Structure of Oxanthromicin (Antibiotic 16–550), a Novel Dimeric Anthrone Peroxide

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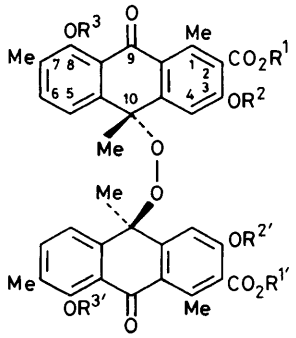
Oxanthromicin, an antibiotic isolated from an *Actinomadura* sp. SCC 1646 fermentation broth, has been assigned a novel dimeric peroxide structure based on chemical transformations and spectral data, and by a single-crystal *X*-ray analysis of a tetramethyl derivative.

Oxanthromicin (**1**), a dimeric anthrone peroxide possessing good *in vitro* activity *vs.* dermatophytes and moderate activity *vs. Candida* spp., is a component of the fermentation broth produced by *Actinomadura* sp. SCC 1646.[1] The antibiotic was isolated by extraction with ethyl acetate followed by h.p.l.c.; subsequent deionization and crystallization afforded pure (**1**) {m.p. 211—213 °C (decomp.); $[\alpha]_D^{26}$ −172.1° (*c* 0.3, EtOH)}.

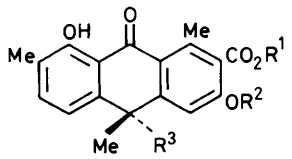
The ^{1}H n.m.r. spectrum† of (**1**) contained six non-exchangeable absorptions, three corresponding to *C*-methyl groups and three to aromatic hydrogens two of which were *ortho*-coupled (δ 6.55, 7.25; *J* 8 Hz). From a total of eighteen resonances in the ^{13}C n.m.r. spectrum,† two were ascribed to carbonyl carbon atoms, twelve to aromatic or olefinic carbon atoms, and four to aliphatic carbon atoms. Of the four aliphatic carbon atoms, one gave a singlet and three gave quartets in the non proton-decoupled spectrum. Only fragments were observed in the mass spectrum of (**1**).

Esterification of (**1**) gave a diester which on further reaction with diazomethane led to tri- and tetra-methyl derivatives (**2**)



(**1**) $R^1 = R^2 = R^3 = R^{1'} = R^{2'} = R^{3'} = H$
(**2**) $R^1 = R^2 = R^{1'} = Me$; $R^3 = R^{2'} = R^{3'} = H$
(**3**) $R^1 = R^2 = R^{1'} = R^{2'} = Me$; $R^3 = R^{3'} = H$
(**4**) $R^1 = R^2 = R^3 = R^{1'} = R^{2'} = R^{3'} = Me$

† Oxanthromicin (**1**): i.r. (KBr) ν_{max} 1620—1650 cm^{-1}; u.v. (MeOH) λ_{max} (log ε) 315(4.36), 354(4.19) nm; ^{1}H n.m.r. (80 MHz, MeOD) δ 1.2 [6H, s, C(10)-, C(10′)-Me], 2.24 [6H, s, C(7)-, C(7′)-Me], 2.85 [6H, s, C(1)-, C(1′)-Me], 6.55 [2H, d, *J* 8 Hz, H(5), H(5′)], 6.7 [2H, s, H(4), H(4′)], 7.25 [2H, d, *J* 8 Hz, H(6), H(6′)]; ^{13}C n.m.r. (25.2 MHz, MeOD): δ 189.3 [C(9), C(9′)], 168.7 (CO_2H), 159.5 [C(3), C(3′) or C(8), C(8′)], 158.2 [C(8), C(8′) or C(3), C(3′)], 148.9 [C(9a), C(9a′) or C(4a), C(4a′)], 141.1 [C(4a), C(4a′) or C(9a), C(9a′)], 139.4 [C(arom.)], 135.8 [C(6), C(6′)], 127.1 [C(arom)], 124.8 [C(arom.)], 119.9 [C(arom.)], 116.7 [C(5), C(5′)], 114.5 [C(arom.)], 112.1 [C(4), C(4′)], 79.7 [C(10), C(10′)], 32.9 [C(10)-, C(10′)-Me], 20.5 [C(1)-, C(1′)-Me], 15.1 p.p.m. [C(7)-, C(7′)-Me]. The spectra of all other compounds reported were in accord with their assigned structures.



(**5**) $R^1 = R^2 = H$; $R^3 = OH$
(**6**) $R^1 = R^2 = R^3 = H$
(**7**) $R^1 = R^2 = Me$; $R^3 = OH$
(**8**) $R^1 = R^2 = Me$; $R^3 = H$

and (**3**), respectively, with the former being an intermediate in the formation of the latter. The dimeric nature of the antibiotic was revealed by the ^1H n.m.r. spectrum of (**2**) which had approximately twice as many absorptions as those of (**1**) and (**3**). Thus, it was apparent that (**1**) comprised two identical units each containing a carboxylic acid function and at least one phenolic group. The presence of a second, strongly hydrogen-bonded phenolic group was evident from the exchangeable absorption at δ 13.6 in the ^1H n.m.r. spectrum of (**3**) and those at δ 13.5 and 13.6 in the spectrum of (**2**). Exhaustive methylation [$(MeO)_2SO_2$–K_2CO_3] of (**3**) yielded hexamethyl derivative (**4**) which gave a molecular ion at *m/z* 738, consistent with a stoicheiometry of $C_{42}H_{42}O_{12}$ derived from analytical data.

Consideration of the proposed stoicheiometry in conjunction with the ^1H and ^{13}C n.m.r. spectra data led to the suggestion that a peroxide group was present in (**1**). This was explored by treating (**1**) or its derivatives under reducing conditions. Reduction of (**1**) with zinc in acetic acid or with palladium on carbon in the presence of hydrogen both gave monomer (**5**) as the major product. Prolonged treatment led to an optically inactive compound, assigned structure (**6**). Similar treatment of (**3**) gave monomers (**7**) and (**8**). That hydrogenolysis occurred was certainly consistent with the presence of a peroxide linkage although treatment with phosphines or potassium iodide did not reduce (**1**). However, support lay in the ^{13}C n.m.r. chemical shift changes accompanying the hydrogenolysis. Thus, the only *C*-methyl group to undergo a significant chemical shift change was that associated with the absorption at δ 34.0 p.p.m. in (**3**) which moved to δ 38.6 p.p.m. in (**7**); this downfield shift was consistent with the removal of a γ effect. Similarly, the resonance at δ 79.8 p.p.m. assigned to the tertiary aliphatic carbon atom in (**3**) was shifted upfield to δ 71.2 p.p.m., consistent with the removal of a β effect; in compound (**8**), the absorption for the corresponding atom occurred at δ 39.1 p.p.m. as expected. In the ^1H n.m.r. spectrum of (**8**), the methyl group appeared as a doublet at δ 1.5.

Although spectral data alone did not allow the aromatic substitution pattern in (**1**) to be established with certainty, they did reveal that the *C*-methyl group at δ 2.38 in the ^1H n.m.r. spectrum of (**3**) was bonded to the ring containing the *ortho* substituted hydrogen atoms and the chelated phenolic group. Thus, irradiation of this methyl resonance caused sharpening of the doublet at δ 7.45, whereas irradiation of the methyl group at δ 2.80 resulted in sharpening of the singlet at δ 6.58. Moreover, the i.r. spectrum of (**2**) was especially informative in this regard in that the ester absorptions occurred at 1730 and 1670 cm^{-1}, whereas that in (**3**) was at 1730 cm^{-1}. Therefore, the phenolic groups that reacted with diazomethane were *ortho* to the ester function. Only on formation of hexamethyl derivative (**4**) did the ketone carbonyl absorption change [from 1625 in (**1**), (**2**), and (**3**) to 1660 cm^{-1} in (**4**)].

As oxanthromicin is optically active, the chirality of both of the monomeric units must be identical. In order to determine

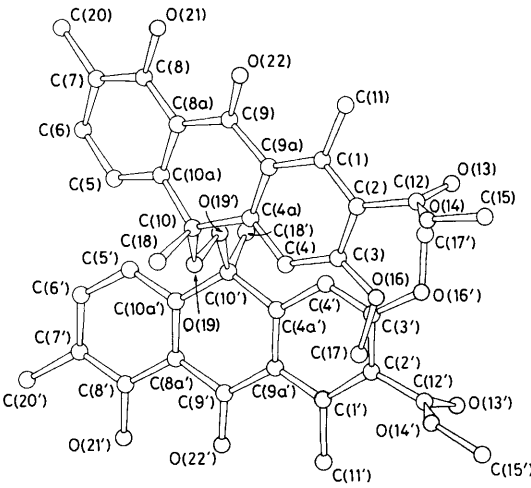


Figure 1. Structure and solid-state conformation of (**3**); hydrogen atoms have been omitted for clarity.

the substitution pattern of the trisubstituted ring unambiguously, an *X*-ray crystal structure analysis of (**3**) was undertaken. *Crystal data:* (**3**), $C_{40}H_{38}O_{12}$, $M = 710.74$, monoclinic, space group $P2_1$, $a = 9.337(4)$, $b = 22.997(10)$, $c = 8.444(3)$ Å, $\beta = 104.61(1)°$, $U = 1754.5$ Å^3, $Z = 2$, $D_c = 1.345$ g cm^{-3}. The structure was solved by direct methods.[2] Hydrogen atoms were located in a difference Fourier synthesis at a late stage in the analysis. Full-matrix least-squares refinement of atomic positional‡ and thermal (anisotropic C, O; isotropic H) parameters converged to R 0.042 over 2520 statistically significant [$I > 2.0\sigma(I)$] reflections recorded on an Enraf-Nonius CAD-3 automated diffractometer[3] (Ni-filtered Cu-K_α radiation; θ–2θ scans, θ_{max} 67°). A view of the solid-state conformation with the atom numbering scheme is provided in Figure 1; the absolute configuration remains to be defined. Both hydroxy groups are involved in intramolecular hydrogen bonds to their adjacent carbonyl groups [O(21)···O(22) 2.52, O(21')···O(22') 2.50 Å].

Since (**1**) is chiral an enzymic oxidation is suggested. To the best of our knowledge, (**1**) constitutes the first known example of such a naturally occurring compound.

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2 P. Main, L. Lessinger, M. M. Woolfson, G. Germain, and J.-P. Declerq, 'MULTAN 76, A System of Computer Programmes for the Automatic Solution of Crystal Structures,' Universities of York and Louvain, 1976.
3 For details, see R. W. Miller and A. T. McPhail, *J. Chem. Soc., Perkin Trans.* 2, 1979, 1527.

‡ Crystallographic calculations were performed on an IBM 3081 computer located at the Triangle Universities Computation Centre, North Carolina. For the least-squares calculations, a locally modified version of the Gantzel, Sparks, and Trueblood UCLA full-matrix program was used. Atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.