STUDIES ON MYCOTRIENIN ANTIBIOTICS, A NOVEL CLASS OF ANSAMYCINS

III. THE ISOLATION, CHARACTERIZATION AND STRUCTURES OF MYCOTRIENOLS I AND II

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Two minor components designated mycotrienols I and II which were concurrently produced with mycotrienins I and II by *Streptomyces rishiriensis* T-23 were isolated and characterized. Like mycotrienins I and II, mycotrienols I and II are also interconvertible *via* redox reaction and their structures have been established as depicted in **3** and **4** on the basis of ^{13}C NMR evidence along with chemical degradation. The biological activities are discussed.

In continuing our studies on mycotrienin antibiotics, we have succeeded in the isolation of two minor components from the culture broth of *Streptomyces rishiriensis* T-23 along with mycotrienins I (**1**) and II (**2**).

Similar to the parent antibiotics **1** and **2**, these compounds are also interconvertible *via* a redox reaction and the names mycotrienols I (**3**) and II (**4**) are given.

In this paper, we describe the isolation, structural elucidation and biological activities of **3** and **4** in detail.

Fig. 1. Structures of mycotrienins and mycotrienols.

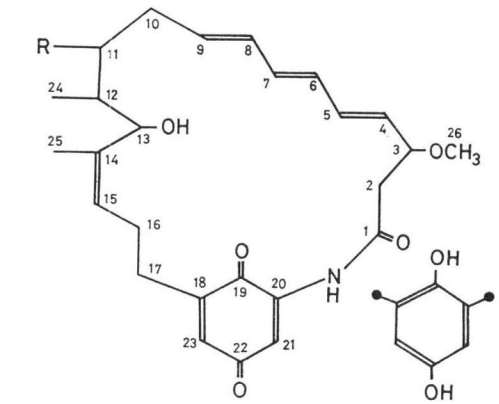
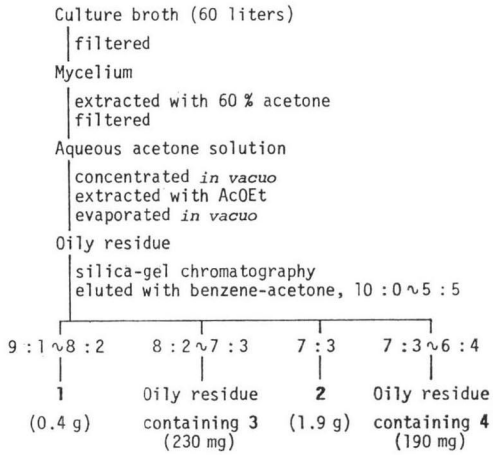


Chart 1. Flow diagram for the isolation of mycotrienins and mycotrienols.



Isolation of **3** and **4**

The same fermentation procedure employed for mycotrienins was carried out for the production of **3** and **4**. Consequently, a crude mixture of mycotrienins was obtained by an extraction of the harvested mycelia with aqueous acetone and reextraction by ethyl acetate followed by evaporation to an oily syrup, as described in the preceding paper[1].

Separation of each component was performed by silica gel column chromatography using a gradient solvent system of benzene - acetone (10: 0 ~ 5: 5) as presented in Chart 1.

Since **4** was readily converted to **3** by oxidation with $FeCl_3$ and reversed by reduction with $Na_2S_2O_4$, further purification was worked out predominantly with **4**.

The oily residue (190 mg) containing **4** was subjected to preparative TLC developed with benzene - chloroform - methanol (3: 7: 3). The appropriate fraction was eluted by methanol and **4** was obtained as an oily substance (32 mg). In order to oxidize **4** to **3**, this material was dissolved in a 1% methanolic $FeCl_3$ solution and stirred for 30 minutes. The reaction mixture was diluted with ethyl acetate and washed with water. After concentration *in vacuo*, the residue was applied to preparative TLC developed with benzene - chloroform - ethyl acetate (1: 1: 1) to give crude **3** as an oily residue. This material was reduced with $Na_2S_2O_4$ in methanol and the reaction mixture was filtered. After dilution with ethyl acetate, the solution was washed with water and evaporated to dryness. The yellow powder thus obtained was dissolved in a small volume of ethyl acetate and **4** was precipitated as a white powder (9 mg) by the addition of hexane. Since **3** could be obtained as a yellow powder by the oxidation of **4**, the isolation of **3** was not performed.

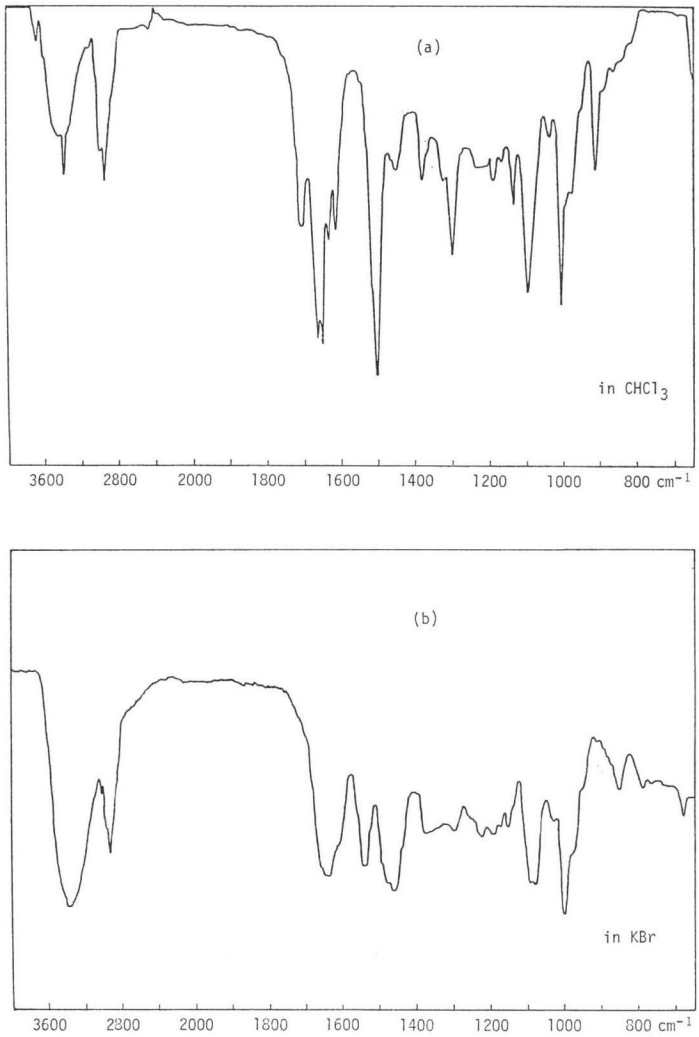
Physicochemical Properties of **3** and **4**

The physicochemical properties of **3** and **4** are summarized in Table 1. The UV spectra show the presence of a triene group in **3** and **4**. Their IR spectra indicate the presence of amide functions (see Fig. 2). The ^1H NMR spectra are shown in Fig. 3 and the ^{13}C NMR spectral data are summarized in Table 2. The thin layer chromatograms of **1**, **2**, **3** and **4** are presented in Fig. 4. **3** is soluble in alcohols, esters and chloroform; slightly soluble in benzene and ethyl ether; insoluble in water, petroleum ether and hexane. **4** is soluble in alcohols and esters; slightly soluble in chloroform, benzene and ethyl ether: insoluble in water, petroleum ether and hexane.

Table 1. Physicochemical properties of mycotrienols.

	Mycotrienol I (**3**)	Mycotrienol II (**4**)
Appearance	Yellow powder	White powder
Mp	94 ~ 95°C	130 ~ 132°C
$[\alpha]_D$ (*c* 1.0, MeOH)	+4.3°	+273°
M.W. (M^+) *m/z*	455	457
Molecular formula	$C_{26}H_{33}NO_6$	$C_{26}H_{35}NO_6$
Elemental analyses	Calcd. Found C 68.57% C 68.26% H 7.25 H 7.67 N 3.08 N 3.06	Calcd. Found C 68.27% C 68.76% H 7.66 H 7.67 N 3.06 N 3.07
UV spectrum λ_{max}^{MeOH} nm (ε)	261 (33,800), 272 (43,300), 282 (33,500), 386 (1,400),	263 (30,800), 273 (39,800) 282 (30,600), 307 (3,300),
IR spectrum ν_{max} cm^{-1}	3340(NH, OH), 1650, 1503(amide) 1003 (triene) in $CHCl_3$	3340(NH, OH), 1645, 1540(amide), 1003 (triene) in KBr

Fig. 2. IR spectra of mycotrienols I (a) and II (b).



Structural Elucidation of **3** and **4**

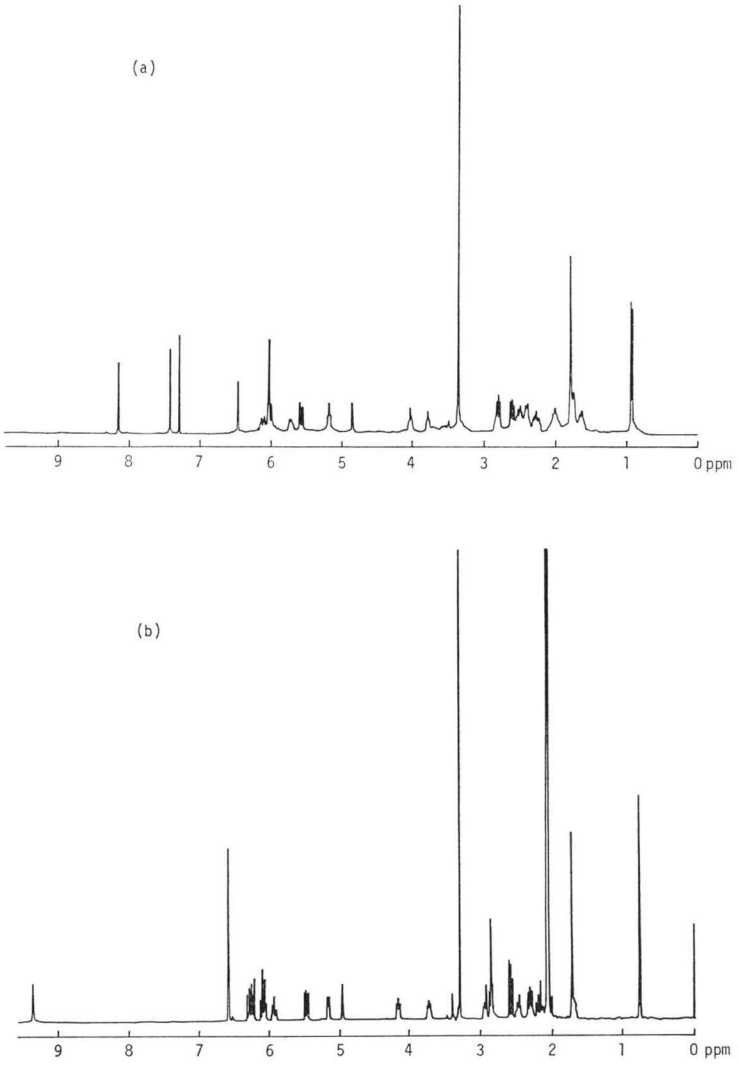
The facile interconversion between **3** and **4** *via* a redox reaction suggests the presence of a quinone nucleus and its dihydro form in **3** and **4**, respectively.

The IR spectra suggested the absence of ester linkages in **3** and **4**, which are present in **1** and **2** (see Fig. 2).

The ^{13}C NMR spectra of **3** and **4** revealed 26 signals as shown in Table 2. Comparison of ^{13}C NMR spectra of **3** and **4** with those of **1** and **2** indicates the absence of ten signals corresponding to the cyclohexanecarbonylalanine unit in the spectra of **3** and **4**. However, all signals expected for the macrocyclic lactam ring were present in the spectra of **3** and **4** (see Table 2).

As shown in Fig. 3, the ^1H NMR spectrum of **3** in $CDCl_3$ shows the absence of the signals due to the α-methine proton (δ_H 4.36), one methyl group (δ_H 1.41) and the cyclohexanecarbonyl protons (δ_H 2.15~1.25) which are observed in the spectrum of **1**[2]. In addition, the chemical shifts of the C-11 oxymethine

Fig. 3. 400 MHz ^{1}H NMR spectra of mycotrienols I in $CDCl_3$ (a) and II in acetone-d_6 (b).



group of **1** (δ_H 4.98 and δ_C 75.2)[2] have moved upfield to δ_H 3.77 and δ_C 72.5 in the ^{1}H and ^{13}C NMR spectra of **3** in $CDCl_3$. These changes were also observed in the ^{1}H and ^{13}C NMR spectra of **4**.

These data indicate that the cyclohexanecarbonylalanine ester group is replaced by a hydroxyl function at C-11 in the molecules of **3** and **4**.

Furthermore, the parent ions were observed at *m/z* 455 and 457 in the mass spectra of **3** and **4**, respectively. These ions correspond to loss of the side chain ester at C-11 of **1** and **2**.

Accordingly, the structures of **3** and **4** have been established as shown in Fig. 1.

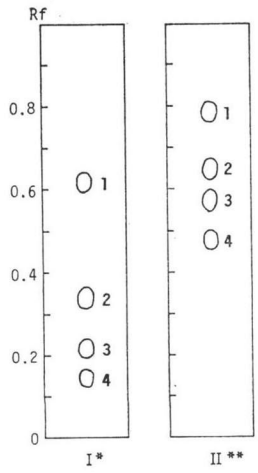
In order to confirm this conclusion, **2** was treated with lithium aluminium hydride in tetrahydrofuran at −23°C to eliminate the C-11 ester residue[3]. Two products thus obtained, *i.e.* 11-*O*-deacylmycotrienins I and II, were completely identical with **3** and **4**, respectively, by ^{1}H and ^{13}C NMR spectral analysis.

Table 2. ^{13}C NMR spectral data for mycotrienins and mycotrienols.

No.	1*	3*	2**	4**	No.	1	3	2	4
C- 1	169.7	169.4	170.3	169.9	C-19	188.2	188.1	141.7	140.8
- 2	44.8	44.6	43.1	43.1	-20	145.4	145.2	127.7	127.5
- 3	79.2	78.8	80.7	80.5	-21	114.5	114.2	108.1	107.6
- 4	131.3	131.8	131.1	132.3[c]	-22	182.5	182.4	151.3	150.9
- 5	133.7	133.6[a]	135.8	135.3[d]	-23	133.1	133.4	116.4	115.5
- 6	129.5	130.5	130.5[b]	130.3[e]	-24	9.6	10.5	9.8	10.5
- 7	133.7	134.1	134.8	135.2[d]	-25	20.5	20.3	21.1	20.9
- 8	133.2	133.4[a]	133.8	132.5[c]	-26	56.6	56.6	56.7	55.9
- 9	129.3	128.7	130.6[b]	128.8[e]	-27	172.9		173.1	
-10	33.0	36.4	33.6	37.3	-28	48.5		49.5	
-11	75.2	72.5	75.4	71.8	-29	17.4		17.2	
-12	39.9	40.7	38.9	40.9	-30	176.6		176.8	
-13	68.0	69.2	68.1	68.8	-31	44.9		44.9	
-14	139.9	139.0	139.8	139.9	-32	29.4[f]		30.0[h]	
-15	122.5	123.3	123.8	123.8	-33	25.6[g]		25.9[i]	
-16	25.6	25.6	27.0	26.7	-34	25.5[g]		26.0[i]	
-17	29.4	29.5	32.3	32.0	-35	25.5[g]		26.1[i]	
-18	137.9	137.8	132.9	132.6	-36	29.3[f]		29.9[h]	

[a,b,c,d,e,f,g,h,i] Assignments may be exchangable.
* δ_C in $CDCl_3$ ** δ_C in pyridine-d_5

Fig. 4. Thin-layer chromatograms of mycotrienins and mycotrienols on silica gel GF_{254} plates.



*Solvent I Chloroform - Ethanol, 15 : 1
**Solvent II Benzene - Chloroform - Methanol, 3 : 7 : 3

Table 3. Cytotoxicities of mycotrienins and mycotrienols against L-5178Y cells.

	M.I.C.
Mycotrienin I	0.8 μg/ml
II	0.4
Mycotrienol I	6.3
II	3.2
Geldanamycin	0.1

The cytotoxicities of mycotrienins and mycotrienols were determined by a procedure identical with that of the bioassay as described in preceding paper[1].

Biological Activities of 3 and 4

Mycotrienols show no antimicrobial activity over a dose range between 10 μg/ml to 100 μg/ml, while mycotrienins show antifungal and antiyeast activities (M.I.C. 4.0~12.5 μg/ml). Mycotrienols are at least 8 times less potent than mycotrienins.

Mycotrienols are also less potent than mycotrienins (about 8 fold) in cytotoxicity, as shown in Table 3.

From these data it is suggested that the cyclohexanecarbonylalanine group is important for biological activities.

Experimental

NMR spectra were obtained on a JEOL FX-400 spectrometer with ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz. Mass spectra were measured on a Hitachi M-80 spectrometer. UV spectra were recorded using a Hitachi 320 spectrometer and IR spectra were taken with a JASCO A-102 infrared spectrophotometer.

Redox Reaction of **3** and **4**

To 2 ml of a MeOH solution containing **3** (5 mg), 2 mg of $Na_2S_2O_4$ was added and the mixture was stirred for 30 minutes at room temperature. The reaction mixture was filtered and diluted with 10 ml of ethyl acetate. After washing with water, the solution was dried over Na_2SO_4 and evaporated to dryness. **4** was obtained as a white powder (4.5 mg).

4 (5 mg) was dissolved in 2 ml of 1% methanolic $FeCl_3$ solution and stirred for 30 minutes at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water. After drying over Na_2SO_4, the solution was concentrated *in vacuo* to give 4.3 mg of **3**.

Degradation of **2** with Lithium Aluminium Hydride[3]

4 (700 mg) was dissolved in 70 ml of dry tetrahydrofuran and kept at −23°C (carbon tetrachloride-dry ice bath). To this solution was added 160 mg of lithium aluminium hydride and the mixture was stirred for 1.5 hours. The reaction mixture was diluted with 100 ml of ethyl acetate and washed with 100 ml of pH 6.8 phosphate buffer. The solution was dried over Na_2SO_4 and evaporated to dryness. The residue was subjected to silica gel column chromatography developed with chloroform - ethanol (25: 1) to give **3** (210 mg) and **4** (50 mg), respectively.

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