Identification of Three New Pterocarpans (6a,11a-Dihydro-6*H*-benzofuro[3,2-*c*][1]benzopyrans) from *Pisum sativum* infected with *Fusarium solani* f. sp. *pisi*

By **Steven G. Pueppke** * and **Hans D. VanEtten**, Department of Plant Pathology, Cornell University, Ithaca, New York 14853, U.S.A.

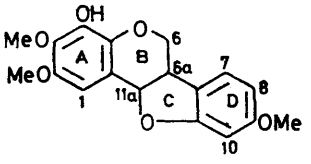
The isolation of three antifungal pterocarpans from *Pisum sativum* epicotyls infected with *Fusarium solani* f. sp. *pisi* is described. By a combination of spectroscopic and degradative techniques the compounds were identified as 4-hydroxy-2,3,9-trimethoxy- (1), 3-hydroxy-2,9-dimethoxy- (2), and 2,3,9-trimethoxy-pterocarpan (3).

THE theory that plants accumulate antifungal compounds called phytoalexins in response to challenge by fungal pathogens was first formulated by Müller and Börger.[1] Phytoalexins produced by plants of the family Leguminosae often have the pterocarpan ring structure as in (1).[2] Previous investigations have demonstrated that garden pea (*Pisum sativum* L.) produces 6a-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (pisatin) [2b] and 3-hydroxy-8,9-methylenedioxypterocarpan (maackiain) [2c] in response to fungal challenge. We now report the identification of three additional pterocarpans from pea epicotyls infected with the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) Snyd. & Hans.

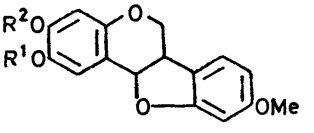
The mass spectra of the compounds, in which the molecular ions are the base peaks but which show little further fragmentation, are like those reported for pterocarpans.[3,4] The n.m.r. spectrum of each compound contains the complex four-spin system which arises from the heterocyclic protons of pterocarpans.[5] Carbonyl stretching absorbance is absent in the i.r. spectrum, but several prominent bands characteristic of aryl ether functionality are present.[6] All three compounds gave a negative Labat reaction,[7] which indicates the absence of methylenedioxy-groups.

Compound (1), $C_{18}H_{18}O_6$ (M^+ 330), is phenolic, as evidenced by positive reaction with $FeCl_3$ and diazotized *p*-nitroaniline. Hydroxy-stretching absorbance is present in the i.r. spectrum. Reaction with diazomethane afforded a monomethylated product (M^+ 344), and reaction with acetic anhydride produced a monoacetylated product (M^+ 372). Neither product retained phenolic character. The n.m.r. spectrum of compound (1) contains the signals of three methoxy-groups and one hydroxy-group.

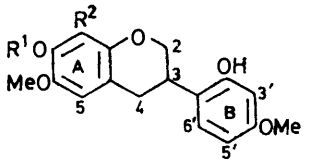
Distinction between ring-A and ring-D substituents of pterocarpans cannot be made on the basis of mass spectra, because any fragment ion may reasonably arise from either ring.[3] However, Nakayama *et al.*[4] have demonstrated that the total substituent mass can be correctly divided between the rings on the basis of mass spectral evidence. For example, if a pterocarpan is known to possess hydroxy-dimethoxy-substitution, distinction can be made between structures (i) requiring the methoxy-groups on one ring and the hydroxy-group



(1)



(2) R^1 = Me, R^2 = H
(3) R^1 = R^2 = Me
(4) R^1 = Me, R^2 = Ac



(5) R^1 = Me, R^2 = OH
(6) R^1 = R^2 = H

on the other and (ii) requiring hydroxy-methoxy-substitution on one ring and a methoxy-group on the other.

In the mass spectrum of compound (1) three of the four possible fragment ions indicating methoxy- and hydroxy-dimethoxy-ring substitution are present.† Fragments consistent with any other substitution pattern are lacking. In the n.m.r. spectrum signals for three of the four aromatic protons appear as an ABX system. These were assigned to C-7, C-8, and C-10 on the basis of correspondence of the observed chemical shifts and coupling constants with those of 9-methoxypterocarpans.[2a,3a,5,‡] The alternative assignment of the ABX protons to C-1, C-2, and C-4 was ruled out by hydrogenolysis of compound (1) to form the corresponding isoflavandiol (5). The major mass spectral fragments of the isoflavandiol (5) (Scheme) confirm that

† *m/e* 148 ($C_9H_8O_2$), 161 ($C_{10}H_9O_2$), and 194 ($C_{10}H_{10}O_4$).

‡ Such comparisons are possible, since it is known that variation in substituents on ring A of pterocarpans does not affect the n.m.r. of ring D protons.[7]

[1] K. O. Müller and H. Börger, *Arb. Biol. Anst. (Reichsaust) Berlin*, 1941, **23**, 189.

[2] (*a*) D. G. Smith, A. G. McInnes, V. J. Higgins, and R. L. Millar, *Physiol. Plant Pathol.*, 1971, **1**, 41; (*b*) D. R. Perrin and W. Bottomley, *J. Amer. Chem. Soc.*, 1962, **84**, 1919; (*c*) A. Stoessl, *Canad. J. Biochem.*, **50**, 107; (*d*) D. R. Perrin, *Tetrahedron Letters*, 1964, 29; (*e*) D. R. Perrin, C. P. Whittle, and T. J. Batterham, *ibid.*, 1972, 1673; (*f*) J. J. Sims, N. T. Keen, and V. K. Honwad, *Phytochemistry*, 1972, **11**, 827.

[3] (*a*) A. Pelter and P. I. Amenechi, *J. Chem. Soc.* (*C*), 1969, 887; (*b*) A. Pelter, P. Stainton, and M. Barber, *J. Heterocyclic Chem.*, 1965, **2**, 262.

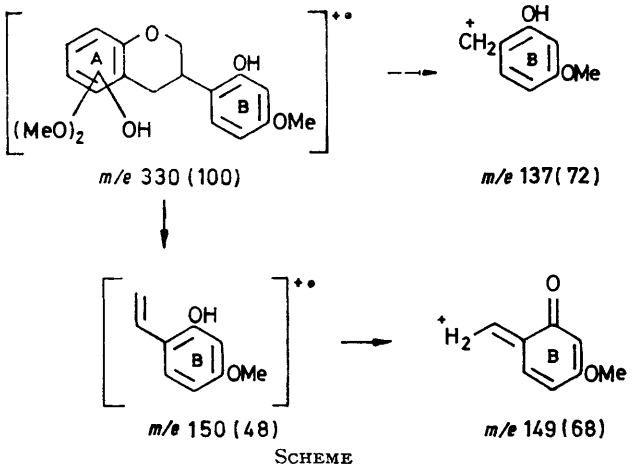
[4] M. Nakayama, S. Eguchi, A. Matsuo, S. Hayashi, S. Hishida, and Y. Kato, *Shitsuryo Bunseki*, 1972, **20**, 239.

[5] K. G. R. Pachler and W. G. E. Underwood, *Tetrahedron*, 1967, **23**, 1817.

[6] L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, *Analyt. Chem.*, 1957, **29**, 904.

[7] S. H. Harper, A. D. Kemp, W. G. E. Underwood, and R. V. M. Campbell, *J. Chem. Soc.* (*C*), 1969, 1109.

ring D of the parent pterocarpan is monomethoxy-substituted.



SCHEME

The problem was thus reduced to determining the positions of the ring-A substituents. Compound (1) reacted positively with 2,6-dichlorobenzoquinone 4-chloroimine (Gibbs reagent),[8] and thus the lone ring-A proton is *para* to the hydroxy-group. This places the methoxy-groups at C-2 and C-3. The position of the proton was determined by comparison of its n.m.r. signal with the calculated chemical shift values of Ballantine and Pillinger,[9] as amended by Pelter and Amenechi.[3a] The

(7) (8)

possible positions of the proton are shown in formulae (7) and (8); the numerals in parentheses are the calculated values. The observed signal at δ 6·61 places the proton at C-1 and indicates that compound (1) is 4-hydroxy-2,3,9-trimethoxypterocarpan.

Compound (2), $C_{17}H_{16}O_5$ (M^+ 300), contains one less methoxy-group than compound (1). It formed a single methylation product (M^+ 314), a single acetylation product (M^+ 342), and possesses an i.r. spectrum similar to that of compound (1). Analysis of the mass spectrum indicated hydroxymethoxy- and methoxy-substitution; signals corresponding to these substituents appear in the n.m.r. spectrum. A negative Gibbs test indicated substitution *para* to the aromatic hydroxy-group.

The aromatic region of the n.m.r. spectrum contains signals for five protons. Three of these appear as an ABX system which is nearly identical to that of compound (1). Assignment of these protons to C-7, C-8, and C-10 (rather than C-1, C-2, and C-4) was verified by preparation of the isoflavandiol (6). Its mass spectrum possesses ring-B fragments analogous to those of the isoflavandiol (5) (Scheme).

The two remaining aromatic singlets could only have arisen from *para*-protons. Comparison with the published spectra of 2,3-oxygenated pterocarpans [10] allowed us to assign the signals to H-4 and H-1, respectively, but the data did not permit us to distinguish between 2-hydroxy-3-methoxy- and 3-hydroxy-2-methoxy-substitution. Choice was made by comparison of the H-1 and H-4 signals of compound (2) with those of its acetate. Data from other pterocarpans indicate that acetylation of an aromatic hydroxy-group induces a greater downfield shift of *ortho*- than of *meta*-protons.[3a,10,11] The phenomenon also occurs with other phenols and their acetates [12] and has been utilized in the structure determination of pterocarpans.[10] The 2-hydroxy-structure requires a greater shift for the H-1 signal of the acetate and the 3-hydroxy-structure requires a greater shift for the H-4 signal of the acetate. The greater observed downfield shift for H-4 (0·16 p.p.m.; *cf.* 0·12 p.p.m. for H-1) is evidence that compound (2) is 3-hydroxy-2,9-dimethoxypterocarpan.

Compound (3), $C_{18}H_{18}O_5$ (M^+ 314), did not form methylation or acetylation products. Its u.v., n.m.r., and mass spectra are identical with those of the mono-*O*-methyl derivative of compound (2), and thus compound (3) is 2,3,9-trimethoxypterocarpan.[13]

Large negative optical rotations ($[\alpha]_D^{23}$ −185, −297, and −228°) were recorded for compounds (1)—(3) respectively, indicating that the compounds possess the (6a*R*,11a*R*) absolute configuration, which is common to laevorotatory pterocarpans.[14]

Preliminary studies indicate that the pterocarpans described here exhibit antifungal activities comparable to those of other known pterocarpans.[15] Results of these studies will be published elsewhere.

EXPERIMENTAL

M.p.s were determined with a Fisher-Johns apparatus. U.v. spectra were recorded with a Bausch and Lomb Spectronic UV200 spectrophotometer, i.r. spectra with a Perkin-Elmer 421 instrument (for films on NaCl crystals), and mass spectra with an A.E.I. MS9 instrument (A.E.I. MS902 for high resolution measurements). N.m.r. spectra of dilute solutions in $CDCl_3$ were recorded at 90 MHz with a Bruker HX90 instrument utilizing the fast Fourier transform technique. Optical rotation measurements were obtained with a Perkin-Elmer 141 polarimeter. T.l.c. was performed with Polygram Sil G-UV_{254} (Brinkmann Instruments) or silica gel with fluorescent indicator 13181 (Eastman Kodak Co.).

[8] F. E. King, T. J. King, and L. C. Manning, *J. Chem. Soc.*, 1957, 563.

[9] J. A. Ballantine and C. T. Pillinger, *Tetrahedron*, 1967, **23**, 1691.

[10] G. J. H. Rall, J. P. Englebrecht, and A. J. Brink, *Tetrahedron*, 1970, **26**, 5007.

[11] (*a*) K. Fukui, M. Nakayama, and T. Harano, *Bull. Chem. Soc. Japan*, 1969, **42**, 233; (*b*) L. Farkas, A. Gottesegen, and M. Nógrádi, *J.C.S. Perkin I*, 1974, 305; (*c*) K. K. Purushothaman, V. N. Kishore, V. Narayanaswami, and J. D. Connolly, *J. Chem. Soc.* (*C*), 1971, 2420.

[12] R. G. Cooke and I. D. Rae, *Austral. J. Chem.*, 1964, **17**, 379.

[13] V. K. Kalra, A. S. Kukla, and T. R. Seshadri, *Indian J. Chem.*, 1967, **5**, 607.

[14] S. Ito, Y. Fujise, and A. Mori, *Chem. Comm.*, 1965, 595.

[15] D. R. Perrin and I. A. M. Cruickshank, *Phytochemistry*, 1969, **8**, 971.

Production and Extraction of Diseased Epicotyls.—Seeds of *Pisum sativum* were planted in flats of steamed soil, which were kept in a glasshouse. After 7 days a spore suspension of *Fusarium solani* f. sp. *pisi* was poured onto the flats of seedlings. The dark lesions which soon appeared on the epicotyls were allowed to enlarge for 15 to 20 days. The plants were then sacrificed, and the excised epicotyls were comminuted in 95% ethanol (4 v/w). After filtration one volume of water was added, and the ethanol was removed under reduced pressure. The aqueous fraction was extracted with chloroform (2 × 4 volumes), and the chloroform was evaporated off under reduced pressure. The residue was redissolved in a small amount of chloroform and subjected to t.l.c. [PhH–EtOAc–Pr^{i}OH (90 : 10 : 1)]. U.v.-absorbing bands 1—4, at R_F 0·31, 0·39, 0·52, and 0·63, respectively, were resolved; several other bands at lower R_F values were not investigated. Band 2 was identified as 6a-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (pisatin) [2b] and was not further examined. Further t.l.c. [PhMe–EtOAc (8 : 1)] of bands 1, 3, and 4 yielded, respectively, compounds (1) (R_F 0·48), (2) (0·68), and (3) (0·84). Yields varied considerably from batch to batch of epicotyls. Typically, 410 g of epicotyls produced 15·4 mg of compound (1), 7·7 mg of (2), 2·6 mg of (3), and 25·0 mg of pisatin.

4-*Hydroxy*-2,3,9-*trimethoxypterocarpan* (6a,11a-*Dihydro*-2,3,9-*trimethoxy*-6H-*benzofuro*[3,2-c][1]*benzopyran*-4-*ol*) (1).—The *compound* crystallized as needles from aqueous acetone; m.p. 141—145°; $[\alpha]_D^{23}$ −185° (EtOH); $\nu_{max.}$ 3420 (OH), 1495, 1595, 1620 (aryl), 1045, 1140, 1275, 1335, and 1470 cm^{-1} (aryl ether); $\lambda_{max.}$ (EtOH) 287 and 290sh nm (log ε 3·82 and 3·80) (Found: M^+, 330·1120. $C_{18}H_{18}O_6$ requires M, 330·1103); *m/e* 330 (100%), 329 (11), 316 (11), 315 (35), 283 (13), 182 (18), 179 (13), 157·5 (13), 148 (15), and 137 (10); δ 7·13 (1H, d, *J* 9 Hz, H-7), 6·61 (1H, s, H-1), 6·44 (1H, d, *J* 2·5 Hz, H-10), 6·44 (1H, q, *J* 9 and 2·5 Hz, H-8), 5·63br (1H, s, OH), and 3·88, 3·84, and 3·73 (9H, 3 × s, OMe).

4′,6,7-*Trimethoxyisoflavan*-2′,8-*diol* (5).—The pterocarpan (1) (0·8 mg) was dissolved in ethanol (9 ml) and 0·2N-hydrochloric acid (1 ml). 10% Palladium–charcoal (5 mg) was added. Hydrogenation at room temperature and pressure was stopped after 30 min. The residue left after removal of solvent and catalyst was subjected to t.l.c. [Et_2O–C_6H_{14} (5 : 1)]. The major *product* (R_F 0·22) showed $\lambda_{max.}$ (EtOH) 282sh and 286 nm, and reacted positively with $FeCl_3$ and 2,6-dichlorobenzoquinone 4-chloroimine (Found: M^+, 332·1253. $C_{18}H_{20}O_6$ requires M, 332·1260); *m/e* 332 (100%), 196 (16), 184 (16), 183 (32), 182 (28), 180 (16), 150 (48), 149 (68), 137 (72), 121 (24).

3-*Hydroxy*-2,9-*dimethoxypterocarpan* (2).—The *compound* crystallized as plates from acetone; m.p. 146—148°; $[\alpha]_D^{23}$ −297° (EtOH); $\nu_{max.}$ 3420 (OH), 1495, 1595, 1620 (aryl), 1030, 1145, 1275, 1340, and 1465 cm^{-1} (aryl ether); $\lambda_{max.}$ (EtOH) 288sh and 292 nm (log ε 3·98 and 4·01) (Found: M^+, 300·0988. $C_{17}H_{16}O_5$ requires M, 300·0993); *m/e* 300 (100%), 299 (18), 285 (23), 161 (10), 150 (10), 149 (10), and 148 (26); δ 7·12 (1H, d, *J* 8·8 Hz, H-7), 6·96 (1H, s, H-1), 6·52 (1H, s, H-4), 6·44 (1H, d, *J* 2·3 Hz, H-10), 6·43 (1H, q, *J* 8·8 and 2·3 Hz, H-8), 5·89br (1H, s, OH), and 3·90 and 3·77 (6H, 2 × s, OMe).

4′,7-*Dimethoxyisoflavan*-2′,6-*diol* (6).—The pterocarpan (2) (0·29 mg) was hydrogenated as described above. The major *product* (R_F 0·38) showed $\lambda_{max.}$ (EtOH) 287 nm, and reacted positively with $FeCl_3$ and 2,6-dichlorobenzoquinone 4-chloroimine (Found: M^+, 302·1142. $C_{17}H_{18}O_5$ requires M, 302·1154); *m/e* 302 (96%), 166 (18), 165 (29), 164 (32), 154 (14), 153 (46), 151 (21), 150 (100), 149 (25), 138 (14), 137 (54), 133 (29), and 131 (14).

2,3,9-*Trimethoxypterocarpan* (3).—The *compound* crystallized as needles from benzene–heptane; m.p. 122—124°; $[\alpha]_D^{23}$ −228° (EtOH); $\nu_{max.}$ 1500, 1600, 1620 (aryl), 1030, 1140, 1270, 1345, and 1450 cm^{-1} (aryl ether); $\lambda_{max.}$ (EtOH) 288sh and 292 nm (log ε 3·96 and 3·98) [lit.,[13] m.p. 121—122°; $\nu_{max.}$ (KBr) 2985, 1623, 1510, 1418, 1387, 1348, 1269, 1195, 1176, 1081, 1042, 960, and 855 cm^{-1}; $\lambda_{max.}$ (MeOH) 290 nm (log ε 3·35)] (Found: M^+, 314·1133. $C_{18}H_{18}O_5$ requires M, 314·1154); *m/e* 314 (100%), 313 (24), 312 (10), 311 (10), 299 (28), 161 (10), and 148 (31); δ 7·12 (1H, d, *J* 8·8 Hz, H-7), 6·97 (1H, s, H-1), 6·47 (1H, s, H-4), 6·45 (1H, d, *J* 2·5 Hz, H-10), 6·44 (1H, q, *J* 8·8 and 2·5 Hz, H-8), and 3·91, 3·86, and 3·78 (9H, 3 × s, OMe).

We thank Dr. J. Henion and Mrs. J. Shea for recording the mass spectra, and Mr. S. Hair for recording the n.m.r spectra. We also thank Dr. M. F. Semmelhack for critical comments and review of the manuscript.

[4/2290 *Received*, 5*th November*, 1974]