Short Communication

The Structures of Myocotrienins I. and II, a Novel Class of Ansamycin Antibiotic

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During the course of screening for antitumor antibiotics, we isolated two metabolites, interconvertible to each other *via* an oxido-reduction reaction from *Streptomyces rishiriensis*.

The reduced form was found to be identical with mycotrienin, an antifungal antibiotic obtained from *Streptomyces* sp.[1] The partial structure of this compound, which was classified into the polyene macrolide group based on its UV absorption, was given briefly by Coronelli *et al.*[1]

In this paper, we wish to report the structures of mycotrienins I and II,[2] and their biosynthesis.

The physicochemical properties of mycotrienins I (**1**) and II (**2**) are as follows; **1**, $C_{36}H_{48}N_2O_8$ [Calcd. C 67.92%, H 7.55, N 4.40, O 20.13; Found C 67.72%, H 7.68, N 4.28, O 20.32], yellow amorphous powder, mp 117°C, in beam EI MS M^+ (m/z) Obs. 636.3439; Calcd. 636.3407, $[\alpha]_D^{25}$ +71° (c=1.0, MeOH), UV λ_{max}^{MeOH} 260 nm (ε38500), 269 (49600), 282 (38800) and 383 (3400), IR $\nu_{max}^{CHCl_3}$ 3450 cm^{-1}, 3380 (NH, OH), 1740, 1180 (ester), 1750, 1660 (quinoid C=O) and 1640 (amide), and **2**, $C_{36}H_{50}N_2O_8$ [Calcd. C 67.71%, H 7.84, N 4.39, O 20.06; Found C 68.06%, H 7.86, N 4.39, O 19.69], white powder, mp 151°C, in beam EI MS M^+ (m/z) Obs. 638.3549; Calcd. 638.3535, $[\alpha]_D^{25}$ +288° (c=1.0, MeOH), UV λ_{max}^{MeOH} 260 nm (ε40800), 270 (52300), 280 (40500) and 310 (5900), IR $\nu_{max}^{CHCl_3}$ 3350 cm^{-1}, 3340 (NH, OH), 1730, 1200 (ester), 1650 and 1535 (amide).

1 was readily converted into **2** by treatment with $Na_2S_2O_4$ and the reverse reaction was accomplished by oxidation with air or $FeCl_3$. This suggested the presence of a quinone nucleus in the molecule. Acid hydrolysis of **1** with 6 N HCl afforded D-alanine.

The 100 MHz ^{13}C-NMR spectrum of **2** in pyridine-d_5 exhibited 36 signals which revealed

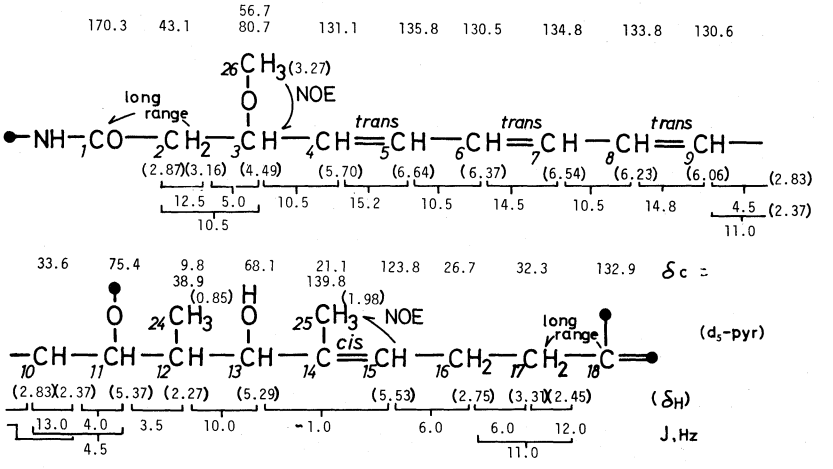

FIG. 1. Structure of Unit A.

the following functional groups; $CH_3 \times 3$, $CH_2 \times 9$, $CH \times 3$, $CH_3O \times 1$, $CH{-}O \times 3$, $CH{=} \times 9$, $C{=} \times 5$, and amide and/or ester carbonyls $\times 3$ (δ_C 170.3, 173.3 and 176.8). Since the two nitrogen atoms contained in **2** did not show any basicity, two of these carbonyl carbons are assigned to amide functions; the remaining one being ascribed to an ester residue. In agreement with this, two amide protons appeared at δ_H 8.78 (1H, bs) and 9.0 (1H, J=7.2 Hz) in addition to two quinone hydroxy signals at δ_H 11.0 and 11.24.

Consecutive proton spin decoupling experiments on **2** (400 MHz in pyridine-d_5) revealed the sequence from C-2 to C-17 as shown in Unit A (see Fig. 1). The NOE observed with the oxymethine signal H-3 (δ_H 4.49) upon irradiation of the methoxy proton (at δ_H 3.27) indicated that C-3 must be connected to the methoxy function. In the ^{1}H-NMR spectrum of **2** taken in $CDCl_3$, H-13 appeared at δ_H 4.78 (δ_H 5.29 in pyridine-d_5), while it moved downfield to δ_H 5.65 in that of tetraacetate **3** [$C_{44}H_{58}N_2O_8$, M^+ (m/z) 806.3961, Calcd. 806.3985, mp 117°C]. On the other hand, the chemical shift of H-11 remained almost unchanged (δ_H 4.96 in **2** and 4.87 in **3** in $CDCl_3$) showing that the oxygen at C-11 is protected by an ester linkage.

In order to extend further this partial structure, use was made of ^{13}C-{^{1}H} long range selective proton decoupling in CD_3OD. Thus, irradiation at δ_H 2.82 (H-2) and 2.91 (H-17) collapsed sp^2 carbons at δ_C 171.5 and 133.1, respectively, affording evidence that C-2 must be combined to the amide carbon (C-1) with C-17 to a quaternary sp^2 carbon (C-18). Thus, the partial structure, Unit A, has been unambiguously established to be as shown.

The structure of Unit B was proved by mass spectral analysis. In the high resolution mass spectrum of **2**, fragment peaks were observed at m/z 83.0836 (C_6H_{11}, Calcd. 83.0859), 111.0776 ($C_7H_{11}O$, Calcd. 111.0809) and 154.1189 ($C_9H_{16}NO$, Calcd. 154.1230). This implies that Unit B is the cyclohexylcarbonyl alanine moiety. In agreement with this partial structure, the treatment of **2** with $NaHCO_3$–MeOH afforded cyclohexylcarbonyl alanine methyl ester [$C_{11}H_{19}NO_3$, mp 68°C, M^+ (m/z) 213.1363], which was completely identical with a synthetic sample prepared by the condensation of cyclohexane carbonyl chloride and L-alanine followed by treatment with diazomethane. Thus, Unit B is connected to C-11 through an ester linkage (*vide supra*).

The remaining carbons in **2** not contained either in Unit A or B are two –C= (δ_C 127.7 and 132.9), two quinone hydroxy groups (δ_C 141.7 and 151.3, δ_H 11.0 and 11.24) and two –CH= (δ_C 108.1 and 116.4, δ_H 7.12, 2H in pyridine-d_5). These two protons were observed as an AB quartet in CD_3OD (δ_H 6.48 and 6.55,

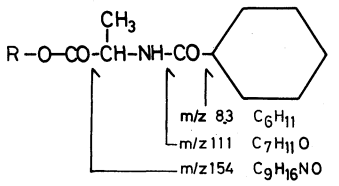

FIG. 2. Structure of Unit B.

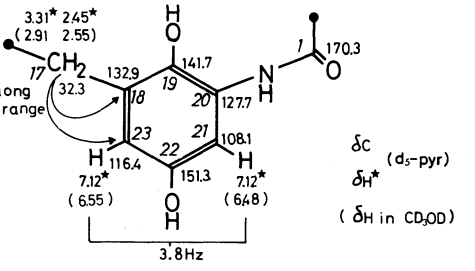

FIG. 3. Structure of Unit C.

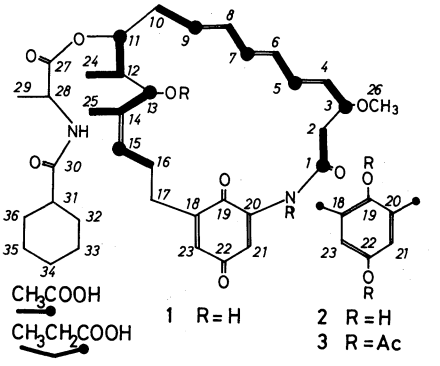

FIG. 4. Structures and Biosynthetic Origin of Mycotrienins.

J=3.7 Hz). These NMR spectral data together with facile interconversion between **1** and **2** indicate that a 2,6-disubstituted *p*-benzoquinone nucleus and its hydroxy form are present in **1** and **2**, respectively (Unit C). The coupling of H-17 (δ_H 2.91) to C-23, and to C-18 in the ^{13}C-NMR spectrum of **2** taken in CD_3OD which had been confirmed by long range selective proton decoupling proved the sequence of C-17 (δ_C 32.5), C-18 (δ_C 133.1) and C-23 (δ_C 116.3). Thus, the only remaining NH group at C-1 in Unit A must be connected to C-20 of the quinone ring. The chemical shifts of these carbons are in good agreement with calculated values.[3] Accordingly, the structure of **2**, and therefore of **1**, has been unambiguously established to be as shown in Fig. 4.

In order to reveal the biosynthetic pathway involved in the formation of **1**, [1-^{13}C] sodium acetate and [1-^{13}C] sodium propionate were separately added to the fermentation broth of *Streptomyces rishiriensis*, and ^{13}C-labelled **2** was isolated from the mycelium.

In the ^{13}C-NMR spectrum of **1** labelled with [1-^{13}C] sodium acetate, the signals due to C-1, 3, 5, 7, 9 and 15 were enriched 10$\sim$20 fold, while [1-^{13}C] propionate was incorporated at C-11 and 13 *ca.* 8 fold as shown in Fig. 4. Thus, **1** is a polyketide in origin. The quinone moiety of **1** may be derived from a C_7-N unit in a similar manner as suggested for rifamycin,[4] geldanamycin[5] and pactamycin.[6] Oshima and Ariga[7] reported that the structure of ω-cyclohexyl carboxylic acid was formed from glucose, *via* the shikimate pathway. Presumably, the same mechanism may be operative in the biosynthesis of **1**.

Mycotrienins I and II are closely related to Macbecins I and II[8] in their structures, however, they are unique among the ansamycin group in that **1** has a 21-membered macrocyclic lactam ring and that a cyclohexylcarbonyl moiety was found for the first time in this group. ω-Cyclohexyl fatty acids have been reported as metabolites of *Curtobacterium pusillum*[9] and a component of asukamycin.[10]

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