THE STRUCTURES OF ATPENINS A4, A5 AND B, NEW ANTIFUNGAL ANTIBIOTICS PRODUCED BY *PENICILLIUM* SP.

HIDETOSHI KUMAGAI, HIROYUKI NISHIDA, NOBUTAKA IMAMURA, HIROSHI TOMODA and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, and School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan

JON BORDNER

Central Research, Pfizer Inc., Groton, Connecticut 6340, U.S.A.

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The structures of atpenins A4, A5 and B, new antifungal antibiotics produced by *Penicillium* sp., have been deduced to be **I**, **II** and **III**, respectively, on the basis of spectroscopic and ^{1}H and ^{13}C NMR spectral data. The molecular structure of atpenin A4 with absolute configurations was finally confirmed to be 2′*S*,4′*S*,5′*S*-5,6-dimethoxy-4-hydroxy-5′-chloro-2′,4′-dimethyl-1′-oxoheptyl-2-hydroxypyridine (**I′**) by the single crystal X-ray crystallographic analysis. The absolute configurations of atpenins A5 and B were also expected to be 2′*S*,4′*S*,5′*R*-**II** (**II′**) and 2′*S*,4′*R*-**III** (**III′**), respectively.

In the course of screening for microbial inhibitors of lipid metabolism, we have discovered triacsins, long chain acyl-CoA synthetase inhibitors produced by *Streptomyces* sp. SK-1894[1,2], and 1233 A (F-244), an inhibitor of HMG-CoA synthase isolated from *Scopulariopsis* sp. F-244[3]. In our continuous screening, a series of antifungal antibiotics named atpenins were isolated from the culture broth of *Penicillium* sp. FO-125[4]. In the previous papers[4,5], the taxonomy of the producing microorganism, production, isolation, physico-chemical and biological properties and the mechanism of action of atpenins were reported. This paper deals with the structural elucidation of these compounds.

Results

Presumed Structures of Atpenins Based on Their Spectral Data

Based on the ^{1}H-^{1}H COSY NMR spectrum of atpenin B ($C_{15}H_{23}NO_5$, λ_{max} nm (ε) 237 (5,600), 270 (3,600), 318 (3,100)), a partial structure **1** was deduced as a side chain in this compound. The catalytic hydrogenation of atpenin B with PtO_2 in the mixture (1 : 1) of acetic acid and trifluoroacetic acid afforded dihydrodeoxo-atpenin B ($C_{15}H_{25}NO_4$, λ_{max} nm 270). Its ^{1}H-^{1}H COSY NMR spectrum proved the existence of the partial structure **2** as a side chain, which was generated *via* hydrogenolysis of a carbonyl conjugated with the aromatic moiety. ^{1}H-^{1}H COSY, ^{1}H-^{13}C long range selective proton decoupling (LSPD) and NOE experiments of this dihydrodeoxo-derivative showed that the aromatic portion remaining posesses of the partial structures **3** and **4**, and the connection between **2** and **4** was also determined as seen in **5** (Fig. 1).

The sum of all the partial structures **2**, **3** and **4** exactly corresponded to the empirical formula of dihydrodeoxo-atpenin B. Taking all the spectral data mentioned above into consideration, the plane structures of dihydrodeoxo-atpenin B and atpenin B are deduced to be **IV** and **III** (Fig. 2), respectively.

Fig. 1. Partial structures of atpenins.

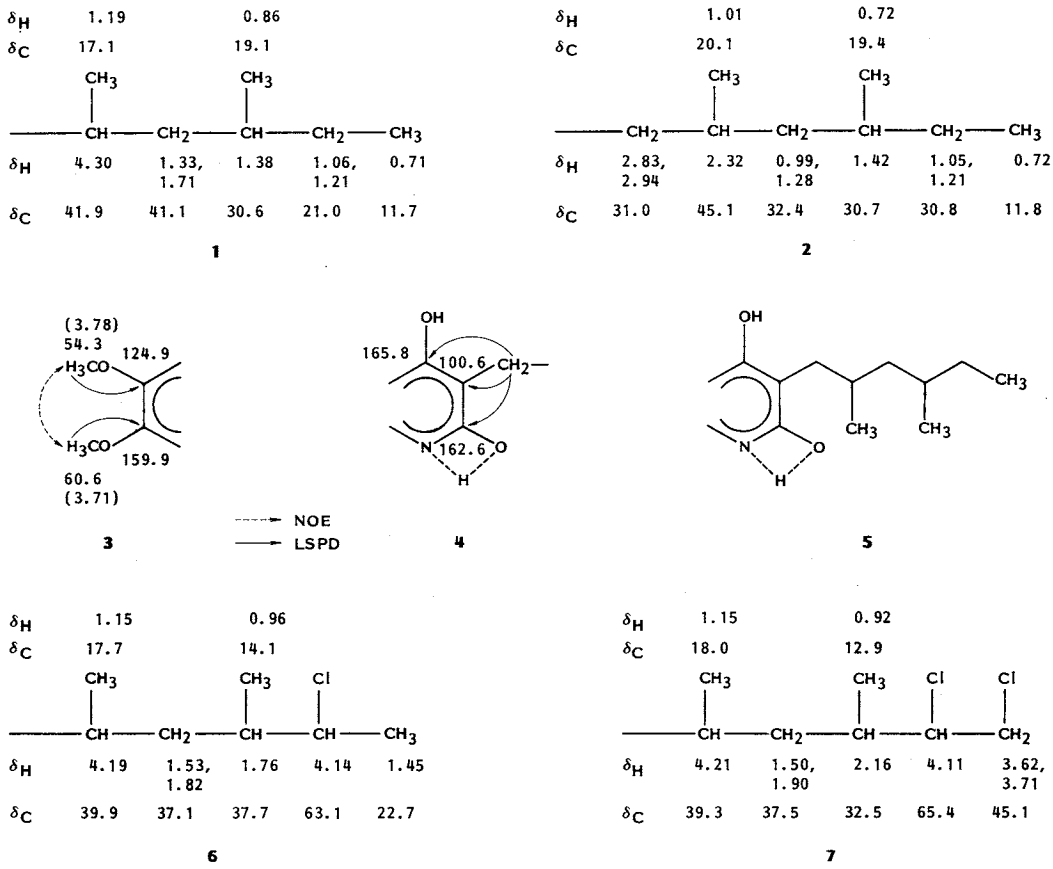
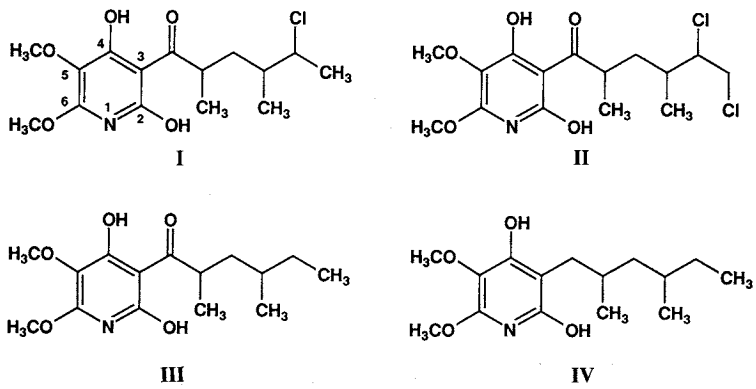


Fig. 2. Structures of atpenins A4 (**I**), A5 (**II**) and B (**III**), and dihydrodeoxo-atpenin B (**IV**) (pyridine form).



UV, IR and NMR spectral data of atpenins A4 ($C_{15}H_{22}NO_5Cl$, λ_{max} nm (ε) 235 (12,200), 267 (8,400), 320 (5,800)) and A5 ($C_{15}H_{21}NO_5Cl_2$, λ_{max} nm (ε) 237 (6,400), 272 (4,400), 320 (3,500)) clearly showed the following facts. 1) The aromatic moieties conjugated with a carbonyl group of atpenins A4 and A5 are the same as that of atpenin B (**III**). 2) The side chains of atpenins A4 and A5 are **6** and **7** (Fig. 1), respectively. Accordingly the plane structures of atpenins A4 and A5 are deduced to be **I** and **II** (Fig. 2).

Table 1. ^{1}H NMR data of atpenins.

Proton	I[a]	II[a]	III[b]	IV[b]
1′-H				2.83 (1H, dd, J=8.5, 12.9), 2.94 (1H, dd, J=6.1, 12.9)
2′-H	4.18 (1H, m)	4.21 (1H, m)	4.30 (1H, tq, J=6.6, 6.8)	2.32 (1H, m)
2′-CH_3	1.15 (3H, d, J=6.8)	1.15 (3H, d, J=6.8)	1.19 (3H, d, J=6.6)	1.01 (3H, d, J=6.6)
3′-H	1.53 (1H, dt, J=6.2, 12.4), 1.82 (1H, m)	1.50 (1H, ddd, J=6.5, 7.3, 13.6), 1.90 (1H, ddd, J=7.1, 8.1, 13.6)	1.33 (1H, m) 1.71 (1H, ddd, J=5.3, 5.9, 12.5)	0.99 (1H, m) 1.28 (1H, m)
4′-H	1.76 (1H, m)	2.16 (1H, dq, J=2.6, 6.5)	1.38 (1H, m)	1.42 (1H, m)
4′-CH_3	0.96 (3H, d, J=6.5)	0.92 (3H, d, J=6.5)	0.86 (3H, d, J=6.4)	0.72 (3H, d, J=6.1)
5′-H	4.14 (1H, dq, J=3.1, 6.7)	4.11 (1H, ddd, J=2.6, 5.9, 8.5)	1.06 (1H, m), 1.21 (1H, m)	1.05 (1H, m), 1.21 (1H, m)
6′-H	1.45 (3H, d, J=6.7)	3.62 (1H, dd, J=8.5, 11.2), 3.71 (1H, dd, J=5.9 11,2)	0.71 (3H, t, J=7.3)	0.72 (3H, t, J=7.3)
5-OCH_3	3.80 (3H, s)	3.77 (3H, s)	3.71 (3H, s)	3.64 (3H, s)
6-OCH_3	4.19 (3H, s)	4.21 (3H, s)	3.78 (3H, s)	3.79 (3H, s)

[a] In $CDCl_3$, 300 MHz. [b] In C_5D_5N, 400 MHz.
J=Hz.

^{1}H and ^{13}C NMR spectral data of atpenins are summarized in Tables 1 and 2.

From all the data described above, the existence of the aromatic ring structures of **I**~**IV** could not be demonstrated positively. So the complete structures were clarified by the single crystal X-ray analysis of atpenin A4.

Table 2. ^{13}C NMR data of atpenins.

Carbon	I[a]	II[a]	III[b]	IV[b]
C-2[c]	161.8	161.9	162.6	157.8
C-3	100.5	100.8	100.6	100.3
C-4[c]	171.1	172.5	165.8	159.3
C-5	121.2	120.9	124.9	125.9
5-OCH_3	61.5	61.6	60.6	60.6
C-6	155.5	155.2	159.9	154.0
6-OCH_3	57.9	58.3	54.3	53.2
C-1′	210.1	209.8	211.7	32.5
C-2′	39.9	39.3	41.9	31.0
2′-CH_3	17.7	18.0	17.1	20.1
C-3′	37.1	37.5	41.1	30.8
C-4′	37.7	32.5	30.6	45.1
4′-CH_3	14.1	12.9	19.1	19.4
C-5′	63.1	65.4	21.0	32.4
C-6′	22.7	45.9	11.7	11.8

[a] In $CDCl_3$, 75 MHz. [b] In C_5D_5N, 100 MHz.
[c] Assignments may be interchanged.

The Single Crystal X-Ray Crystallographic Analysis of Atpenin A4

In order to confirm the complete structures of atpenins, a single crystal X-ray crystallographic analysis of atpenin A4, recrystallized from diethyl ether - *n*-hexane mixture, was carried out at Central Research, Pfizer Inc. The analytical data are summarized in Table 3. The asymmetric unit somewhat unusually contained four molecules.

A trial structure was obtained by direct methods. This trial structure was refined without problems. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R-index was 0.061.

The phenolic hydrogens deserve special comment. In all molecules we prepared, the phenolic group in the position 4 (O18) had a hydrogen that was strongly hydrogen bonded to O8 in the side chain. The

phenolic group at position 2 (O17) was strongly hydrogen bonded to a nitrogen in a neighboring moleclule. In fact, a close examination of difference maps indicated significant electron density within bonding distance of both the nitrogen and the oxygen. An attempt was made to further study the locations of these acidic hydrogens. However, because of the large size of this structure, least squares refinement of these hydrogens using fractional occupancy was not successful. This lack of success illustrates the limits of X-ray diffraction when dealing with hydrogens in a large structure. Aside from the above mentioned feature, a final difference Fourier revealed no missing or misplaced electron density.

Table 3. Single crystal X-ray crystallographic analysis.

Crystal parameters	
Formula	$C_{15}H_{22}NO_5Cl$ (331.8)
Crystallization media	*n*-hexane and diethyl ether
Crystal size (mm)	$0.28 \times 0.52 \times 0.54$
Cell dimensions	$a=15.484(3)$ Å
	$b=16.415(4)$ Å
	$c=27.193(6)$ Å
	$\alpha=90.00°$
	$\beta=90.00°$
	$\gamma=90.00°$
	$V=6912(2)$ Å^3
Space group	$P2_12_12_1$ with $Z=4$
Molecules/unit cell	16
Density calc (g/cm^3)	1.28
Linear absorption factor (cm^{-1})	21.6
Refinement parameters	
No. of reflections	3,942
Nonzero reflections ($I>3.0\sigma$)	3,171
R-index[a]	0.061
GOF[b]	1.50
R weighted[c] (full data)	0.0734 correct enantiomer, 0.0827 other enantiomer
Scale factor	1.228(1)
Secondary extinction factor	none

[a] R-index $=\Sigma||F_o|-|F_c||/\Sigma|F_o|$.

[b] GOF $=[\Sigma w(F_o^2-F_c)^2/(m-s)]^{1/2}$ where $w=[\sigma^2(F)+|g|F^2]^{-1}$ $g=0.00100$.

[c] R weighted $=[\Sigma w(|F_o-F_c|)^2/\Sigma wZF_o]^{1/2}$ where $w=[\sigma^2(F)+|g|F^2]^{-1}$ $g=0.00100$.

Fig. 3. The molecular structure of two molecules of atpenin A4 in the asymmetric unit.

The dotted lines indicate interactions between hydrogens and basic atoms. Hydrogen positions were not refined.

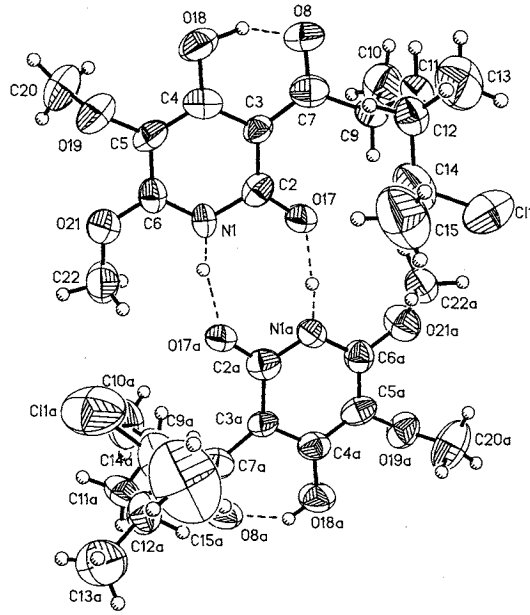
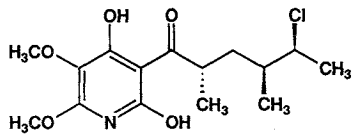
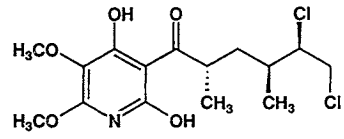


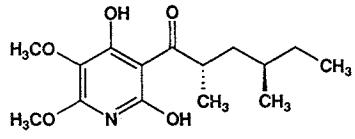
Fig. 4. Absolute configurations of atpenins A4 (I'), A5 (II') and B (III') (pyridine form).



I'



II'



III'

The refined structure was plotted using the SHELXTL plotting package (Fig. 3). The positions of the hydrogens on O17 and O18 were taken from the final difference map and were not refined. Distances to these hydrogens would therefore be unreliable.

Absolute Configurations of Atpenins

The absolute configurations of atpenin A4 were clarified to be 2′*S*,4′*S*,5′*S*-**I** (**I′**) as seen in Fig. 4. During the course of fermentation, the production of atpenin B occurred first and then was followed by those of atpenins A4 and A5. In the case that the configurations of the precursors are preserved, the absolute configurations of atpenins A5 and B are expected to be 2′*S*,4′*S*,5′*R*-**II** (**II′**) and 2′*S*,4′*R*-**III** (**III′**) (Fig. 4), respectively.

Discussion

The structures of atpenins A4, A5 and B were determined as described above. Frequently 2(1*H*)-pyridinone tautomer structures have been adopted for this kind of compounds such as ilicicolin H[6,7] and 2(1*H*)-pyridinone[8]. However, the 2-hydroxypyridine type of structure was obtained from the X-ray crystallographic analysis of atpenin A4. The tautomerism between 2(1*H*)-pyridinone type and 2-hydroxypyridine type structures of these compounds awaits further investigation.

Experimental

UV spectra were recorded on a Shimadzu UV-240 spectrometer and IR spectra on a Jasco A-102 spectrometer. MS spectra were measured on a Jeol JMS DX-300. NMR spectra were recorded on a Varian XL-400 NMR spectrometer with ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz.

Hydrogenation of Atpenin B

A solution of atpenin B (25 mg) in a mixture of 0.5 ml of acetic acid and 0.5 ml of TFA, was stirred under hydrogen atmosphere in the presence of PtO_2 (10 mg) at room temperature for 0.5 hour until hydrogen-uptake ceased. After filtrating the catalyst, the organic solvent was evaporated *in vacuo*. The residue was purified first on preparative TLC and finally by HPLC to give dihydrodeoxo-atpenin B as white powder. EI-MS *m/z* 283 $(M)^+$, UV λ_{max} nm 204, 225 (sh) and 270. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

Single Crystal X-Ray Analysis

A representative crystal was surveyed and a 1A data set (maximum sin $\theta/\lambda = 0.5$) was collected on a Nicolet R3m/μ diffractometer. The asymmetric unit contained four molecules. Atomic scattering factors were taken from the International Tables for X-Ray Crystallography[9]. All crystallographic calculations were facilitated by the SHELXTL[10] system. All diffractometer data were collected at room temperature. Pertinent crystal, data collection, and refinement parameters are summarized in Table 3.

The absolute configuration was determined by the method of HAMILTON[11], IBERS and HAMILTON[12] (see Table 3).

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