

Takanawaenes, Novel Antifungal Antibiotics Produced by *Streptomyces* sp. K99-5278

II. Structure Elucidation

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The structures of takanawaenes A, B and C, novel antifungal antibiotics produced by *Streptomyces* sp. K99-5278, were elucidated by various spectroscopic analyses including UV and NMR, and spectrometric analyses including MS. They have the common skeleton of a 28-membered pentaene macrolide.

Takanawaenes A, B and C and compound AB023a were isolated as antifungal antibiotics from the culture broth of *Streptomyces* sp. K99-5278^{1,2)} (Fig. 1). The fermentation, isolation and their biological properties are described in the preceding paper¹⁾. We report herein the structure elucidation of takanawaenes A, B and C.

Materials and Methods

Materials

Takanawaenes A, B and C and compound AB023a were purified from the culture broth of *Streptomyces* sp. K99-5278 as described in the preceding paper¹⁾.

General Experimental Procedures

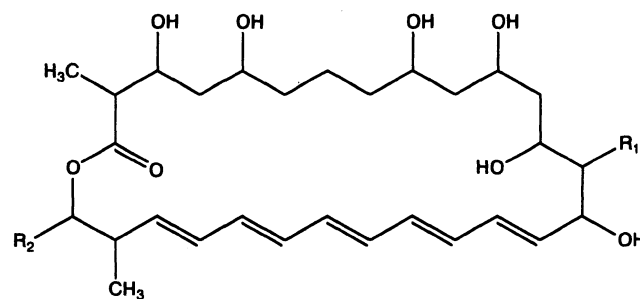
Optical rotations were recorded with a JASCO DIP-370 digital polarimeter. Melting points were measured with a Yanaco micro melting apparatus. FAB-MS spectrometry was conducted on a JEOL JMS-AX505H spectrometer. UV and IR spectra were measured with a Beckman DU640 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. The various NMR spectra were measured with Varian XL-400 spectrometers.

Results

Physico-chemical Properties of Takanawaenes

Physico-chemical properties of takanawaenes A, B and C are summarized in Table 1. The data of AB023a is also shown for comparative purpose. Similarity in their data indicates that they are structurally related. All takanawaenes, like AB023a²⁾, show the same absorption

Fig. 1. Structures of takanawaenes A, B and C and compound AB023a.



Takanawaene A :	R ₁ = H	R ₂ = CH ₃
Takanawaene B :	R ₁ = CH ₃	R ₂ = CH ₂ CH ₃
Takanawaene C :	R ₁ = CH ₃	R ₂ = CH(CH ₃) ₂
AB023a :	R ₁ = CH ₃	R ₂ = CH ₃

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Table 1. Physico-chemical properties of takanawaenes A, B and C and compound AB023a.

	Takanawaene A	Takanawaene B	Takanawaene C	AB023a
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder
Melting point	160	155	156	162
$[\alpha]_D^{25}$	-15.9 (c 0.1, CH ₃ OH)	-63.9 (c 0.1, CH ₃ OH)	-53.9 (c 0.1, CH ₃ OH)	-29.9 (c 0.1, CH ₃ OH)
Molecular formula	C ₃₀ H ₄₈ O ₈	C ₃₂ H ₅₂ O ₈	C ₃₃ H ₅₄ O ₈	C ₃₁ H ₅₀ O ₈
Molecular weight	536	564	578	550
HR-FAB-MS m/z (M+Na) ⁺				
Calcd	559.3246	587.3559	601.3716	573.3403
Found	559.3247	587.3561	601.3717	573.3396
UV $\lambda_{\text{max}}^{\text{CH}_3\text{OH}}$ nm	306, 319, 334, 352	306, 319, 335, 352	306, 319, 335, 352	306, 319, 334, 352
IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	3415, 2937, 1726, 1010	3405, 2937, 1726, 1006	3390, 2935, 1727, 1008	3400, 2937, 1726, 1008
Solubility				
Soluble	DMSO, CH ₃ OH CHCl ₃ , ethyl acetate (slightly)	DMSO, CH ₃ OH CHCl ₃ , ethyl acetate (slightly)	DMSO, CH ₃ OH CHCl ₃ , ethyl acetate (slightly)	DMSO, CH ₃ OH CHCl ₃ , ethyl acetate (slightly)
Insoluble	<i>n</i> -Hexane, H ₂ O	<i>n</i> -Hexane, H ₂ O	<i>n</i> -Hexane, H ₂ O	<i>n</i> -Hexane, H ₂ O

maxima at 352, 334~335, 319 and 306 nm in the UV spectrum, strongly suggesting that they have a conjugated pentaene in the structures. Their IR spectra showed absorptions at about 3400 and 1726 cm⁻¹, suggesting the presence of hydroxy and carbonyl groups.

Structure Elucidation of Takanawaene A

The molecular formula of takanawaene A was determined to be C₃₀H₄₈O₈ on the basis of HRFAB-MS measurement (m/z , found 559.3247, calcd 559.3246 for C₃₀H₄₈O₈Na [M+Na]⁺). The ¹³C NMR spectrum (in DMSO-*d*₆) showed 30 resolved signals, which were classified into three methyl carbons, seven methylene carbons, seven *O*-methine carbons, two methine carbons, ten *sp*² methine carbons and one carbonyl carbon by analysis of DEPT spectra. The ¹H NMR spectrum (in DMSO-*d*₆) showed three methyl signals, ten *sp*² methine signals, seven methylene signals, seven *O*-methine signals, two methine signals, and six hydroxy signals. The connectivity of proton and carbon atoms was established by the ¹³C-¹H HMQC spectrum as shown in Table 2. Analysis of the ¹H-¹H COSY and ¹³C-¹H HMBC spectra revealed the two partial structures **I** and **II** (Fig. 2).

The ¹³C-¹H long range couplings of ²*J* and ³*J* observed in the ¹³C-¹H HMBC experiments (Fig. 3) gave the following evidence. 1) The cross peaks from 28-H₃ (δ 1.02) to C-1 (δ 173.7), C-2 (δ 45.9) and C-3 (δ 71.8), from 2-H (δ 2.24) to C-1, C-3, C-4 (δ 41.6) and C-28 (δ 12.7), from 3-H (δ 3.76) to C-2, C-4 and C-5 (δ 71.0), from 4-H (δ 1.28) to C-2 and C-3, from 6-H₂ (δ 1.10) to C-4, C-7 (δ 22.4) and C-8 (δ 38.2), from 8-H₂ (δ 1.08) to C-6 (δ 38.9) and C-7, from 10-H₂ (δ 1.25) to C-8 and C-9 (δ 70.8) from 11-H (δ 3.74) to C-9, from 12-H (δ 1.20) to C-11 (δ 68.8), from 14-H₂ (δ 1.60) to C-12 (δ 43.7), C-13 (δ 65.4), C-15 (δ 68.6) and C-16 (δ 137.7), from 15-H (δ 4.08) to C-16 and C-17 (δ 129.8), and from 16-H (δ 5.57) to C-15 supported the partial structure **I**. 2) The cross peaks from 17-H (δ 6.11) to C-15 and C-16 and from 15-H to C-17 suggested that the partial structure **I** extends to the pentaene moiety from the double bond between C-16 and C-17. 3) The cross peaks from 25-H (δ 5.82) to C-26 (40.4), C-27 (δ 73.4) and C-30 (δ 16.0), from 26-H (δ 2.32) to C-24 (δ 129.7), C-25 (δ 136.3), C-27, C-30 and C-31 (δ 18.8), from 30-H₃ (δ 0.98) to C-25, C-26 and C-27, from 27-H (δ 4.62) to C-25, C-26 and C-30, and from 31-H (δ 1.16) to C-26 and C-27, supported the partial structure **II**. Furthermore, it was presumed that the partial structure **II** also extends to the

Table 2. ^1H and ^{13}C NMR chemical shifts of takanawaenes A, B and C.

Carbon No.	Takanawaene A		Takanawaene B		Takanawaene C	
	^{13}C chemical shifts (ppm) ^a	^1H chemical shifts (ppm) ^b	^{13}C chemical shifts (ppm) ^a	^1H chemical shifts (ppm) ^b	^{13}C chemical shifts (ppm) ^a	^1H chemical shifts (ppm) ^b
C-1	173.7		174.2		174.2	
C-2	45.9	2.24 (1H, m)	46.0	2.28 (1H, m)	45.9	2.28 (1H, m)
C-3	71.8	3.76 (1H, br m)	71.9	3.71 (1H, br m)	71.8	3.72 (1H, br m)
C-4	41.6	1.28 (2H, m)	41.6	1.28 (2H, m)	41.7	1.28 (2H, m)
C-5	71.0	3.33 (1H, br m)	71.5	3.42 (1H, br m)	71.4	3.42 (1H, br m)
C-6	38.9	1.10 (2H, m)	38.8	1.20 (2H, m)	38.8	1.20 (2H, m)
C-7	22.4	0.82 (1H, m) 1.60 (1H, m)	22.5	0.81 (1H, m) 1.70 (1H, m)	22.5	0.81 (1H, m) 1.70 (1H, m)
C-8	38.2	1.08 (2H, m)	38.6	1.20 (2H, m)	40.1	1.20 (2H, m)
C-9	70.8	3.49 (1H, br m)	71.4	3.49 (1H, br m)	71.4	3.49 (1H, br m)
C-10	43.9	1.25 (2H, m)	43.5	1.20 (2H, m)	43.5	1.24 (2H, m)
C-11	68.8	3.74 (1H, br m)	69.5	3.79 (1H, br m)	69.5	3.78 (1H, br m)
C-12	43.7	1.20 (2H, m)	39.2	1.20 (2H, m)	38.3	1.20 (2H, m)
C-13	65.4	3.33 (1H, m)	67.6	3.40 (1H, m)	67.7	3.40 (1H, m)
C-14	44.2	1.60 (2H, m)	45.4	1.60 (1H, m)	45.4	1.60 (1H, m)
C-15	68.6	4.08 (1H, m)	74.5	3.58 (1H, m)	74.5	3.59 (1H, m)
C-16	137.7	5.57 (1H, dd, $J = 15.20, 7.69$ Hz)	136.7	5.54 (1H, dd, $J = 15.02, 8.61$ Hz)	136.7	5.54 (1H, dd, $J = 15.20, 8.75$ Hz)
C-17	129.8	6.11 (1H, m)	130.4	6.06 (1H, m)	130.4	6.06 (1H, m)
C-18	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-19	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-20	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-21	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-22	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-23	133-131.3	6.23 (1H, m)	133-131.2	6.21 (1H, m)	132.9-131.1	6.20 (1H, m)
C-24	129.7	6.10 (1H, m)	129.6	6.06 (1H, m)	129.7	6.06 (1H, m)
C-25	136.3	5.82 (1H, dd, $J = 15.20, 6.78$ Hz)	136.1	5.83 (1H, dd, $J = 15.20, 5.83$ Hz)	136.1	5.80 (1H, dd, $J = 15.01, 6.69$ Hz)
C-26	40.4	2.32 (1H, m)	38.1	2.38 (1H, m)	35.9	2.45 (1H, m)
C-27	73.4	4.62 (1H, m)	77.7	4.59 (1H, td, $J = 8.42, 3.30$ Hz)	80.1	4.57 (1H, dd, $J = 8.60, 3.84$ Hz)
C-28	12.7	1.02 (3H, d, $J = 6.96$ Hz)	13.8	1.07 (3H, d, $J = 7.47$ Hz)	13.8	1.09 (3H, d, $J = 6.96$ Hz)
C-29			10.5	0.85 (3H, d, $J = 6.95$ Hz)	10.5	0.85 (3H, d, $J = 6.96$ Hz)
C-30	16.0	0.98 (3H, d, $J = 6.95$ Hz)	15.8	0.99 (3H, d, $J = 6.77$ Hz)	15.6	0.97 (3H, d, $J = 6.78$ Hz)
C-31	18.8	1.16 (3H, d, $J = 6.23$ Hz)	25.3	1.45 (1H, m) 1.70 (1H, m)	28.7	1.93 (1H, m)
C-32			9.2	0.79 (3H, t, $J = 7.42$ Hz)	19.7	0.81 (3H, d, $J = 5.86$)
C-33					15.4	0.82 (3H, d, $J = 6.60$)

a) Chemical shifts are shown with reference to DMSO- d_6 as 39.5 ppm. b) Chemical shifts are shown with reference to DMSO- d_6 as 2.50 ppm.

pentaene moiety from the double bond between C-24 and C-25. 4) The cross peak from 27-H to C-1 suggested that the partial structures **I** and **II** are jointed as shown in Fig. 3. Taking the molecular formula and the degree of unsaturation into consideration, it was concluded that the pentaene moiety should be between C-16 and C-25 (Fig. 3). Taken together, the structure of takanawaene A was elucidated as shown in Fig 1. All the data assigned here is very reasonable in comparison with the data of compound AB023a reported previously²⁾.

Structure Elucidation of Takanawaene B

The molecular formula ($\text{C}_{32}\text{H}_{52}\text{O}_8$) of takanawaene B is C_2H_4 bigger than that of takanawaene A. The spectral data of ^1H and ^{13}C NMR of takanawaene B is similar to that of takanawaene A (Table 2). Different points are as follows; 1) $^{29}\text{CH}_3$ - ^{14}CH - sequence in the partial structure **III** (instead of the partial structure **I** for takanawaene A) was defined from the ^1H - ^1H COSY experiment. The cross peaks from 29- H_3 (δ 0.85) to C-12 (δ 39.2), C-14 (δ 45.4) and C-15 (δ 74.5) and from 14-H (δ 1.60) to C-29 (δ 10.5) in the ^{13}C - ^1H HMBC experiment supported the partial structure **III**

Fig. 2. Partial structures I and II of takanawaene A.

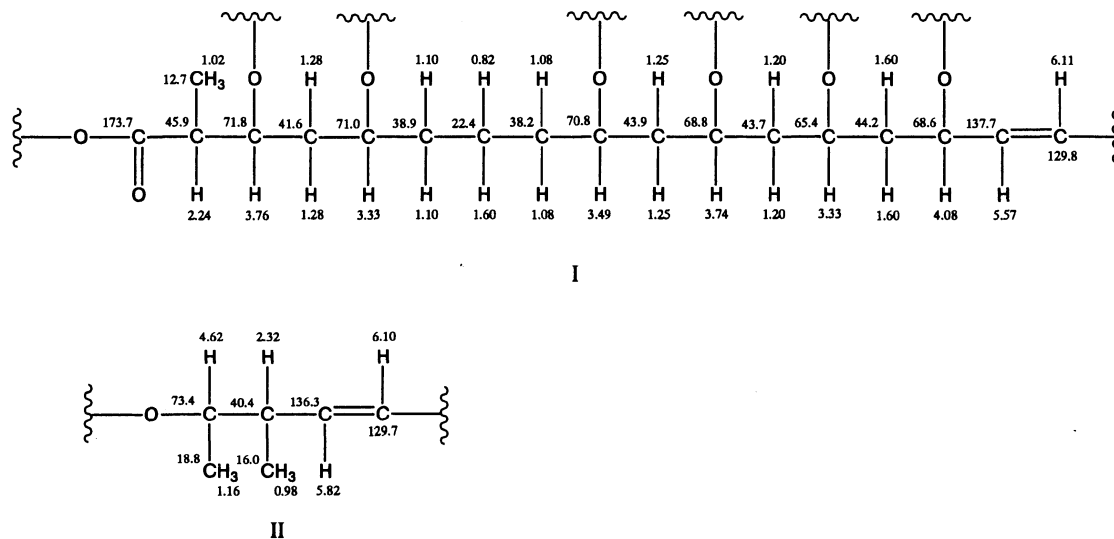
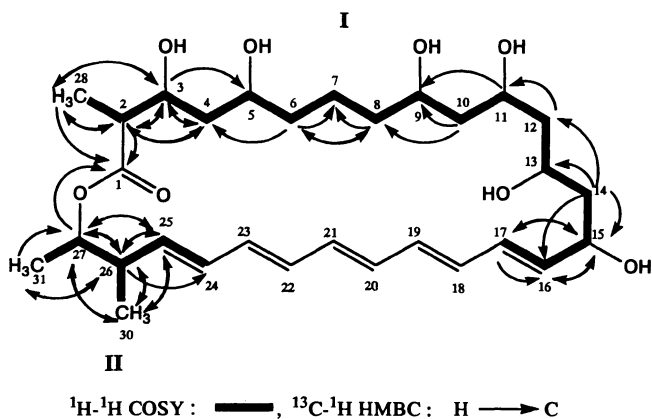
Fig. 3. ^1H - ^1H COSY and ^{13}C - ^1H HMBC experiments of takanawaene A.

Fig. 4. Partial structures III and IV of takanawaene B.

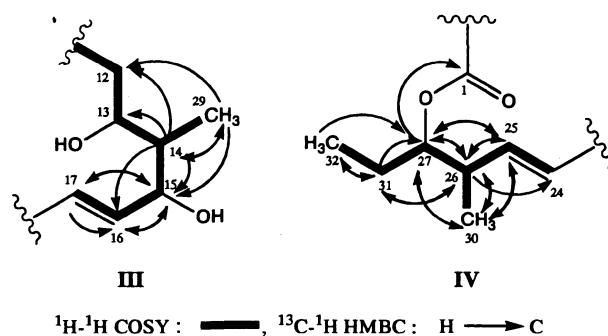
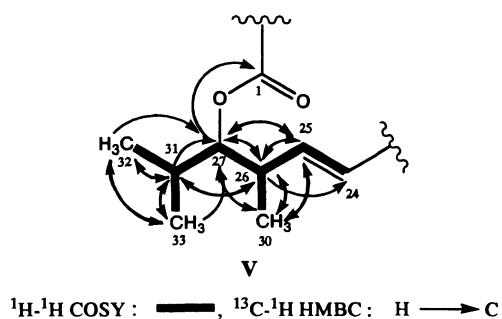


Fig. 5. Partial structure V of takanawaene C.



(Fig. 4). $^2\text{ }^{32}\text{CH}_3\text{-}^{31}\text{CH}_2\text{-}^{27}\text{CH-}$ sequence in the partial structure IV (instead of the partial structure II for takanawaene A) was determined from the ^1H - ^1H COSY experiment. The cross peaks from 32- H_3 (δ 0.79) to C-31 (δ 25.3) and C-27 (δ 77.7) and from 31- H_2 (δ 1.45, 1.70) to C-32 (δ 9.2) and C-27 in the ^{13}C - ^1H HMBC experiment supported the partial structure IV (Fig. 4). Thus, the structure of takanawaene B was elucidated as shown in Fig. 1.

Structure Elucidation of Takanawaene C

The molecular formula ($C_{33}H_{54}O_8$) of takanawaene C is CH_2 bigger than that of takanawaene B. $^{32}CH_3-^{31}CH(^{33}CH_3)-^{27}CH-$ sequence in the partial structure **V** (instead of the partial structure **IV** for takanawaene B) was defined from the $^1H-^1H$ COSY experiments (Fig. 5). The cross peaks from 31-H (δ 1.93) to C-26 (δ 35.9), C-27 (δ 80.1), C-32 (δ 19.7) and C-33 (δ 15.4), from 32- H_3 (δ 0.81) to C-31 (δ 28.7), C-27 and C-33, and from 33- H_3 (δ 0.82) to C-31, C-27 and C-32 in the $^{13}C-^1H$ HMBC experiments supported the partial structure **V**. Thus, the structure of takanawaene C was elucidated as shown in Fig. 1.

Discussion

Nitrogen- and sugar-free pentaene macrolides with a 28-membered ring fall into three groups: 1) The best characterized methylpentaenes, where one end (C-16) of the pentaene moiety is substituted with a methyl group, include filipins^{3,4}, fungichromin⁵, elizabethin⁶ and chainin⁷. They are all produced by *Streptomyces* spp. 2) Strevertenes⁸ recently discovered and polyene I⁹ are new pentaenes with a carboxylic acid at C-14 of the 28-membered ring. Polyene I was produced by *Streptomyces* sp., but strevertenes were produced by a different actinomycete, *Streptoverticillium* sp. The absolute stereochemistries of strevertenes were reported. 3) Takanawaenes described in this paper and AB023s have the simple pentaene with no methyl residue at C-16 and no carboxylic acid at C-14. They are produced by *Streptomyces* spp.

Thus, this series of compounds has the common 28-membered pentaene and shows the diversity in side chains at C-2 (proton, methyl or ethyl), C-14 (proton, methyl or carboxylic acid), C-16 (proton or methyl) and C-27

(methyl, ethyl or isobutyl) and hydroxylation sites.

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