STUDIES ON MYCOTRIENIN ANTIBIOTICS, A NOVEL CLASS OF ANSAMYCINS

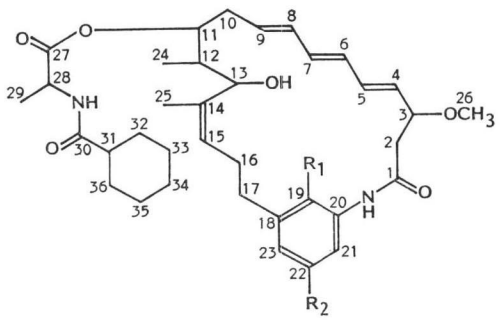
V. ISOLATION AND STRUCTURE DETERMINATION OF NOVEL MYCOTRIENIN CONGENERS

Sir:

Mycotrienins are unique benzoquinoid ansamycin antibiotics produced by *Streptomyces rishiriensis* T-23. In our previous paper we described the isolation, characterization and structural elucidation of mycotrienins I (**1**) and II (**2**)[1,2] (see Fig. 1), and two minor components, mycotrienols I and II[3].

Independently, ZEECK *et al.* isolated closely related compounds named ansatrienins A and B[4], except for the stereochemistry of alanine. They also reported the isolation of two minor components, ansatrienins A_2 and A_3, wherein the cyclohexyl group of ansatrienin A is replaced by a 2-methylbutyryl group and an isovaleryl group, respectively[5].

Fig. 1. Structures of mycotrienins.



2 $R_1=R_2=OH$
3 $R_1=OH$, $R_2=OCH_3$
4 $R_1=H$, $R_2=OH$

Further screening for minor components of mycotrienins resulted in the isolation of two metabolites named 22-*O*-methylmycotrienin II and 19-deoxymycotrienin II. We wish to report the isolation and structural elucidation of these minor components in this article.

The same fermentation procedure employed for mycotrienins[1] was repeated for the production of these two minor compounds. Consequently, a crude mixture of mycotrienins was obtained by extraction of the harvested mycelia with aqueous acetone and reextraction by ethyl acetate followed by evaporation to an oily syrup. Further purification of the minor components was performed by the method summarized in Fig. 2.

The production yields of 22-*O*-methylmycotrienin II (**3**) and 19-deoxymycotrienin II (**4**) were 0.27 mg/liter and 0.5 mg/liter, respectively.

3; $C_{37}H_{52}N_2O_8$, SIMS, $[MH]^+$ m/z 653, in beam EIMS $[M-H_2O]^+$ m/z observed 634.3664; calcd 634.3616, $[\alpha]_D^{20}$ +373° (*c* 0.045, MeOH), UV λ_{max}^{MeOH} 262 nm (ε 44,100), 272 (57,600), and 281 (43,700), IR ν_{max}^{KBr} 3400, 2930, 1720, 1685, 1675, 1650, 1615, 1250 cm^{-1}, was obtained as a white powder which melted at 128°C.

Fig. 2. Isolation procedure of mycotrienins.

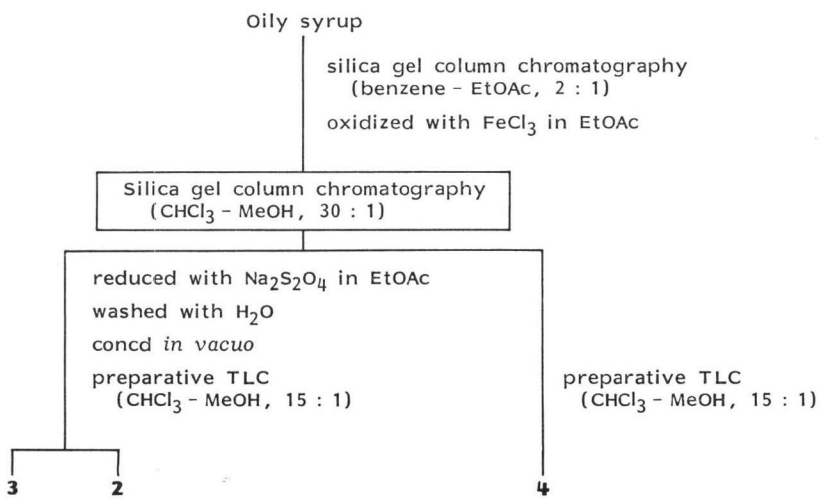


Table 1. ^{13}C NMR chemical shifts of **2**, **3** and **4** in $CDCl_3$.

No.	2	3	4	No.	2	3	4
C-1	169.7 s*	169.7 s	168.8 s	C-20	125.5 s	125.0 s	138.4 s
2	43.1 t	43.0 t	43.6 t	21	107.5 d	106.0 d	105.9 d
3	79.6 d	79.1 d	78.9 d	22	149.2 s	152.5 s	157.3 s
4	129.6 d	129.4[a] d	130.7[d] d	23	115.8 d	115.1 d	111.0 d
5	134.9 d	134.6[b] d	134.1[e] d	24	9.6 q	9.6 q	9.9 q
6	129.1 d	129.2[a] d	129.3[d] d	25	20.3 q	20.2 q	20.4 q
7	134.4 d	134.4[b] d	133.7[e] d	26	56.6 q	56.6 q	56.7 q
8	133.9 d	133.6 d	133.4[e] d	27	173.3 s	173.1 s	172.9 s
9	129.5 d	129.3[a] d	129.5[d] d	28	48.7 d	48.5 d	48.6 d
10	33.7 t	33.8 t	33.2 t	29	17.7 q	17.8 q	17.6 q
11	75.8 d	75.3 d	75.5 d	30	176.9 s	176.4 s	176.7 s
12	39.0 d	38.9 d	39.4 d	31	45.1 d	45.1 d	44.9 d
13	68.7 d	68.4 d	68.4 d	32	29.4 t	29.4 t	29.4 t
14	137.8 s	138.0 s	138.2 s	33	25.7 t	25.6 t	25.6 t
15	124.3 d	124.2 d	124.8 d	34	25.6 t	25.6 t	25.6 t
16	26.6 t	26.5 t	25.6 t	35	25.6 t	25.6 t	25.6 t
17	31.7 t	32.2 t	33.2 t	36	29.4 t	29.4 t	29.4 t
18	132.7 s	133.2 s	144.0 s	22-OCH_3		55.8 q	
19	141.1 s	142.2 s	112.2 d				

[a,b,c,d]: Assignments may be exchanged.
*: Multiplicities in the off-resonance decoupling.

Fig. 3. The ^1H NMR spectrum of **4** (400 MHz, $CDCl_3$).

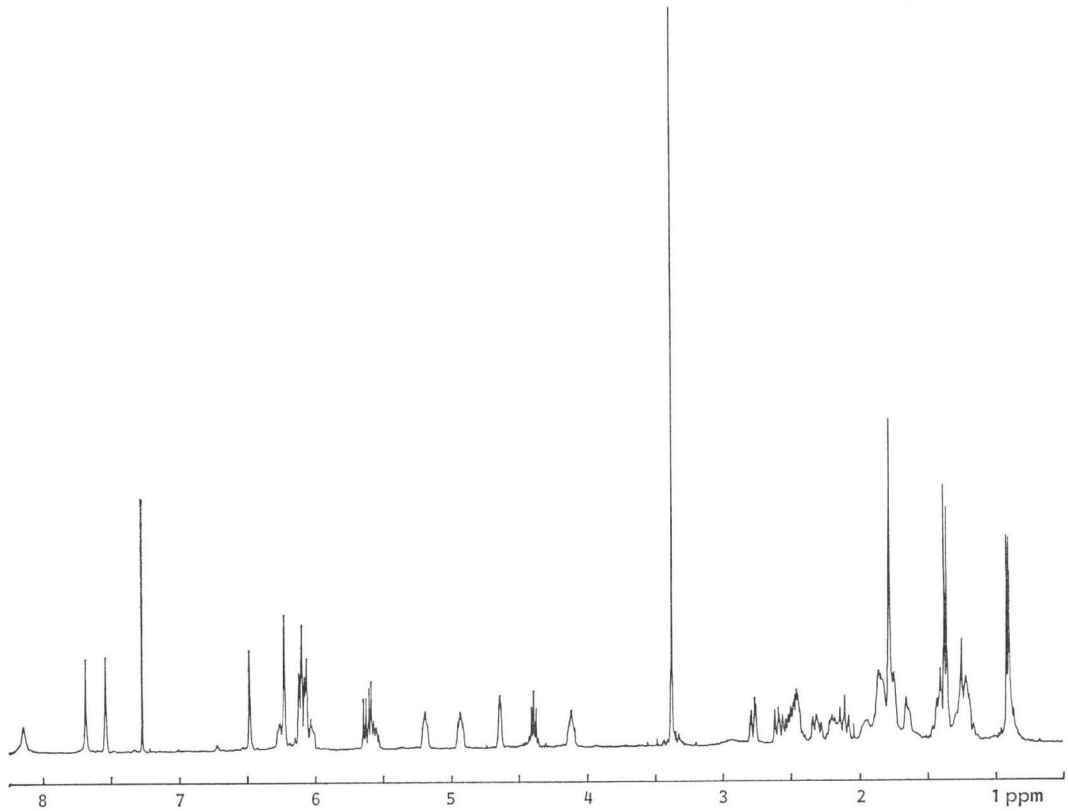
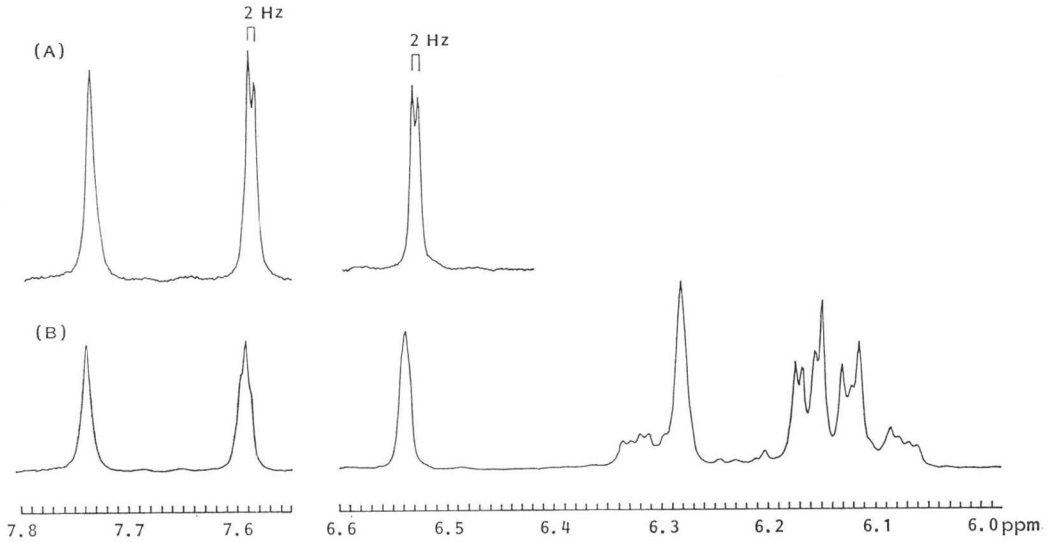


Fig. 4. Partial ^1H NMR spectra of 19-deoxymycotrienin II (**4**) in $CDCl_3$.
(A) Decoupling spectrum irradiating at δ_H 6.28, (B) Nondecoupling spectrum.



It has a larger molecular formula than **1**, differing by possession of an extra CH_2 group. The 50 MHz ^{13}C NMR spectrum of **3** in $CDCl_3$ is very close to that of **2** as shown in Table 1. The most significant difference observed was the appearance of a new methoxy signal at δ_C 55.8 in addition to 36 signals common to **2**. In 400 MHz ^1H NMR spectrum of **3** in $CDCl_3$, a new methoxy signal was observed at δ_H 3.72. Upon irradiation of these methoxy protons, a nuclear Overhauser effect was observed with two aromatic protons at δ_H 6.49 and δ_H 6.66 (H-21,23). These data indicate that **3** is the 22-*O*-methyl derivative of **2**. This structure was confirmed by ^{13}C-{^1H} long-range selective proton decoupling irradiating at methoxy protons (δ_H 3.72), whereupon sp^2 carbon C-22 (δ_C 152.5) collapsed to a sharp signal. Furthermore, oxidation of **3** with $FeCl_3$ in EtOAc did not give any oxidized product, supporting that a phenolic hydroxyl group in **3** is replaced by a methoxy group.

Thus, the structure of **3** has been elucidated as 22-*O*-methylmycotrienin II (Fig. 1).

4; $C_{36}H_{50}N_2O_7$, SIMS, $[MH^+]$ m/z 623, diacetate of **4**, $[M]^+$ m/z 706.4015 ($C_{40}H_{54}N_2O_9$, calcd 706.3831), $[\alpha]_D^{20}$ +151° (*c* 0.169, MeOH), UV λ_{max}^{MeOH} 260 nm (ε 27,600), 271 (33,000) and 282 (25,400), IR ν_{max}^{KBr} 3400, 2930, 1730, 1650, 1640, 1600, 1200 cm^{-1}, was obtained as a white powder which melted at 135°C.

Compound **4** contains one less oxygen atom than **2**. The 50 MHz ^{13}C NMR spectrum of **4** in $CDCl_3$ revealed 36 signals as shown in Table 1. Comparison of the ^{13}C NMR spectra of **2** and **4** shows that one non protonated sp^2 carbon signal of **2** (δ_C 141.1) is replaced by a new methine sp^2 carbon resonance in **4** (δ_C 112.2). In the 400 MHz ^1H NMR spectra in $CDCl_3$, **2** shows two aromatic proton signals at δ_H 6.49 (H-23) and 6.59 (H-21), while **4** shows three aromatic signals at δ_H 6.28, 6.54, and 7.59 (Fig. 3). Irradiation at δ_H 6.28 changed the other two aromatic broad triplet signals to broad doublets (J=2.0 Hz) as shown in Fig. 4. This proves that the three aromatic protons in **4** are located at C-19, C-21 and C-23. Furthermore, in contrast to **2**, **4** was not oxidized by $FeCl_3$. These data indicate that the hydroxyl group at C-19 in **2** is replaced by hydrogen in **4**. The chemical shifts of the aromatic carbons in **4** are comparable with the following calculated values[6]; C-18 145.5 ppm, C-19 112.6 ppm C-20 139.6 ppm, C-21 105.1 ppm, C-22 155.6 ppm, C-23 110.9 ppm.

Thus, the structure of **4** has been determined as 19-deoxymycotrienin II (Fig. 1).

The cytotoxicities of **3** and **4** were tested against L-5178Y cells *in vitro* by the method as described in the previous paper[1]. The minimum inhi-

bitory concentrations of **3** and **4** were almost identical with each other (0.11 μg/ml). It should be noted that these two compounds are three to four times as active as **2**, while 22-*O*-β-D-glucopyranosylmycotrienin II was 2-fold less active than **2** as reported in our recent paper[7]. These results suggest that the substituent pattern on the benzene nucleus in mycotrienins is important to their biological activity.

During preparation of the manuscript of this work, we were informed from Dr. K. KOMIYAMA that they had isolated trienomycin A, an antibiotic which is identical with **4** (see the accompanying communication[8]).

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