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ISOLATION AND X-RAY CRYSTAL STRUCTURES OF ASTELLOLIDES A AND B, SESQUITERPENOID METABOLITES OF ASPERGILLUS VARIECOLOR

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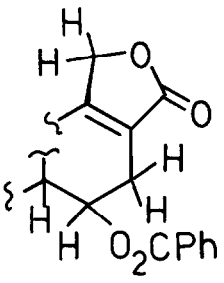
Abstract. Two sesquiterpenoid metabolites, astellolides A and B, have been isolated from cultures of Aspergillus variecolor. Their structures have been elucidated by X-ray crystallography and spectroscopic methods.

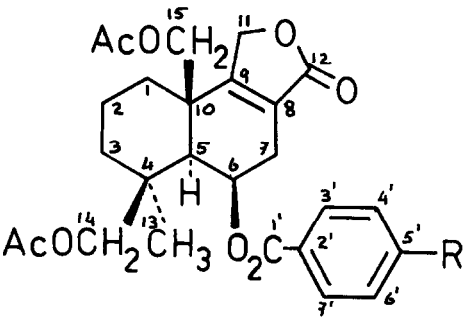
In the course of biosynthetic[1] and structural[2] studies of andibenin and related metabolites of Aspergillus variecolor we obtained a mutant of the andibenin producing strain, 212KI69, which lacked the usual abundant polyketide derived mycelial pigments.[3] We now wish to report the isolation and structures of two biosynthetically significant sesquiterpenoid metabolites from extraction and chromatography of the fermentation liquors of this mutant strain.

The major metabolite astellolide A, $C_{26}H_{30}O_8$ (mass spec. and analysis), formed colourless prisms from ethyl acetate with m.p. 213-214°C, showed $[\alpha]_D^{25}$-8°, and u.v. maxima at 221 (log Σ4.30) and 275 (3.02) nm. The 360MHz ^{1}H n.m.r. spectrum showed signals at δ1.15, (tertiary methyl); 1.90 and 2.11 (both 3H, s), 3.99 and 4.38 (both 1H, d, J 12Hz) and 4.88 and 4.97 (both 1H, d, J11Hz) assignable to two tertiary acetoxy methyls; and 5.95 (1H, m), 8.02 (2H, d, J8Hz), 7.57 (1H, t, J8Hz), 7.47 (2H, t, J8Hz) assignable to the benzoate of a secondary alcohol. The corresponding carbon signals were readily identified in the 25MHz ^{13}C n.m.r. spectrum (Table). Extensive spin-decoupling experiments showed that the methine proton at δ5.95 which was coupled to a methine proton at δ1.82 (1H, d, J1.5Hz) was also coupled (J5.0 and 2.0Hz) to allylic methylene protons at 2.72 (2H, m). This allylic methylene in turn showed a homo-allylic coupling to two doublets of triplets at δ4.81 and 5.97 (each 1H, J17 and 2.5Hz) corresponding to an oxygen bearing allylic methylene. No other allylic protons were present. From the chemical shifts and consideration of the remaining functionality, this oxymethylene and the tetrasubstituted double bond (δc 122.5 and 165.6) must be linked with the remaining acyl carbon (δc173.0) to form an α,β-unsaturated γ-lactone and so partial structure (A) can be deduced. After allowing for the acetate and benzoate carbons, the remaining carbon skeleton is C_{15} and tricyclic, and the carbons not accounted for in the above functions consist of three aliphatic

methylenes and two aliphatic quaternary carbons. These features can best be accommodated in a substituted confertifolin structure as indicated in (1).



(A)



(1) R = H
(2) R = OH

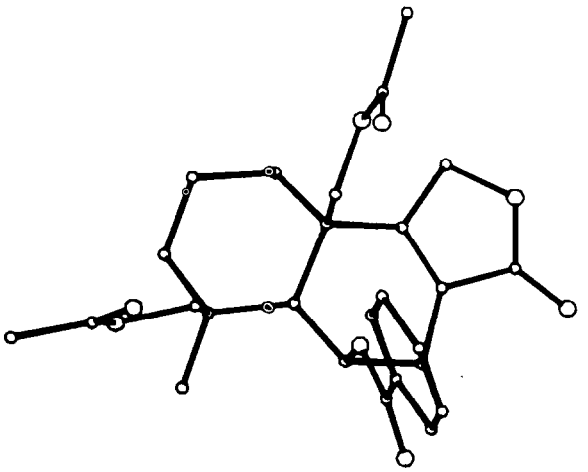
The coupling constants of the 6-proton to the 5- and 7-protons indicate that H-6 is equatorial and thus the benzoate is in the axial position. The C-methyl can be placed at the 4-equatorial position, rather than the 4- or 10-axial positions by virtue of its high chemical shift value[5] and comparison with the shifts observed for C-methyls and acetoxymethyls in a variety of terpenoid compounds e.g. in podocarpol acetate[5] the 4-equatorial methyl and 4-axial acetoxymethylene carbons resonate at 27.4 and 67.0 p.p.m. respectively whereas in andalusol 6,18-diacetate[6] the 4-axial methyl and 4-equatorial acetoxymethylene resonate at 17.9 and 74.1 p.p.m. respectively. Thus all the n.m.r. data are consistent with structure (1) for astellolide A. This has been confirmed by a single crystal X-ray diffraction study.[7]

Crystal data: $C_{26}H_{30}O_8$, M=470, clear colourless orthorhombic crystals, space group $P2_12_12_1$ (No.19), a = 11.023 (3), b = 14.426 (4), c = 15.045 (5) Å, U = 2392 Å^3, Z = 4, Dc = 1.31 g cm^{-3}; MoKα radiation (λ = 0.71069 Å, μ = 0.58 cm^{-1}). Intensity data were collected on a Nonius CAD 4 diffractiometer (to 2θ = 50°). Of 2436 unique reflections, 1631 had $I > 3\sigma(I)$. Multan 77[8] was used to solve the structure which was refined isotroptically to a R value of 0.086 using SHELX[9], hydrogen atoms were included in calculated positions. The structure of the molecule is illustrated in the Figure by a PLUTO[10] drawing.

Astellolide B, $C_{26}H_{30}O_9$, gave colourless rods from ethyl acetate m.p. 251-253°C. Its spectroscopic properties differed from astellolide A, only in signals that were readily attributable to having a p-hydroxybenzoate function in place of the benzoate of astellolide A. Thus ^{1}H n.m.r. showed signals at δ6.84 and 7.87 (each 2H, d, J8Hz) and the aromatic ring carbons in the ^{13}C n.m.r. spectrum appeared at 161.3 (s), 131.3 (2xd), 115.6 (2xd), 120.6 (s); so astellolide B has structure (2).

Table ^{13}C n.m.r. chemical shifts (p.p.m. from Me_4Si) and multiplicities observed for astellolide A (1) and astellolide B (2).

Carbon	(1)	(2)
1	31.6t	31.7t
2	17.9t	17.9t
3	37.0t	37.0t
4	37.7s	37.7s
5	53.9d	54.1d
6	66.6d	66.0d
7	29.0t	29.1t
8	122.5s	122.8s
9	165.6s	165.6s
10	40.5s	40.5s
11	65.9t	66.0t
12	173.0s	173.4s
13	27.7q	27.8q
14	66.9t	67.1t
15	71.3t	71.4t
$CH_3\underline{C}O$	170.4s	171.1s
	170.0s	170.3s
$\underline{C}H_3CO$	20.9q	21.0q
	20.5q	20.6q
1'	165.9s	165.6s
2'	129.5s	120.6s
3'7'	129.5d	131.3d
4'6'	128.6d	115.6d
5'	133.3d	161.3s



Figure

Previous studies have indicated that andibenin is formed by alkylation of a bis-C-methylated tetraketide precursor by farnesylpyrophosphate. It is significant that this mutant strain which is apparently impaired in polyketide production should divert the farnesylpyrophosphate to sesquiterpenoid production. Mycophenolic acid, the important antitumour metabolite of Penicillium brevicompactum is biosynthesised by a pathway[11] analogous to that proposed for andibenin. It is thus interesting to note that the pebrolides with similar structures to the astellolides have also been reported as metabolites of P.brevicompactum.[12]

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