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THE BIOSYNTHESIS OF ASPERUGIN IN *ASPERGILLUS RUGULOSUS*[1]

J. A. BALLANTINE, V. FERRITO and C. H. HASSALL
Department of Chemistry, University College of Swansea, Swansea, Wales

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Abstract—Asperugin C, $C_{23}H_{32}O_4$, has been identified as 2,4-dihydroxy-5-(*trans–trans*-farnesyloxy)-6-methylbenzaldehyde (XI) It is probably a shunt metabolite in the biosynthetic pathway leading to asperugin (II.) Several stages in the biosynthesis of asperugin have been proposed

IT HAS been established that mutation of *Aspergillus rugulosus* I.M.I. 84338 gave rise to various strains which produced different phenolic metabolites.[2–5] It was envisaged that the investigation of the structures of related compounds produced by these blocked mutants would make it possible to propose a biosynthetic pathway leading to the more complex members of this series. The series of metabolites includes arugosin (I), asperugin (II), averufin (III) and sterigmatocystin (IV).

In the course of these studies, a compound, $C_{23}H_{32}O_4$, which we have named asperugin C, was isolated from the mycelium of a blocked mutant, which no longer produced asperugin. Asperugin C gave colour reactions for a phenol and the u v. spectrum (Table 1) showed similarities to that of asperugin B. Absorption bands at 3300 (broad) and 1630 cm^{-1} in the i r. spectrum were attributed to a strongly hydrogen-bonded *ortho*-hydroxy carbonyl system.[6] Signals at τ — 2·12 and 0·02 in the ^1H NMR spectrum (Table 2) supported this assignment and indicated that a formyl group was involved.[3] In addition, the groups ArH, $ArCH_3$, ArOH (non-chelated) and Ar-O-(*trans–trans*-farnesyl)[7] were indicated by the analysis of the ^1H NMR spectrum This and similar spectroscopic evidence relating to the monomethyl derivative (VI) and a degradation product (VII), formed from (VI) by the action of acetic acid, led to the formulation of the partial structure (V) for arugosin C.

TABLE 1 U V SPECTRA OF ASPERUGIN C DERIVATIVES

	λ_{max} nm (log ϵ)-ethanol	λ_{max} nm ethanol-NaOH
Asperugin C (XI)	215 (3 99), 240 (4 03), 291 (4 15), 343 (3 77)	214, 256, 350
Acid Cleavage Product (VII)	214 (3 80), 245 (4 04), 289 (4 08), 368 (3 69)	
o-Orsellinaldehyde (XVI)	220 (4 07), 233 (3 95), 291 (4 20), 324 sh (3 84)	208, 253, 341
Asperugin B (XVIII)	209 (4 31), 258 (4 15), 293 (3 87), 330 (3 82)	211, 287, 386

[1] This is Part XXIII of this series 'Biosynthesis of Phenols', previous paper E R CATLIN and C H HASSALL, *J Chem Soc* (C), 460 (1971)
[2] J A BALLANTINE, C H HASSALL and G JONES, *J Chem Soc.* 4672 (1965)
[3] J A BALLANTINE, C H HASSALL, B D JONES and G JONES, *Phytochem* 6, 1157 (1967).
[4] J A BALLANTINE, C H HASSALL and B D JONES, *Phytochem* 7, 1529 (1968)
[5] J A BALLANTINE, D J FRANCIS, C H HASSALL and J L C WRIGHT, *J Chem Soc.* 1175 (1970).
[6] D. W CAMERON, P M SCOTT and LORD TODD, *J Chem Soc* 42 (1964)
[7] R B BATES, D M GALE and B J GRUNER, *J Org Chem.* **28**, 1086 (1963)

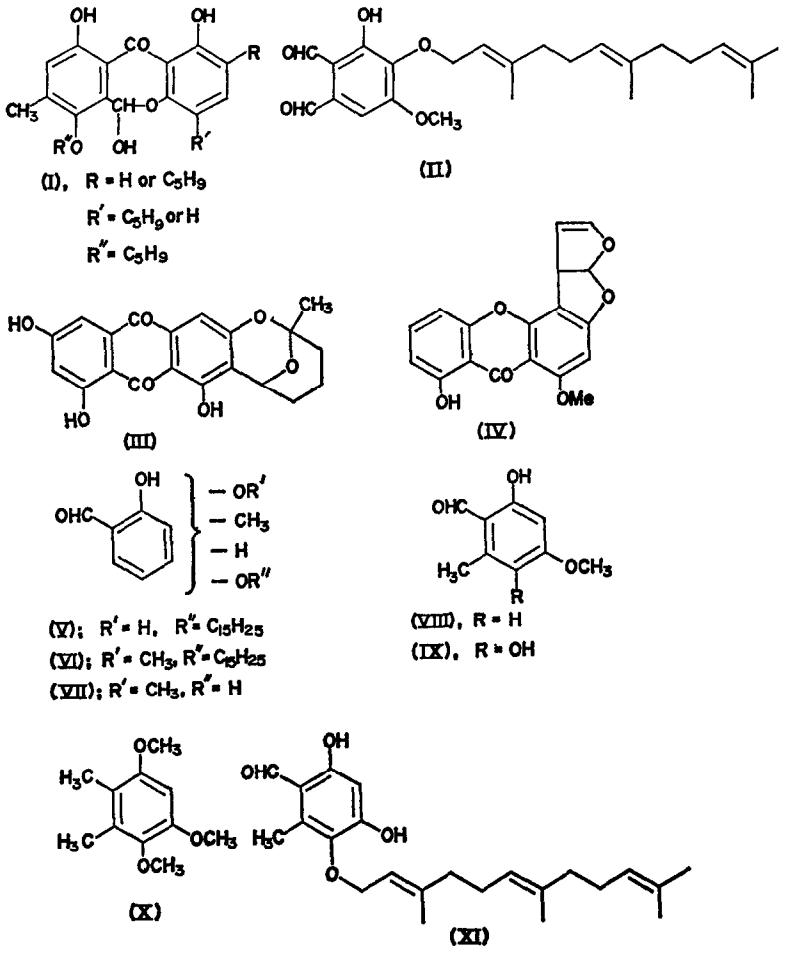


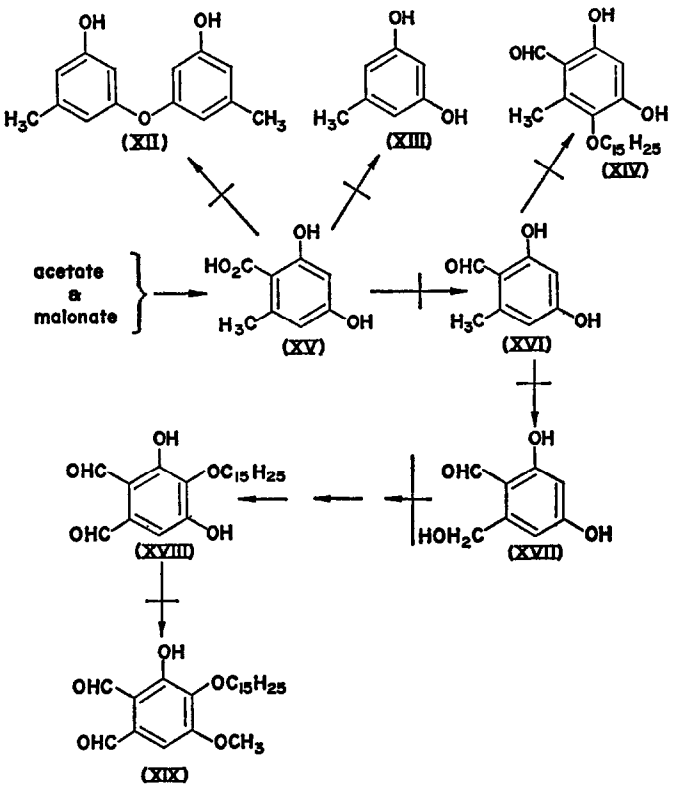
TABLE 2 H NMR CHARACTERISTICS OF ASPERUGIN C DERIVATIVES (τ VALUES)

Assignments	Protons	Multiplicity (J in Hz)	Asperugin C (XI)	Asperugin B (XVIII)	Monomethyl derivative (VI)	Acid cleavage product (VII)
Chelated ArOH	1*	s	−2 12	−3 13	−2 39	−2 20
ArCHO	1	s	—	−0 81	—	—
ArCHO	1	s	−0 02	−0 04	−0 04	−0 04
ArOH	1*	s	3 68	very broad	—	4 68
ArH	1	s	3 76	2 96	3 75	3 74
Ar—O—CH_2—CH=	1	t	4 52	4 80	4 50	—
—CH=	2	m	4 97	4 80	4 94	—
Ar—O—CH_2—CH=	2	d (7)	5 70	5 10	5 66	—
$ArOCH_3$	3	s	—	—	6 14	6 11
$ArCH_3$	3	s	7 50	—	7 55	7 58
=C—CH_2—CH_2—C=	8	m	7 94	8 00	7 98	—
—$C(CH_3)$=	6	s	8 32	8 34	8 35	—
—$C(CH_3)$=	6	s	8 40	8·41	8 41	—

* Exchangeable with deuterium oxide.

It seemed probable from knowledge of the structures of other metabolites of *A. rugulosus*, that asperugin C was a hydroxy-derivative of *o*-orsellinaldehyde (XVI). This was confirmed by showing that the product (VII), formed by acid-catalysed cleavage of mono-O-methylarugosin C, was identical with the product of hydroxylation of everinaldehyde (VIII) by the Elbs persulphate reaction. Furthermore, it was established that the likely *para* hydroxylation had occurred in this reaction, to produce the hydroxyhydroquinone (IX). The synthetic product was methylated and reduced to give 1,2,5-trimethoxy-3,4-dimethylbenzene (X) which was identical with material prepared through 2,3-dimethyl-1, 4-benzoquinone by Thiele acetylation, followed by *O*-methylation.

This evidence established that arugosin C was 2,4-dihydroxy-5-(*trans–trans*-farnesyloxy)-6-methylbenzaldehyde (XI).



The identification of asperugin C and other metabolites of blocked mutants of *A. rugulosus* which had lost the ability to produce asperugin, has made it possible to postulate several stages in the biosynthesis of asperugin (XIX) and related compounds. It is suggested that four of the metabolites which have been identified: *o*-orsellinic acid (XV),[8] *o*-orsellinaldehyde (XVI),[4] *ω*-hydroxy-*o*-orsellinaldehyde (XVII),[4] and asperugin B (XVIII)[3] are on the pathway to asperugin (XIX) whereas asperugin C (XIV), orcinol (XIII),[8] and the diphenyl ether (XII)[4] are shunt metabolites. There are various precedents[9,10] for the

[8] G. Jones, University of Wales Ph.D thesis (1964)
[9] G Pettersson, *Acta Chem Scand* **18**, 1428 (1964).
[10] G Pettersson, *Acta Chem Scand* **19**, 543 (1965)

hydroxylation of a resorcinol to a hydroxyhydroquinone nucleus and for enzyme-catalysed decarboxylation of *o*-orsellinic acid.[11] There is, as yet, no specific evidence relating to the biosynthesis of the diphenyl ether (XII) but the orientation of substituents suggests that it may be derived from *o*-orsellinic acid.

EXPERIMENTAL

All m ps were determined on a Kofler hot-stage microscope U v spectra were measured with a Unicam SP 800 spectrophotometer I r spectra were determined with a Perkin-Elmer 257 spectrophotometer. ^{1}H NMR spectra were obtained at 60 MHz with a Perkin-Elmer R10 instrument and at 100 MHz with a Varian HA100 instrument. Mass spectra were measured at 70 ev with an A E I MS-9 spectrometer. Accurate mass measurements were performed relative to fragment ions of heptacosofluorotributylamine R_f values refer to TLC chromatograms as previously described [2] Light petroleum had b p 60–80°

Extraction and Purification of Asperugin C

A white, fluffy, proline-requiring mutant (A R M 884) derived in two mutational steps from *A rugulosus* I M I strain 84338 was grown from a spore syspension for 21 days at 25° on a low-N medium[12] to which Marmite (1 g/l l) and proline (10 mg/l l) had been added The stationary cultures were in 500 flat-sided bottles (*ca* 1 l capacity) each containing 200 ml of medium The mycelium was collected on a wire mesh strainer, macerated with an equal volume of H_2O and extracted with Et_2O (5 × 500 ml for each 1 l of macerate) After drying, the solvent was removed to furnish a dark brown viscous residue (28 g) which was dissolved in Et_2O (500 ml) and acidic impurities were removed by extraction with Na_2CO_3 Phenolic compounds were extracted into N-NaOH (500 ml) which was washed with Et_2O (750 ml), the aqueous layer acidified to pH 2 with HCl and extracted with Et_2O (2 × 250 ml) After drying, the extract was evaporated *in vacuo* to give a dark brown, viscous residue (412 g) which was chromatographed on silica gel (450 g, 200–300 mesh) by gradient elution. Elution with benzene yielded fractions containing asperugin C (XI) (1 1 g), colourless plates from light petroleum, m p 54° (R_f 0 76, purple spot) [Found C, 74 6, H, 8 4%, M (mass spectrometry) 372 2300 ± 0 0017 $C_{23}H_{32}O_4$ requires C, 74·2, H, 8 7%, M, 372 2300], ν_{max} (film) 3300 broad (OH), 2960 (CH), 2930 (CH), 2860 (CH) and 1630 (CO) cm^{-1}

Methylation of Asperugin C

Asperugin C (100 mg) was methylated in the usual way with acetone (2 ml) K_2CO_3 (35 mg) and MeI (50 mg) for 8 hr under reflux The *product* (VI) was obtained as a viscous oil (92 mg) (R_f 0 80 orange-brown). [Found M (mass spectrometry), 386 2457 ± 0 0017 $C_{24}H_{34}O_4$ requires M, 386 2457] ν_{max} (film) 3200 broad (OH), 2960 (CH), 2920 (CH), 2840 (CH) and 1635 (CO) cm^{-1}

Cleavage of Methoxy-asperugin C with Acetic Acid

Methoxy-asperugin C (92 mg) was treated with a mixture of a few drops of N-HCl in HOAc (2 ml) for 12 hr at 25°. Removal of the solvent left a residue which was crystallized from light petroleum to yield the *phenolic cleavage product* (VII) as light yellow prisms (22 mg) m p. 163–165° [Found C, 59 3, H, 5·5%, M (mass spectrometry), 182 $C_9H_{10}O_4$ requires C, 59 3, H, 5 5%, M, 182] ν_{max} (KBr) 3340 broad (OH) and 1635 (CO) cm^{-1}.

Elbs Perusulphate Oxidation of 2-Hydroxy-4-methoxy-6-methylbenzaldehyde

2-Hydroxy-4-methoxy-6-methylbenzaldehyde[13] (100 mg), syspended in 10% NaOH (1 2 ml), was treated with a saturated aqueous solution of $K_2S_2O_8$ (175 mg) for 3 hr at below 20°. After acidification the *product* (IX) was isolated as yellow plates (47 mg) from light petroleum, m p 164° [Found C, 59 3; H, 5 5%; M (mass spectrometry), 182 $C_9H_{10}O_4$ requires C, 59 3, H, 5 5%, M, 182], which were identical (i r, u v, ^{1}H NMR, mass spectra and mixed m p) with the phenolic cleavage product of methoxy-asperugin C

Methylation and Hydrogenation of the Elbs Persulphate Oxidation Product

The above oxidation product (200 mg) was methylated in the usual way with excess Me_2SO_4 (600 mg), K_2CO_3 (550 mg) in acetone (20 ml) for 8 hr under reflux in N_2 The *tri-O-methyl ether* aldehyde derivative (150 mg) was obtained as colourless microcrystals after sublimation, m p 102° [Found C, 62 7 H, 6 8%, M (mass spectrometry), 210 $C_{11}H_{14}O_4$ requires C, 62 8; H, 6 7%, M, 210], $\tau_{(CDCl_3)}$ −0 7 (1H, s, ArC$\underline{H}$O), 3 58 (1H), s, Ar$\underline{H}$), 6 02 (3H, s, ArOC$\underline{H}_3$), 6 08 (3H, s, ArOC$\underline{H}_3$), 6 25 (3H, s, ArOC$\underline{H}_3$) and 7 45 (3H, s, ArC$\underline{H}_3$)

[11] R BENTLEY in *Biogenesis of Antibiotic substances* (edited by Z VANEK and Z HOSTALEK), p 241 Czechoslovak Academy of Sciences, Prague (1965)
[12] C H HASSALL and K LAWRENCE, *J Gen Microbiol* **35**, 483 (1964).
[13] A ROBERTSON and R J STEPHENSON *J Chem Soc* 1390 (1932)

The tri-*O*-methyl aldehyde derivative (100 mg), in EtOH (20 ml) was mixed with 10% Pd/C (50 mg) and shaken with H_2 (1 atmos) for 12 hr at 20°. The *tri-O-methyl-o-xylene* product (X) was obtained as colourless prisms (42 mg) by sublimation at 33°/0 05 mm, m p 64° [Found C, 67 0, H, 8·4%; M (mass spectrometry), 196. $C_{11}H_{16}O_3$ requires C, 67·3· H, 8·2%; M, 192], λ_{max} (EtOH), 231 and 285 nm (log ϵ 3 66 and 3 48) $\tau_{(CDCl_3)}$ 3·58 (1H, s, ArH), 6 12 (3H, s, $ArOCH_3$), 6 20 (3H, s, $ArOCH_3$), 6·25 (3H, s, $ArOCH_3$), 7·78 (3H, s, $ArCH_3$) and 7 90 (3H, s, $ArCH_3$)

3,4,6-*Trimethoxy*-1,2-*dimethylbenzene*

3,4,6-Triacetoxy-1,2-dimethylbenzene[8] (200 mg) and Me_2SO_4 (1 ml) were added in N_2 to de-aerated 10% NaOH (20 ml) and the mixture refluxed for 4 hr. On cooling a solid separated which was collected and purified by sublimation at 43°/0 05 mm 3,4,6-*Trimethoxy*-1,2-*dimethylbenzene* was obtained as colourless prisms (65 mg) m p. 65°. [Found. C, 67 3; H, 8 5%; M (mass spectrometry), 196 $C_{11}H_{16}O_3$ requires C, 67 3; H, 8·2%; M, 196], identical (u v, i r, ^{1}H NMR, mass spectra and mixed m p.) with the product from methylation and hydrogenation of the Elbs persulphate product.

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