	C_2H_5OH, Ethyl acetate	C_2H_5OH, Ethyl acetate
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane
Color reaction		
Positive:	50% H_2SO_4	50% H_2SO_4

spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer.

Results

Physico-chemical properties of deacetylravidomycin M are summarized in Table 1, where those of deacetylravidomycin are also shown for comparative purposes. Similarity in their data indicates that they are structurally related. Absorption at 1716 cm^{-1} in the IR spectrum suggests the presence of a carbonyl group[9] in the structure.

The ^{13}C NMR spectrum (DMSO-*d*) of deacetylravidomycin M showed 28 resolved peaks (Table 2), which were classified into two methyl, two *O*-methyl, two *N*-methyl, four *O*-methine, one *N*-methine, five sp^2 methine, eleven sp^2 quaternary and one carbonyl carbon by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 29 proton signals (Table 2). One downfield broad singlet signal (δ 9.85) suggested the presence of a phenol OH proton. The connectivity of proton and carbon atoms (Table 2) was also established by the HMQC spectrum. From the ^1H-^1H COSY spectrum, two partial structures, $-C^2H=C^3H-$ and $-C^{1'}H-C^{2'}H-C^{3'}H-C^{4'}H-C^{5'}H-C^{6'}H_3$, were determined (Fig. 2). ^{13}C-^1H long range couplings of ^2J and ^3J observed in the HMBC experiment (Fig. 3) gave the following evidence: 1) The long range couplings from H-1′ (δ 5.66) to C-5′ (δ 75.09), from H-5′ (δ 4.13) to C-1′ (δ 79.61), from 3′-NCH_3a (δ 3.35) to C-3′ (δ 80.36) and 3′-NCH_3b (δ 57.55), and from 3′-NCH_3b (δ 3.20) to C-3′ and 3′-NCH_3a (δ 55.13) showed the presence of hexosamine moiety containing the partial structure II (Fig. 2). A fragment ion peak (*m/z* 173) of FAB-MS also supported the presence of the amino sugar moiety. The proton coupling constants, 8.8 Hz between H-1′ and H-2′ (δ 4.60), 10.0 Hz between H-2′ and H-3′ (δ 3.50), 2.5 Hz between H-3′ and H-4′ (δ 4.06) and ~0 Hz between H-4′ and H-5′, indicated that the hexose is an α-altropyranoside, which was also

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

Carbon No.	Deacetylravidomycin M		Deacetylravidomycin	
	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]
C-1	153.68		153.72	
1-OH		9.85 (1H, brs)		9.86 (1H, brs)
C-2	111.68	6.97 (1H, d, J =8.3 Hz)	112.00	6.98 (1H, d, J =8.3 Hz)
C-3	129.63	7.85 (1H, d, J =8.3 Hz)	129.70	7.85 (1H, d, J =8.3 Hz)
C-4	125.31		125.41	
C-4a	125.11		125.11	
C-4b	141.80		142.22	
C-6	159.69		159.64	
C-6a	121.48		122.02	
C-7	121.20	7.74 (1H, d, J =1.5 Hz)	119.12	7.97 (1H, d, J =1.5 Hz)
C-8	140.47		138.91	
C-9	119.05	7.44 (1H, d, J =1.5 Hz)	114.84	7.72 (1H, d, J =1.5 Hz)
C-10	156.97		157.50	
10-OCH_3	56.57	4.063 (3H, s)	56.81	4.14 (3H, s)
C-10a	120.81		122.88	
C-10b	113.61		113.50	
C-11	101.79	8.43 (1H, s)	101.81	8.47 (1H, s)
C-12	151.80		151.93	
12-OCH_3	56.29	4.077 (3H, s)	56.38	4.09 (3H, s)
C-12a	115.21		115.46	
C-13	21.10	2.47 (3H, s)	135.18	6.92 (1H, dd, J =17.8, 11.2 Hz
C-14			117.36	5.48 (1H, d, J =11.2 Hz)
				6.13 (1H, d, J =17.8 Hz)
C-1'	79.61	5.66 (1H, d, J =8.8 Hz)	79.53	5.67 (1H, d, J =8.3 Hz)
C-2'	66.70	4.60 (1H, dd, J =10.0, 8.8 Hz)	66.76	4.58 (1H, dd, J =10.0, 8.3 Hz)
C-3'	80.36	3.50 (1H, dd, J =10.0, 2.5 Hz)	80.41	3.53 (1H, dd, J =10.0, 2.5 Hz)
3'-NCH_3a	55.13	3.35 (3H, s)	55.44	3.35 (3H, s)
3'-NCH_3b	57.55	3.20 (3H, s)	57.27	3.22 (3H, s)
C-4'	68.01	4.06 (1H, d, J =2.5 Hz)	68.05	4.08 (1H, d, J =2.5 Hz)
C-5'	75.09	4.13 (1H, q, J =6.5 Hz)	75.13	4.12 (1H, q, J =6.5 Hz)
C-6'	16.80	1.02 (1H, d, J =6.5 Hz)	16.79	1.02 (1H, d, J =6.5 Hz)

a) Each sample was dissolved in DMSO-*d*. Chemical shifts are shown with reference to DMSO-*d* as 39.5 ppm. b) Chemical shifts are shown with reference to DMSO-*d* as 2.48 ppm.

supported by the ^{13}C NMR chemical shifts[3,7,10]. 2) The long-range couplings of ^{2}J or ^{3}J were observed from 1-OH (δ 9.85) to C-1 (δ 153.68), H-2 (δ 6.97) to C-4 (δ 125.31) and C-12a (δ 115.21), H-3 (δ 7.85) to C-1, C-2 (δ 111.68) and C-4a (δ 125.11), from H-11 (δ 8.43) to C-4b (δ 141.80), C-10b (δ 113.61), C-12 (δ 151.80) and C-12a, and from 12-OCH_3 (δ 4.077) to C-12. Furthermore, the long-range couplings of ^{4}J were observed from H-3 to C-4b and C-12a, and from H-11 to C-4a, supporting the presence of the naphthalene moiety within which is embedded the partial structure I. 3) The long-range couplings from H-1′ to C-3, C-4 and C-4a, and from H-3 to C-1′ showed that the α-altropyranose is attached to the C-4 of the naphthalene skeleton *via* the *C*-glycoside linkage. 4) The long range couplings from H-7 (δ 7.74) to C-6a (δ 121.48), C-8 (δ 140.47), C-9 (δ 119.05), C-10a (δ 120.81) and C-13 (δ 21.10), from H-9 (δ 7.44) to C-7 (δ 121.20), C-8, C-10 (δ 156.97), C-10a and C-13, from 10-OCH_3 (δ 4.063) to C-10 and from H_3-13 (δ 2.47) to C-7, C-8 and C-9 showed the presence of the benzene moiety. 5) The long-range couplings of ^{2}J or ^{3}J from H-11 to C-10a and ^{4}J from H-7 and H-9 to C-10b were observed, indicating that the benzene moiety is linked to the naphthalene moiety.

In addition to the degree of unsaturation, the position of the pyran ring (Fig. 3) was elucidated by the following reasons; 1) the long-range couplings were observed from

Fig. 2. Partial structures of deacetylravidomycin M.

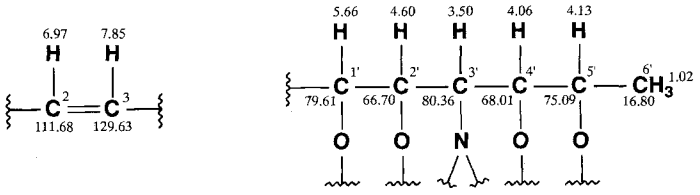

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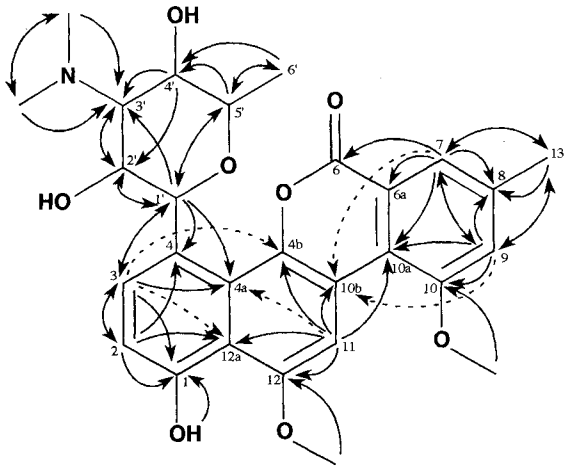
Fig. 3. HMBC experiments of deacetylravidomycin M.



HMBC (^2J or ^3J): H ⟶ C, HMBC (^4J): H ⟶ C

H-7 to C-6 (δ 159.69) in the HMBC experiments, 2) the ^{13}C chemical shifts of the sp^2 quaternary C-6a (δ 121.48) indicated that it should be linked directly to carbons, and 3) the remaining oxygen atom should be attached to C-6 and C-4b due to the ^{13}C chemical shifts.

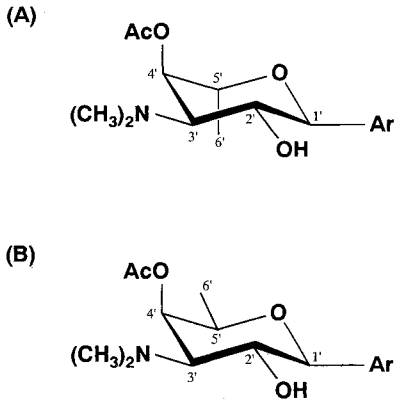
Taken together, the structure of deacetylravidomycin M was elucidated as shown in Fig. 1. The assignments are reasonable from comparison with those of deacetylravidomycin (Table 2). The vinyl residue at the C-8 of deacetylravidomycin is replaced by the methyl one in deacetylravidomycin M.

Discussion

Deacetylravidomycin[1~3], also known as ravidomycin[7,10~12], was reported as a new family of antitumor antibiotics possessing an aromatic *C*-glycoside. Other members of this family include chrysomycins[13,14] and gilvocarcins[6], which incorporate the same aromatic aglycon attached to different glycosidic substituents. As elucidated in this paper deacetylravidomycin M is the analog of deacetylravidomycin in which a methyl substituent replaces the C-8 vinyl residue of deacetylravidomycin. The structural difference is important for eliciting inhibition of IL-4-induced signal transduction by deacetylravidomycin M[8].

Recently, the total synthesis of ravidomycin was achieved to establish the relative and absolute stereochemistry[15]. The relative stereochemistry of C-5′ proton in the hexosamine moiety of ravidomycin, originally reported by FINDLAY *et al.*[16] (Fig. 4A), was revised, and the absolute stereochemistry was concluded as shown in Fig. 4B[15]. To confirm the stereochemistry of this moiety, FUTAGAMI *et al.* recommended an NOE experiment since the conclusive NOE between C-3′ and C-5′ protons was observed in ravidomycin[15]. Members of the ravidomycin family possess the same carbon skeleton of the hexosamine moiety, and are expected to share the similar biosynthetic mechanism, possibly giving the same stereochemistry to their hexosamine moieties. Therefore, it is plausible that deacetylravidomycin M also shares the same stereochemistry as shown in Fig. 4B. However, NOE was not observed between C-3′ and C-5′ protons for deacetylravidomycin M. Further study may be necessary to give a clear conclusion for the stereochemistry.

Fig. 4. Stereochemistry of the hexosamine moiety in ravidomycin.



(A), The relative stereochemistry originally reported by FINDLAY *et al.*[16]. (B), The absolute stereochemistry concluded from the synthetic ravidomycin by FUTAGAMI *et al.*[15].

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