Isolation and Structure (*X*-Ray Analysis) of the Orsellinate of Armillol, a New Antibacterial Metabolite from *Armillaria mellea*

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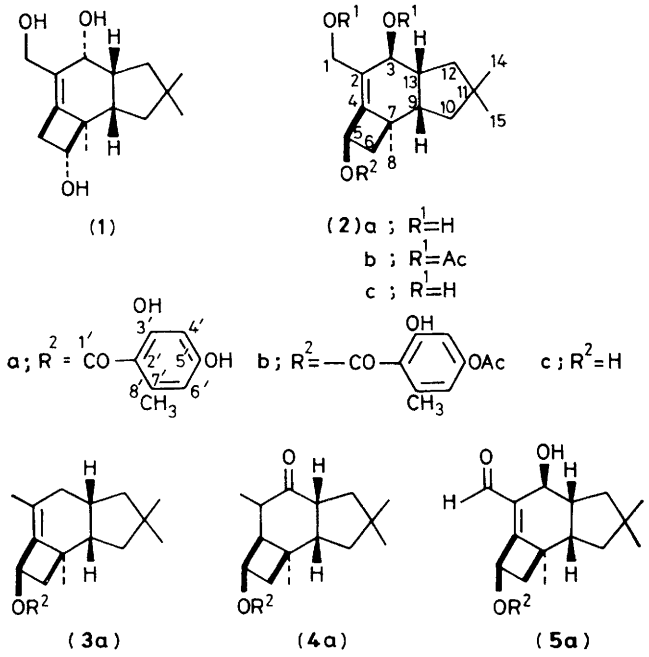
The structure of the orsellinate of armillol (**2a**), a new member of the protoilludane group and one of several sesquiterpenoid esters isolated from *Armillaria mellea*, was established by spectral and chemical means and confirmed by single-crystal *X*-ray analysis of its oxidation product, the hydroxy-aldehyde (**5a**).

A number of Basidiomycetes are known to produce biologically active metabolites, the majority of which have their structures established. Among these are *Clitocybe illudens*, which produces in addition the inactive illudol (**1**),[1] *Fomes annosus*, the source of fomannosin[2] and fomannoxin,[3] and *Armillaria mellea*, the extracts of which have been extensively reported to have antibacterial and antifungal activities.[4] Investigation of the mycelium of *A. mellea* (strain 619) has now led to the isolation of a group of sesquiterpenoid aryl esters. We herein report the structural elucidation of the major metabolite, the orsellinate of armillol (**2a**).†

The mycelium produced from a three week-old culture of *A. mellea* grown on potato dextrose medium (3 l) was extracted with methanol. Addition of water to the methanolic extract followed by extraction with diethyl ether gave a brown

† Fomannosin numbering.[5]



oil (10 g) which was fractionated by Sephadex LH-20 and flash chromatography (Kiselgel 60H). The major component (1.4 g) eluted with n-hexane–acetone (3:1) was crystallised from benzene to give armillyl orsellinate (**2a**). The molecular formula of (**2a**) ($C_{23}H_{30}O_6$) was established by chemical-ionisation mass spectrometry with $[M + NH_4]^+$ at *m/e* 420 and a characteristic fragmentation ion at *m/e* 217 due to loss of a molecule of orsellinic acid and of water from the MH^+ ion. The electron-impact mass spectrum showed the base peak at *m/e* 151 $[Ar(OH)_2MeCO]^+$. Compound (**2a**) had m.p. 91—93 °C: $[\alpha]_D^{20}$ −126.9° (*c* 1.1, MeOH); i.r. ($CHCl_3$) ν_{max} 1645 cm^{-1} (ester); u.v. (MeOH) λ_{max} 263 (ε 14 400) and 300 nm (ε 5820). The 400 MHz ^1H n.m.r. spectrum showed signals due to three aliphatic and one aromatic methyl groups (δ 0.98, 1.07, 1.13, and 2.47) and decoupling experiments allowed the assignments of the other protons: δ 1.07, 1.3, 1.4, and 1.8 (4H, 4 × dd, H-10 and H-12), 1.99 and 2.70 (2H, 2 × dd, *J* 7.0, 7.6, and 11.5 Hz, H-6), 2.4—2.55 (2H, m, H-9, H-13) 4.23 (1H, dd, *J* 9.0 and 2.0 Hz, H-3), 4.18 and 4.38 (2H, 2 × d, *J* 13.0 Hz, H-1), 5.98 (1H, ddd, *J* 7.0, 7.6, and 2.0 Hz, H-5), 6.14 (1H, d, *J* 2.2 Hz, H-4′), and 6.22 (1H, d, *J* 2.2 Hz, H-6′).

The ^{13}C n.m.r. spectrum was consistent with the proposed structure (**2a**), the relevant signals being at δ 170.2 (s, ester), 58.5 (t, C-1), 74.4 (d, C-3), 69.8 (d, C-5), and three triplets at 40.7, 46.1, and 46.2 p.p.m.; in addition eight resonances due to sp^2 carbon atoms were observed. Acetylation of (**2a**) gave a triacetate (**2b**), an oil; $[\alpha]_D^{20}$ −93.6° (*c* 0.55, MeOH); u.v. (MeOH) λ_{max} 252 (ε 8264) and 310 nm (ε 3351), having the 3′-OH free [like the natural product (**2a**), it gave a red-brown colour with alcoholic $FeCl_3$]. The 400 MHz ^1H n.m.r. spectrum showed three additional methyl groups due to the acetoxy-groups, downfield shifts of the aromatic protons (δ 6.49 and 6.62), a well defined low-field AB quartet (δ 4.55 and 4.7, *J* 12.8 Hz, H-1), and a downfield shift for the 3-H resonance (δ 5.39).

Methanolysis of (**2a**) gave methyl orsellinate (m.p. 142 °C) and the non-crystalline sesquiterpene triol, named armillol (**2c**). The ^1H n.m.r. spectrum of the latter displayed signals for CH_2OH at δ 4.43, 3-H at δ 4.06, and 5-H at δ 4.95; the remaining signals corresponded closely in chemical shift to those assigned to armillyl orsellinate. The observed significant upfield shift for 5-H with respect to the natural product (**2a**) proved the attachment point for the ester moiety. The ^{13}C n.m.r. spectrum fully supported the structure of armillol.

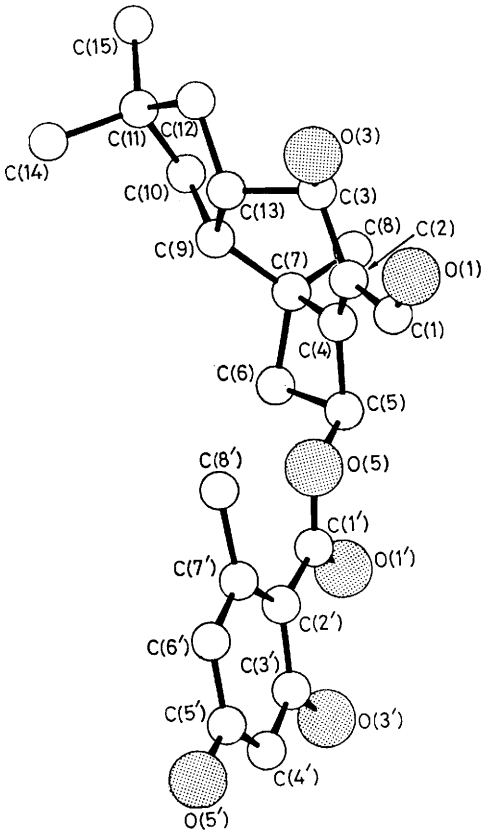


Figure 1. Molecular structure of the hydroxyaldehyde (**5a**) [in the enantiomeric form of formula (**5a**)].

Catalytic hydrogenation of (**2a**) led to two products (**3a**) and (**4a**). The major, (**3a**), $C_{23}H_{30}O_4$ (M^+ at *m/e* 370); m.p. 140—141 °C, λ_{max} (MeOH) 265 (ε 15 644) and 301 nm (6044) arises from hydrogenolysis of both the primary and secondary alcohols, confirming the allylic nature of these functions. Its 400 MHz ^1H n.m.r. spectrum showed the presence of a vinyl methyl group (δ 1.57) and no evidence for alcohol groups on C-2 and C-3. The minor product (**4a**), $C_{23}H_{30}O_5$, (M^+ at *m/e* 386) is a keto-ester. The i.r. spectrum showed an additional carbonyl band at 1710 cm^{-1}; c.d. in dioxan: Δ_ε + 0.94 (318 nm); its ^1H n.m.r. spectrum displayed a signal for a secondary methyl group at δ 0.94 (*J* 6.8 Hz) and two protons at C-2 (δ 3.12 m) and C-4 (δ 3.04, dd, *J* 7.2 and 7.6 Hz). This compound closely resembles one of the hydrogenation products of illudol.[1]

Manganese dioxide oxidation of (**2a**) gave a mixture of compounds from which the β-hydroxy-aldehyde (**5a**) was isolated; double m.p. at *ca.* 90 °C and 140—142 °C $[\alpha]_D^{20}$ −108.1° (*c* 1.1, MeOH) $C_{23}H_{28}O_6$ (M^+ at *m/e* 400); u.v. (MeOH) λ_{max} 222 (ε 16 552), 262 (ε 19 310), and 306 nm (ε 4452). The 400 MHz ^1H n.m.r. spectrum of (**5a**) revealed four methyl groups, three aliphatic (δ 1.0, 1.14, and 1.21) and one aromatic (δ 2.49), in addition to δ 1.25 (1H, dd, *J* 12.2 and 9.6 Hz, 10-H), 1.42 (1H, dd, *J* 12.0 and 9.8 Hz, H-12), 1.53 (1H, dd, *J* 12.0 and 6.2 Hz, H-12), 1.94 (1H, dd, *J* 12.2 and 7.2 Hz, H-10), 2.1 and 2.82 (2H, 2 × dd, *J* 11.6, 7.0, and 11.6, 8.8 Hz, H-6), 4.43 (1H, dd, *J* 2.8 and 7.0 Hz, H-3), 6.30 (1H, ddd, *J* 11.6, 8.8, and 2.8 Hz, H-5), 6.24 and 6.3 (2H, 2 × d. *J* 2.8 Hz, H-4′ and -6′), and 9.84 (*CHO*).

Unequivocal proof for the structure and relative stereochemistry of the aldehyde (**5a**) and hence the natural product (**2a**) was provided by single-crystal *X*-ray analysis. Crystals

of (**5a**) were obtained as a 1:1 solvate from benzene. *Crystal data:* orthorhombic, space group $P2_12_12_1$ ($Z = 4$), $a = 25.346(4)$, $b = 12.934(4)$, $c = 190.8(4)$ Å. 1817 independent structure factors [$I > 2\sigma(I)$] were scanned on an automatic diffractometer using graphite-monochromatised Cu-K_α radiation. The structure was solved by direct methods and refined to a final R value of 7.7% with anisotropic thermal parameters. (All H atoms were located from difference Fourier syntheses and were assigned with isotropic thermal factors). A view of the molecular conformation is shown in Figure 1.‡ Armillol is isomeric with illudol and like the latter possesses a *cis*-fused hydrindane skeleton but differs in the configuration of the hydroxy-group at C-3 and in the position and configuration of the hydroxy group on the cyclobutane ring.[6]

Armillol is a new member of the group of compounds which most probably arise biogenetically from a protoilludyl cation. This route has been proved for fomannosin[5] and proposed for illudol and its related compounds.[5]

The bioassays were carried out using conventional antibiotic discs. Armillyl orsellinate (**2a**) showed strong antibacterial activity against gram-positive bacteria, such as *Bacillus subtilis* 5262 and *Staphylococcus aureus* 209 P (minimum value, 5.6 γ per disc). The oxidation product (**5a**) was even more active (minimum value, 2.4 γ per disc). The compounds did not inhibit the growth of gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*).

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‡ The 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.

References

1 T. C. Morris, M. S. R. Nair, and M. Anchel, *J. Am. Chem. Soc.*, 1967, **89**, 4562; M. F. Semmelhack, S. Tomoda, and K. M. Hurst, *ibid.*, 1980, **102**, 7567; P. D. Cradwick and G. A. Sim, *Chem. Commun.*, 1971, 431.
2 C. Bassett, R. T. Sherwood, J. A. Kepler, and P. B. Hamilton, *Phytopathology*, 1967, **57**, 1046.
3 M. Hirotani, J. O'Reilly, D. M. X. Donnelly, and J. Polonsky, *Tetrahedron Lett.*, 1977, 651.
4 K. A. Oduro, D. E. Munnecke, J. J. Sims, and N. T. Keen, *Trans. Br. Mycol. Soc.*, 1976, 195, and references therein.
5 D. S. Cane and R. S. Nachbar, *J. Am. Chem. Soc.*, 1978, **100**, 3208.
6 An orsellinate of a neoilludol derivative has been isolated from *A. mellea:* J. J. Sims, personal communication.