Structure Determination of Simaomicins α and β,† Extremely Potent, Novel Anticoccidal Agents produced by *Actinomadura*

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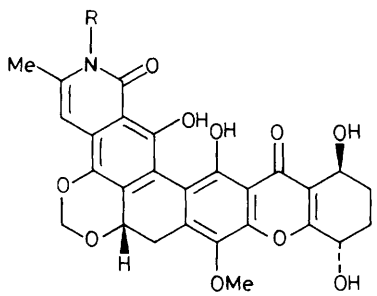
The structures of simaomicins α (**1**) and β (**2**), novel polycyclic xanthone-type antibiotics produced by *Actinomadura madura* subspecies *simaoensis*, were determined by *X*-ray crystallography and spectroscopic methods.

Structures have been determined for two novel extremely potent anticoccidial antibiotics, simaomicins α (**1**) and β (**2**), derived from *Actinomadura madura* subspecies *simaoensis*.[1] At the optimal dosage of 1 p.p.m. in the diet of chickens, simaomicin α is the most potent non-synthetic broad spectrum anticoccidial ever reported.[2] Simaomicins α and β contain a xanthone unit and are structurally related to a small family of polycyclic antibiotics including lysolipins,[3] albofungins,[4] cervinomycins,[5] and actinoplanones.[6] The structures of the simaomicins are unique with respect to all other members of this family in that they have a methylenedioxy ring in line with the xanthone rather than the pyridone unit. All members have a methylenedioxy ring except the cervinomycins. Of this family, only the simaomicins have been reported to have anticoccidial activity.

A fermentation of the micro-organism in a complex medium was filtered and the filtrate was extracted with dichloromethane. The extract was concentrated and chromatographed on a column of silica gel eluted with dichloromethane. Fractions containing simaomicin were then purified by reverse phase preparative h.p.l.c. to obtain the pure components. Simaomicins α and β were isolated as yellow crystalline compounds by this purification process.

High resolution fast atom bombardment mass spectrometry indicated molecular formulae of $C_{28}H_{25}NO_{10}$ and $C_{27}H_{23}NO_{10}$, respectively.‡ The polycyclic aromatic structure of these compounds was suggested from the ^{13}C n.m.r. data, the u.v. chromophores, and the degrees of unsaturation; however, it was somewhat surprising to observe only one aromatic proton (δ_H 6.70, s) in the ^1H n.m.r. spectrum considering the number of aromatic rings. Other obvious features from the ^1H and ^{13}C n.m.r. data of simaomicin α were the presence of one OCH_3 (δ_H 3.88, s; δ_C 61.61, q), one NCH_3 (3.62, s; 30.44, q), and one CCH_3 (2.45, s; 20.36, q) group.§ Simaomicin α crystallized from a chromatographic solvent



(**1**) R = Me
(**2**) R = H

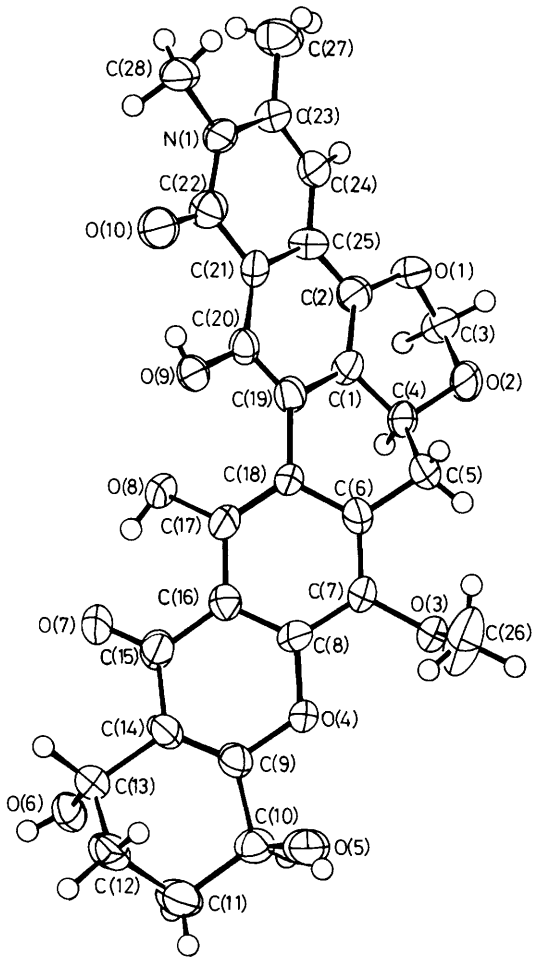


Figure 1. ORTEP drawing of simaomicin α (**1**); the absolute configuration has not been determined.

† Previously designated LL-D42067 α and β (see ref. 1).

‡ Simaomicin α (**1**): $C_{28}H_{25}NO_{10}$ (MH^+, *m/z* 536.1537, calc. 536.1522); simaomicin β (**2**): $C_{27}H_{23}NO_{10}$ (MH^+, *m/z* 522.1400, calc. 522.1400).

§ Simaomicin α (**1**): $[\alpha]_D^{26}$ +836 (*c* 0.3, dimethylformamide); $\lambda_{max.}$ (MeOH) (log ε) 253 (4.55), 320 (4.11), 379 sh (4.36), 395 nm (4.40); $\nu_{max.}$ (KBr) 3450, 1650, 1598, 1543, 1470, 1440, 1260, 1195, 1020 cm^{-1}; ^1H n.m.r. ($CDCl_3$, 300 MHz): δ (Me_4Si) 1.88 (2H, m), 2.34 (2H, m), 2.45 (3H, CMe, s), 2.58 (1H, m), 3.62 (3H, NMe, s) 3.72 (1H, dd, J^a 4.64, J^b 14.22 Hz), 3.88 (3H, OMe, s), 4.80 (2H, m), 5.08 (1H, m), 5.32 (1H, OCH_2O, d, *J* 5.81 Hz), 5.55 (1H, OCH_2O, d, *J* 5.81 Hz), 6.70 (1H, s), 12.76 (1H, OH, s), 13.58 (1H, OH, s); ^{13}C n.m.r. (CD_3SOCD_3, 75 MHz): δ (Me_4Si), 20.4 (CH_3), 25.4 (CH_2), 25.8 (CH_2), 29.0 (CH_2), 30.4 (CH_3), 58.5 (CH), 61.6 (CH_3), 63.3 (CH), 71.7 (CH), 90.4 (CH_2), 100.0 (CH), 109.2 (s), 109.7(s), 110.9 (s), 113.7 (s), 119.0 (s), 125.8 (s), 126.6 (s), 134.9 (s), 135.3 (s), 136.1 (s), 141.3 (s), 147.9 (s), 151.1 (s), 152.5 (s), 165.4 (s), 165.6 (s), 182.3 (s).

system of acetonitrile, water, and acetic acid, and *X*-ray crystallography of a yellow platelike crystal revealed the structure and relative stereochemistry of simaomicin α (Figure 1).¶ Simaomicin β had essentially an identical u.v. chromophore and ^{1}H and ^{13}C n.m.r. spectrum to simaomicin α except that it lacked the ^{1}H n.m.r. singlet and a ^{13}C n.m.r. quartet corresponding to the NCH_3 group. This information, along with the difference in molecular formulae, indicated that simaomicin β was the des-*N*-methyl analogue of simaomicin α. It also showed that simaomicin β was in the same tautomeric form as simaomicin α without enolization of the pyridone ring.

¶ *Crystal data for* (**1**): $C_{28}H_{25}NO_{10}.2H_2O$, M = 571.54, orthorhombic, space group $P2_12_12_1$, a = 15.810(4), b = 17.808(3), c = 8.972(2) Å, U = 2526.3 Å^3, Z = 4, D_c = 1.50 g cm^{-13}, λ (Cu-K_α) = 1.54184 Å, μ (Cu-K_α) = 10.2 cm^{-1}, F (000) = 1200. The structure was solved by direct methods and refined by full-matrix least-squares methods. A total of 3091 reflections were collected using the ω–2θ scan technique to 2θ = 150° on an Enraf-Nonius CAD4 computer controlled κ axis diffractometer equipped with a graphite crystal, incident beam monochromator. Using 350 reflections (minimum E of 1.50) and 5617 relationships, a total of 20 phase sets were produced. 2941 Reflections were unique, and 2109 with $I > 3\sigma(I)$ were used in the refinement; final R 0.049, R_w 0.063. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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