# STRUCTURE OF TRIENOMYCIN A, A NOVEL CYTOCIDAL ANSAMYCIN ANTIBIOTIC

Sir:

In the preceding paper, we reported the production, isolation, physico-chemical properties and a preliminary study of the biological activities of trienomycin A, together with the taxonomy of the producing organism, *Streptomyces* sp. No. 86-16[1].

This communication deals with the structural elucidation of trienomycin A (**1**) mainly from its NMR spectral analysis.

The molecular formula and molecular weight of **1** were established as $C_{36}H_{50}N_2O_7$ and 622 respectively through a combination of high resolution mass spectrometry (HR-MS) (found: 622.3607; calcd for $C_{36}H_{50}N_2O_7$: 622.3615)[1] and elementary analysis (found: C 67.52, H 7.98, N 4.28, O 20.21; calcd for $C_{36}H_{50}N_2O_7 \cdot H_2O$: C 67.47, H 8.18, N 4.37, O 19.97.)

Because of the existence of absorption maxima at $\lambda_{max}^{MeOH}$ 260 (ε 42,900), 271 (55,300) and 282 nm (40,700) in the UV spectrum and $\nu_{max}^{KBr}$ 1000 $cm^{-1}$ in the IR spectrum it was suggested that a triene moiety is present in the structure of **1**[2].

In the IR spectrum of **1** other than $\nu_{max}^{KBr}$ 1000 $cm^{-1}$ (triene) described above, absorption maxima at $\nu_{max}^{KBr}$ 3400 (NH, OH), 1730 and 1205 (ester) and 1650 and 1540 $cm^{-1}$ (amide) were observed.

The IR and UV data of **1** are quite similar to those of mycotrienins I (**2**) and II (**3**)* and mycotrienols I and II[3~6], and the molecular formula of **1** is similar to those of **2** and **3** ($C_{36}H_{48}N_2O_8$ and $C_{36}H_{50}N_2O_8$ respectively).

It was reported that **2** and **3** were interconvertible by treating with $Na_2S_2O_4$ (from **2** to **3**) and $FeCl_3$ (from **3** to **2**) respectively[3], but **1** did not show such features and only the starting material was recovered when **1** was treated with $Na_2S_2O_4$ or $FeCl_3$.

In the UV spectra of **2** and **3**, UV maxima attributed to quinone (**2**, $\lambda_{max}^{MeOH}$ 383 nm (ε 3,400)) or hydroquinone (**3**, $\lambda_{max}^{MeOH}$ 310 nm (ε 5,900)) moieties has been reported[3], but such absorption maxima could not been observed in the UV spectrum of **1**.

In the $^{13}C$ NMR spectrum of **1**, 36 signals (7 × singlets, 16 × doublets, 9 × triplets and 4 × quartets) were observed. The total number of carbons and the numbers of triplets and quartets of **1** were the same as those of **2** and **3** and three lower field signals of **1** ($\delta$ 170.8, 173.7 and 179.2) were similar to the carbonyl signals of **2** ($\delta$ 169.7, 172.9 and 176.6) and **3** ($\delta$ 170.3, 173.1 and 176.8). However, in the $^{13}C$ NMR spectrum of **2**, two additional carbonyl signals ($\delta$ 182.5 and 188.2) which had been assigned to the quinone group were observed. Because no such characteristic additional quinone carbonyl signals were observed in the $^{13}C$ NMR spectrum of **1**, further $^{13}C$ NMR spectral comparisons were conducted between **1** and **3** (Table 1).

Three methyl signals ($\delta$ 10.2, 17.2 and 20.8) and a methoxyl signal ($\delta$ 56.6) and nine methylene signals [$\delta$ 26.7 (2×C), 26.9, 30.5 (2×C), 30.8, 33.7, 37.3 and 44.8] in the $^{13}C$ NMR spectrum of **1** were similar to those of **3** [$\delta$ 9.8, 17.2 and 21.1 (methyls), $\delta$ 56.7 (methoxyl) and $\delta$ 25.9, 26.0, 26.1, 27.0, 29.9, 30.0, 32.3, 33.6 and 43.1 (methylenes)], respectively. Three methine

* ZEECK and his associates[7] reported the structures of ansatrienins A and B which have the same structures as mycotrienins I (**2**) and II (**3**), respectively, except for the stereochemistry of alanine.

Fig. 1. Structures of trienomycin A (**1**) and mycotrienins I (**2**) and II (**3**).

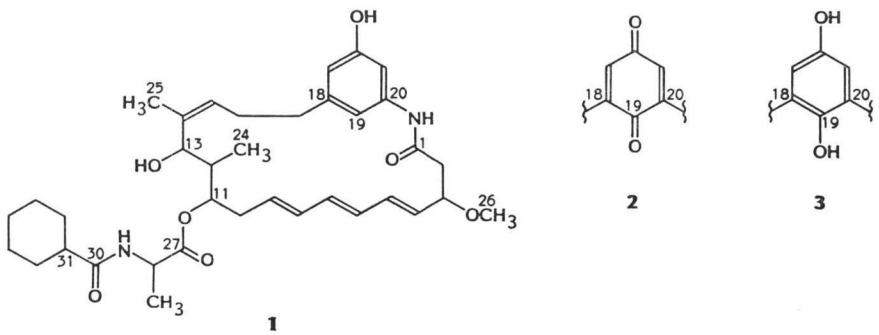

Table 1. $^{13}C$ NMR spectra of trienomycin A (**1**) (in $CD_3OD$) and mycotrienins I (**2**) and II (**3**) (in $CDCl_3$).

| No. | Trienomycin A | Mycotrienin I[3,6] | Mycotrienin II[3,6] |
|---|---|---|---|
| 1 | 170.8*(s**) | 169.7 (s) | 170.3 (s) |
| 2 | 44.8 (t) | 44.8 (t) | 43.1 (t) |
| 3 | 81.6 (d) | 79.2 (d) | 80.7 (d) |
| 4 | 132.5 (d)[a]*** | 131.3 (d) | 131.1 (d) |
| 5 | 135.2 (d)[b] | 133.7 (d) | 135.8 (d) |
| 6 | 131.0 (d)[a] | 129.5 (d) | 130.5 (d)[a] |
| 7 | 135.0 (d)[b] | 133.7 (d) | 134.8 (d) |
| 8 | 134.6 (d)[b] | 133.2 (d) | 133.8 (d) |
| 9 | 130.5 (d)[a] | 129.3 (d) | 130.6 (d)[a] |
| 10 | 33.7 (t) | 33.0 (t) | 33.6 (t) |
| 11 | 76.4 (d) | 75.2 (d) | 75.4 (d) |
| 12 | 40.4 (d) | 39.9 (d) | 38.9 (d) |
| 13 | 69.7 (d) | 68.0 (d) | 68.1 (d) |
| 14 | 139.7 (s)[c] | 139.9 (s) | 139.8 (s) |
| 15 | 125.9 (d) | 122.5 (d) | 123.8 (d) |
| 16 | 30.8 (t) | 25.6 (t) | 27.0 (t) |
| 17 | 37.3 (t) | 29.4 (t) | 32.3 (t) |
| 18 | 140.2 (s)[c] | 137.9 (s) | 132.9 (s) |
| 19 | 112.9 (d)[d] | 188.2 (s) | 141.7 (s) |
| 20 | 144.9 (s)[c] | 145.4 (s) | 127.7 (s) |
| 21 | 107.2 (d) | 114.5 (d) | 108.1 (d) |
| 22 | 158.6 (s) | 182.5 (s) | 151.3 (s) |
| 23 | 113.4 (d)[d] | 133.1 (d) | 116.4 (d) |
| 24 | 10.2 (q) | 9.6 (q) | 9.8 (q) |
| 25 | 20.8 (q) | 20.5 (q) | 21.1 (q) |
| 26 | 56.6 (q) | 56.6 (q) | 56.7 (q) |
| 27 | 173.7 (s) | 172.9 (s) | 173.1 (s) |
| 28 | 50.0 (d) | 48.5 (d) | 49.5 (d) |
| 29 | 17.2 (q) | 17.4 (q) | 17.2 (q) |
| 30 | 179.2 (s) | 176.6 (s) | 176.8 (s) |
| 31 | 45.9 (d) | 44.9 (d) | 44.9 (d) |
| 32 | 30.5 (t) | 29.4 (t)[a] | 30.0 (t)[b] |
| 33 | 26.7 (t) | 25.6 (t)[b] | 25.9 (t)[c] |
| 34 | 26.9 (t) | 25.5 (t)[b] | 26.0 (t)[c] |
| 35 | 26.7 (t) | 25.5 (t)[b] | 26.1 (t)[c] |
| 36 | 30.5 (t) | 29.3 (t)[a] | 29.9 (t)[b] |

* $\delta_C$ relative to TMS.
** Multiplicity in off-resonance spectrum.
*** a~d: Assignments may be interchanged.

signals of **1** ($\delta$ 69.7, 76.4 and 81.6) corresponded to the doublet signals of **3** ($\delta$ 68.1, 75.4 and 80.7) which had been assigned to each of the methine carbons bearing oxygen atoms and three other methines of **1** ($\delta$ 40.4, 45.9 and 50.0) were in good agreement with the methines of **3** ($\delta$ 38.9, 44.9 and 49.5). In addition, the eight $sp^2$ signals of **1** [$\delta$ 125.9, 130.5, 131.0, 132.5, 134.6, 135.0 and 135.2 (each d) and $\delta$ 139.7 (s)] were basically coincident with those of **3** [$\delta$ 123.8, 130.5, 130.6, 131.1, 133.8, 134.8 and 135.8 (each d) and 139.8 (s)].

On the other hand, in the $^1H$ NMR spectra of **1** and **3** two doublet methyl signals ($\delta$ 1.15 and 1.58), a broad singlet methyl signal ($\delta$ 2.03) and a methoxyl signal ($\delta$ 3.26) of **1** were similar to those observed in the $^1H$ NMR spectrum of **3** [$\delta$ 0.85 and 1.57 (each d), $\delta$ 1.98 (br s) and $\delta$ 3.27 (s)]. Seven $sp^2$ signals [$\delta$ 5.34, 5.82 (2×H), 6.19, 6.22, 6.31 and 6.50] and three methine signals ($\delta$ 4.43, 5.13 and 5.34) of **1** were coincident with the three methine signals next to the oxygen atom of **3** [$\delta$ 5.50, 5.70, 6.06, 6.23, 6.37, 6.54 and 6.64 ($sp^2$ signals) and $\delta$ 4.49, 5.29 and 5.37 (methine signals)]. In addition, a methine signal at $\delta$ 4.77 of **1** was basically coincident with a methine signal of an alanine moiety ($\delta$ 4.79) of **3**.

From all of the accumulated data described above, it was concluded that **1** possesses the same ansa moiety as **3** including the *N*-hexahydrobenzoylalanine moiety.

The existence of the *N*-hexahydrobenzoylalanine moiety was also verified by the examination of HR-MS of **1**, *i.e.*, *m/z* 154.1226 ($C_9H_{16}NO$, calcd 154.1231), 111.0821 ($C_7H_{11}O$, calcd 111.0809) and 83.0889 ($C_6H_{11}$, calcd 83.0860) were completely consistent with the fragment peaks reported for the *N*-hexahydrobenzoylalanine moiety of **3** (*m/z* 154.1189, 111.0776 and 83.0836)[5].

In the $^{13}C$ NMR spectra of **1**, thirty signals out of thirty-six signals have been assigned to the ansa and *N*-hexahydrobenzoylalanine moieties respectively and six carbon signals of **1**, *i.e.*, $\delta$ 107.2 (d), 112.9 (d), 113.4 (d), 140.2 (s), 144.9 (s) and 158.6 (s) have not been discussed. The six signals of **3** [$\delta$ 108.1 (d), 116.4 (d), 127.7 (s), 132.9 (s), 141.7 (s) and 151.3 (s)] were assigned to the *p*-hydroquinone moiety. It was shown that **1** has an extra doublet in this moiety instead of a singlet bearing the phenolic OH group in **3**. This change is coincident with the fact that **1** has one less oxygen than **3**.

In the $^1H$ NMR spectrum of **1**, three aromatic signals [$\delta$ 6.83, 7.09 and 7.77 (each 1H, dd)] which are coupled ($J$=1.5~2 Hz) were observed instead of two such signals [$\delta$ 7.12 (2H)] in the $^1H$ NMR spectrum of **3**.

From this evidence, it was concluded that **1** possesses a 1,3,5-trisubstituted partial structure.

Because it was shown that **1** does not have a hydroquinone moiety like **3**, the fact that **1** was

not interconvertible by treating with $Na_2S_2O_4$ or $FeCl_3$ can be clearly explained.

From all of the accumulated data described above, the structure of trienomycin A was concluded to be **1**.

Trienomycin A (**1**) is closely related to mycotrienins I (**2**) and II (**3**)[3~5] in its structure. However, it is unique among the benzenoid ansamycin group in that **1** does not have a *p*-quinone or *p*-hydroquinone moiety in the structure. The benzenoid moiety of **1** is somewhat similar to those of maytansinoids[8].

During the preparation of this manuscript, Dr. H. SETO personally mentioned us that *Streptomyces rishiriensis* T-23 also produced **1**[9].

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## References

1) UMEZAWA, I.; S. FUNAYAMA, K. OKADA, K. IWASAKI, J. SATOH, K. MASUDA & K. KOMIYAMA: Studies on a novel cytocidal antibiotic, trienomycin A. Taxonomy, fermentation, isolation, physico-chemical and biological characteristics. J. Antibiotics 38: 699~705, 1985
2) CORONELLI, C.; R. C. PASQUALUCCI, J. E. THIEMANN & G. TAMONI: Mycotrienin, a new polyene antibiotic isolated from *Streptomyces*. J. Antibiotics, Ser. A 20: 329~333, 1967
3) SUGITA, M.; Y. NATORI, T. SASAKI, K. FURIHATA, A. SHIMAZU, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. I. Taxonomy, fermentation, isolation and properties of mycotrienins I and II. J. Antibiotics 35: 1460~1466, 1982
4) SUGITA, M.; K. FURIHATA, H. SETO, N. ŌTAKE & T. SASAKI: The structures of mycotrienins I and II, a novel class of ansamycin antibiotic. Agric. Biol. Chem. 46: 1111~1113, 1982
5) SUGITA, M.; T. SASAKI, K. FURIHATA, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. II. Structure elucidation and biosynthesis of mycotrienins I and II. J. Antibiotics 35: 1467~1473, 1982
6) SUGITA, M.; Y. NATORI, N. SUEDA, K. FURIHATA, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. III. The isolation, characterization and structures of mycotrienols I and II. J. Antibiotics 35: 1474~1479, 1982
7) DAMBERG, M.; P. RUSS & A. ZEECK: Die Konstitution der fungistatischen Ansamycin-antibiotica Ansatrienin A und B. Tetrahedron Lett. 23: 59~62, 1982
8) REIDER, P. J. & D. M. ROLAND: Maytansinoids. *In* The Alkaloids. Vol. XXIII. *Ed.*, A. BROSSI, pp. 71~156, Academic Press, Inc., Orland, 1984
9) HIRAMOTO, S.; M. SUGITA, C. ANDŌ, T. SASAKI, K. FURIHATA, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. V. Isolation and structure determination of novel mycotrienin congeners. J. Antibiotics 38: 1103~1106, 1985