Phytochemistry, 1967, Vol. 6, pp. 1157 to 1159. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

THE BIOSYNTHESIS OF PHENOLS—XII.[1]

ASPERUGIN B, A METABOLITE OF *ASPERGILLUS RUGULOSUS*

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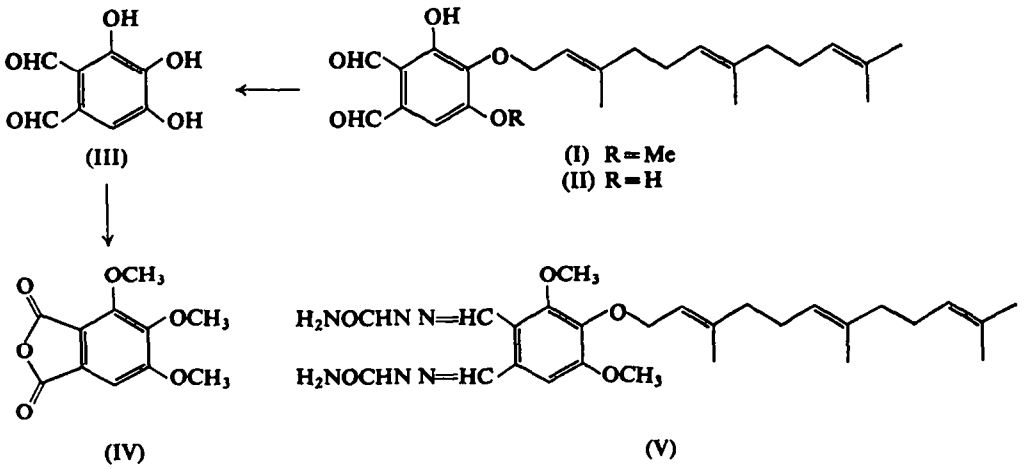
(*Received* 1 *March* 1967)

Abstract—Asperugin B, $C_{23}H_{30}O_5$, a metabolite of a mutant strain of *Aspergillus rugulosus* which carries the genetic marker "fluffy", has been identified as 4-O-(*trans,trans*-farnesyl)-3,5-dihydroxyphthalaldehyde (II)

IN OUR earlier studies utilising *Aspergillus terreus*,[2] it was shown that the elucidation of the structures of related metabolites of a series of blocked mutants made it possible to outline the course of biosynthesis of complex products of secondary metabolism. A related investigation of *A. rugulosus* has led to the isolation of a variety of mutants with different capabilities for synthesising phenolic metabolites. One of these mutants differed from the wild-type in producing a phenolic compound which has been named asperugin and has been assigned the molecular structure (I).[3] Moreover, it was established that there was a 1:1 relationship between the production of asperugin and the formation of fluffy aerial mycelium by this mutant.[4]



(III)

(I) R = Me
(II) R = H

(IV)

(V)

[1] Part XI of this series: C. H. HASSALL and T. E. WINTERS, *J. Chem. Soc.* (1967) In press.
[2] R. F. CURTIS, P. C. HARRIES, C. H. HASSALL and J. D. LEVI, *Biochem. J.* **90**, 43 (1964).
[3] J. A. BALLANTINE, C. H. HASSALL and G. JONES, *J. Chem. Soc.* 4671 (1965).
[4] C. H. HASSALL and K. LAWRENCE, *J. Gen. Microbiol.* **35**, 483 (1964).

Careful fractionation of the mixture of phenols produced by this mutant with "fluffy" morphology led to the isolation of both asperugin and another compound which we have named asperugin B. It soon became apparent that asperugin, $C_{24}H_{32}O_5$, and asperugin B, $C_{23}H_{30}O_5$, were closely related in structure. Both compounds gave disemicarbazones and both were cleaved by acid-washed silica gel to give a hydrocarbon and a phenolic fraction. The differences in the NMR spectra of asperugin and asperugin B (Table 1) could be attributed to the presence of a methoxyl group in the former but not in the latter. This was confirmed by Zeisel determinations.

TABLE 1. NMR CHARACTERISTICS OF ASPERUGIN AND ASPERUGIN B

Asperugin			Asperugin B	
Chemical Shift (τ)*	No. of Protons	Assignment	Chemical Shift (τ)	No. of Protons
−2·4 (s)	1	Chelated Ar—O*H*	−3·31 (s)	1
−0·75 (s)	1	Ar—C*H*O	−0·81 (s)	1
−0·04 (s)	1	Ar—C*H*O	0·04 (s)	1
—	—	Ar—O*H*	very broad peak	1
2·95 (s)	1	Ar—*H*	2·96 (s)	1
4·80 (m)	3	*H*C=	4·80 (m)	3
5·35 (d, J = 8 c/s)	2	Ar—O—CH_2—CH=	5·10 (d, J = 8 c/s)	2
5·98 (s)	3	Ar—O—CH_3	—	—
7·97 (m)	8	=C—CH_2—CH_2—C=	8·00 (m)	8
8·34 (s) 8·41 (s)	12	—C(=)— CH_3	8·34 (s) 8·41 (s)	12

* s = singlet, d = doublet, m = multiplet.

The phenol produced from asperugin B, by acid-catalysed cleavage, has been identified as (III) by comparison with authentic fomecin B;[5] furthermore, methylation, oxidation and sublimation gave the anhydride (IV) which was identical with synthetic material.

It has been established that the point of attachment of the farnesyl sidechain of asperugin B is the same as in asperugin. Treatment of the disemicarbazones of both compounds with diazomethane gave identical products (V). This was in accord with the observation that asperugin B could not have two adjacent phenolic hydroxyl groups; unlike catechols, it was not retarded when chromatography utilised paper impregnated with sodium borate. This evidence defines the structure of asperugin B as (II).

EXPERIMENTAL

All melting points were determined on a Kofler hot stage-microscope and are uncorrected. U.V. spectra were measured on a Unicam SP 800 spectrophotometer. I.R. spectra were obtained using Perkin-Elmer Model 257 and 137 spectrophotometers. NMR spectra were determined at 60 Mc/s. with a Perkin-Elmer Model R-10 instrument. Mass spectra were measured on an A.E.I. Model MS9 spectrometer. Thin layer chromatograms were run on Kieselgel G (Merck) using benzene:dioxan:acetic acid (90:25:4 v/v)[6] and the spots were made visible by spraying with diazotised *o*-dianisidine and ammonia.[7] Light petroleum had b.p. 60–80°.

[5] T. C. MCMORRIS and M. ANCHEL, *Can. J. Chem.* **42**, 1595 (1964).
[6] G. PASTUKA, *Z. Anal. Chem.* **179**, 355 (1961).
[7] L. REIO, *J. Chromatog.* **1**, 338, (1958).

Asperugin B

Aspergillus rugulosus, mutant (w_1 pro_1 f_1)[4] was cultivated and the mixture of metabolites produced by the organism (37 g) was subjected to separation by countercurrent distribution as previously described.[3] Early fractions (30–95) were found to contain impure asperugin B (4·7 g, R_f 0·59—black spot), which was purified by chromatography on silica gel (250 g, 200–300 mesh, L. Light and Co., Ltd.) using gradient elution. Elution with light petroleum/ether (9:1 v/v) furnished asperugin B (2·01 g), as an unstable yellow gum. Repeated chromatography of asperugin B was necessary to produce chromatographically pure material. (Found: M, by mass spectrometry, 186·2093. $C_{23}H_{30}O_5$ requires 186·2093); ν_{max} (CCl_4) 3490 (OH), 2920, 2845, 1700 (ArCHO), 1640 (hydrogen bonded CHO), 1583, 1499, 1442, 1390, 1375, 1344, 1300, 1282, 1213, 1178, 1125, 1065, 918, 852, 697 cm^{-1}, λ_{max} 209, 258, 293, 330 nm ($\log \epsilon$ 4·31, 4·15, 3·87, 3·82).

During paper chromatography on Whatman No. 1 paper impregnated with sodium borate,[8] asperugin B showed no retardation R_f (untreated paper) 0·91; R_f (borate paper) 0·96.

Asperugin B (108 mg) formed a disemicarbazone (95 mg) under standard conditions. Recrystallisation from ethanol furnished white microcrystals, m.p. 210–211° (decomp.). (Found: C, 60·1; H, 7·6; N, 16·5. $C_{25}H_{36}N_6O_5$ requires: C, 60·0; H, 7·3; N, 16·8%). The i.r. spectrum (KBr) contained peaks at 3470 (OH), 3360 (NH) and 1680 (C=N) cm^{-1}.

Acid-catalysed Cleavage of Asperugin B

Asperugin B (350 mg) in light petroleum (50 ml) was applied to a column of acid treated silica gel[3] (20 g) and subjected to gradient elution. The first fractions eluted with light petroleum-benzene contained the side-chain cleavage products as a hydrocarbon oil.[cf. 3] Elution of the column with benzene-ethyl acetate (1:1 v/v) afforded a yellow solid (175 mg) which upon recrystallisation from ethyl acetate gave the *phenolic cleavage product of asperugin B* as yellow needles, m.p. 241° (decomp.). (Found: C, 53·0; H, 3·0; OCH_3 absent. Calc. for $C_8H_6O_5$: C, 52·8; H, 3·3%); λ_{max} 211, 263, 300 and 330 (broad) nm ($\log \epsilon$ 4·15, 4·03, 3·81 and 3·74); τ (deuteroacetone) −0·84 (1 proton, s, ArC*H*O), −0·09 (1 proton, s, ArC*H*O), 2·73 (1 proton, s, ArH) and 7·90 (2 or 3 protons exchangeable with acetone). The i.r. spectrum showed peaks at 3180 (OH), 1690 (aromatic CHO) and 1635 (hydrogen bonded CHO) cm^{-1}.

Methylation and Oxidation of the Phenolic Cleavage Product

The phenolic cleavage product (150 mg), dry acetone (20 ml), anhydrous K_2CO_3 (600 mg) and dimethyl sulphate (0·45 ml) were refluxed for 2 hr in nitrogen. After the removal of inorganic solids by filtration the solution was evaporated *in vacuo*. The residual oil was taken up in hot, 5% KOH (4 ml) and heated with 3% $KMnO_4$ (10 ml) at 60–65° for 1 hr. The resulting solution was acidified and extracted with ethyl acetate (5 × 250 ml). Evaporation of the solvent gave the *dicarboxylic acid* as a dark yellow, oily residue (120 mg). Sublimation of the diacid at 120°/0·05 mm gave a white sublimate, which was heated cautiously to its melting point, cooled and resulblimed at 100°/0·06 mm to give the *anhydride*, m.p. 145°. (Found: C, 55·6; H, 4·25. Calc. for $C_{11}H_{10}O_6$: C, 55·45; H, 4·2%); ν_{max} 2940, 2842, 1836 (anhydride C=O), 1770 (anhydride C=O), 1615, 1478, 1416, 1348, 1262, 1151, 1134, 1018, 976, and 900 cm^{-1}. Mixed m.p. determinations and comparison of i.r. spectra established that this anhydride was identical with a synthetic specimen of 3,4,5-trimethoxyphthalic anhydride, for which Alimchandani and Meldrum[9] record a m.p. of 147°.

This indicated that the phenolic cleavage product was 3,4,5-trihydroxyphthalaldehyde, a compound which had already been isolated from a fungus and named fomecin B.[5] Mixed m.p. determination and comparison of infrared and NMR spectra established that our product from asperugin B was identical with fomecin B, a sample of which was kindly supplied by Dr. T. C. McMorris.

Disemicarbazone of Di-O-methylasperugin B

The disemicarbazone of asperugin (400 mg) was treated with CH_2N_2 in ether at 0° for 48 hr. Removal of the solvent gave a yellow solid which was recrystallised from ethanol-water to furnish the disemicarbazone of di-O-methylasperugin B as pale yellow microcrystals (324 mg), m.p. 175°. (Found: C, 61·1; H, 7·6; N, 16·0. $C_{27}H_{40}N_6O_5$ requires: C, 61·3, H, 7·6; N, 15·9%).

Mixed m.p. determination and comparison of i.r. and NMR spectra of this compound with those of the product obtained when the disemicarbazone of asperugin was methylated under similar conditions, established that the two derivatives were identical.

Acknowledgements—We are grateful to Dr. J. D. Levi and Mr. J. S. Lewis for the cultivation of *A. rugulosus*. We acknowledge, with thanks, funds from the Science Research Council of Great Britain for two research studentships (B.D.J. and G.J.) and for the purchase of NMR and mass spectrometers.

[8] L. JURD, *J. Chromatog.* **4**, 369 (1960).
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