Phytochemistry, 1975, Vol. 14, pp. 2081–2082. Pergamon Press. Printed in England.

PHYTOCHEMICAL REPORTS

TERREIN AND OTHER METABOLITES OF *PHOMA* SPECIES*

ANDREW W. DUNN,† IAN D. ENTWISTLE‡ and ROBERT A. W. JOHNSTONE†

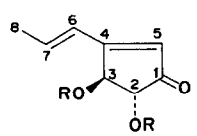
† The Robert Robinson Laboratories, The University, Liverpool L69 3BX, ‡ Shell Research Ltd., Sittingbourne, Kent, England

(*Revised Received* 31 *March* 1975)

Key Word Index—*Phoma*; fungi; terrein; sphaeropsidin (epoxydon); fatty acids.

During studies into fungal toxins, we attempted to isolate sphaeropsidin from cultures of Phoma NRRL 3188 according to the reported procedure [1]. Although the extracts were found to cause wilting of clover, the test plant, no spaeropsidin could be obtained but we isolated terrein, a substance previously reported to occur in *Aspergillus terreus* [2], *fischeri* [3], *stellatus* [4] and *Penicillium raistrickii* [5]. The structure (**1**; R = H) of terrein has been deduced from chemical degradation [5–7] and we have confirmed this structure on the basis of ^{13}C and ^{1}H NMR studies. A synthesis of racemic terrein was reported recently [8].

The mother liquors from the cultivation of *Phoma* NRRL 3188 [9] were extracted to yield a mixture of metabolites, a principle component of which was terrein. The mycelium was extracted to give mannitol and a mixture of fatty acid glycerides. The latter were saponified and the free acids esterified with diazomethane before analysis by GLC.



(**1**) Terrein R = H

PMR and CMR spectra of terrein and its diacetate (**1**; R = Ac) are given in the experimental together with assignments of proton and carbon atoms. The PMR spectrum shows clearly the arrangement of protons at C-6,7,8 (**1**) and the protons at C-2,3 must bear a *trans* relationship since the coupling constant is small. The CMR spectrum showed only seven peaks instead of the eight required by structure (**1**; R = H). On removing the off-resonance decoupling to protons, two peaks remained singlets and were readily assigned to C-1 and C-4 and one peak (C-8) split into a quartet; the remaining peaks separated into doublets. Two carbons (C-5,6) must have accidentally identical chemical shifts (extra large signal at δ 125·3) since there is no symmetry in the molecule. To confirm this, the CMR spectrum of terrein diacetate was obtained when it found that the signals for C-5,6 had separated and appeared at δ 124·5, 128·1.

GLC of the fatty acid methyl esters showed that the principle acids were palmitic, stearic, oleic, and linoleic and UV spectroscopy indicated that presence of a small amount of conjugated, polyunsaturated acid.

EXPERIMENTAL

Fungal culture. Phoma NRRL 3188 was grown for 17 days at 25° in shake culture on the following medium: glucose (2% w/v), malt extract (0·2%; Oxoid), mycological peptone (0·2%; Oxoid), potassium dihydrogen orthophsophate (0·2%), $MgSO_4$ (0·2%) in dist. H_2O. The mycelium and aq liquors were separated by filtration.

Terrein. This was initially isolated by the cumbersome procedure described for the isolation of sphaeropsidin [1]. Subsequent isolations were as follows. The mother liquors (10 l.) were evaporated under red. pres. to a small bulk (*ca.* 250 ml) and were then extracted with EtOAc (6 × 250 ml). Combined extracts were evaporated to yield a brown oil (900 mg) which was leached with hot C_6H_6 (3 × 50 ml). The C_6H_6 extracts were evaporated and the residual brown solid was chromatographed on Si gel (50 g; elution with EtOAc to yield terrein (400 mg; colourless crystals from CH_2Cl_2) mp 121–122°; $[\alpha]_D^{27}$ +155° (*c* = in H_2O); λ_{max}^{EtOH} 273 nm (ϵ = 30000) 345 nm (ϵ = 125); ν_{max}^{nujol} 3320, 3160, 1690, 1635, 1570 cm^{-1}; mass spectrum, *m/e* 154 (M), 139 (M–Me), 121 (M–Me–H_2O); found: C, 62·52; H, 6·55. Calc. for $C_8H_{10}O_3$: C, 62·32; H, 6·54%. PMR (D_2O): δ 2·17 (3H, doublet, *J* 6 Hz, $C\underline{H}_3$–CH

* Part 5 in the series "Fungal metabolites", for part 4 see Howard, C. C. and Johnstone, R. A. W. (1974) *J. Chem. Soc. Perkin I.*

CH–), 4·50 (1H, doublet, J 2·5 Hz, –CO.CHOH.CHOH–, 5·08 (1H, doublet, J 2·5 Hz, –CO.CHOH.CHOH–), 6·38 (1H, singlet, –C=CH–), 6·71 (1H, doublet, J 17 Hz, trans CH=CH–), 7·09 (1H, octet, J 17 and 6 Hz, Me CH=CH–), in the presence of Na_2CO_3, the peak at δ 5·08 disappeared (keto-enolic H). CMR (D_2O); the peak patterns after removal of the off-resonance proton decoupler are shown in parenthesis: δ 19·7 (C-8; quartet), 77·0 (C-3; doublet), 81·3 (C-2; doublet), 125·3 (C-5,6; doublet), 143·9 (C-7; doublet), 171·4 (C-4; singlet), 204·9 (C-1; singlet). Terrein in Ac_2O and pyridine gave the diacetate as a pale yellow viscous oil, mass spectrum, m/e 238 (M), 196 (M–CH_2CO), 135 (M–CH_2CO–Me CO_2H). CMR ($CDCl_3$): δ 19·5 (C-8), 20·5 and 20·8 (CH_3CO-, CH_3CO), 74·7 (C-3), 78·2 (C-2), 124·5 and 128·1 (C-5,6), 140·3 (C-7), 165·1 (C-4), 196·9 (C-1), 170·2 ($MeCO$, CH_3CO).

Mannitol and glycerides. Crushed, dried mycelium was extracted continuously with MeOH for 16 hr. After evaporation of the MeOH, residue was leached with 40–60° petrol to give a mixture of glycerides and a residue (A). The glycerides were refluxed with KOH in MeOH for 30 min to give free fatty acids which were methylated by brief treatment with CH_2N_2. The mixture of methyl esters was investigated before and after catalytic hydrogenation by GLC on a column (1·5 m × 3 mm) of celite coated with 10% EGSS-X at 200° and a N_2 flow of 45 ml/min. UV of the original glyceride mixture showed λ_{max}^{EtOH} 271 nm ($E_{1\,cm}^{1\%}$ = 8). Residue (A) was crystallized from EtOH to give mannitol, mp 165–166°; ν_{max}^{nujol} 3250 cm^{-1}; hexaacetate, mp 121–122°.

Acknowledgements—The authors thank the SRC and Shell Research Ltd., for financial support (AWD).

REFERENCES

1. Coats, J. H., Herr, M. E. and Herr, R. R. U.S. Patent, 3,585, 111.
2. Raistrick H. and Smith, G. (1935) *Biochem. J.* **29**, 606.
3. Misawa, M., Nara, T., Nakayama, K. and Kinochita, S. (1962), *Nippon Nogeikagaku Kaishi*, **36**, 699; (1965) C.A. **62**, 2213.
4. Qureshi, I. H., Kamal, A., Noorani, R., Aziz, S. and Husain, S. A. (1968) *Pak. J. Sci. Ind. Res.* **11**, 367.
5. Grove, J. F. (1954) *J. Chem. Soc.* 4693.
6. Clutterbuck, P. W., Raistrick, H. and Renter, F. (1937) *Biochem. J.* **31**, 987.
7. Barton, D. H. R. and Miller, E. (1955) *J. Chem. Soc.* 1028.
8. Auerbach, J. and Weinrab, S. M. (1974) *J. Chem. Soc., Chem. Somm.* 298.
9. Obtained from ARS Culture Collection Investigations, USDA.

Phytochemistry, 1975, Vol. 14, pp. 2082–2083. Pergamon Press. Printed in England.

3,6,8-TRIHYDROXY-1-METHYLXANTHONE—AN ANTIBACTERIAL METABOLITE FROM *PENICILLIUM PATULUM*

DOUGLAS BROADBENT, RICHARD P. MABELIS and HARRY SPENCER

Imperial Chemical Industries Ltd., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Chesire, SK10 4TG, England

(*Received* 19 *February* 1975)

Key Word Index—*Penicillium patulum*; fungus; norlichexanthone; 3,6,8-Trihydroxy-1-methylxanthone; biosynthesis; griseofulvin.

It is known that *Penicillium patulum* produces the antifungal antibiotic griseofulvin along with the biosynthetically related metabolites griseoxanthone C and griseophenones A, B and C.

We reinvestigated this organism because it was giving antibacterial activity and isolated griseofulvin, griseoxanthone C and griseophenone C along with 3,6,8-trihydroxy-1-methylxanthone, which was the only antibacterial metabolite (MIC vs *Clostridium welchii* = 25 ppm). It was identified from its UV and IR spectra, which were characteristic of other fungal xanthones [1]; its NMR spectrum (Ar–Me, 2 ArH as an Abqu J 2 Hz, 2 ArH as a multiplet, absence of O Me); and because on treatment with CH_2N_2 both it and griseoxanthone C were converted into the same product, 1-hydroxy-3,6-dimethoxy-8-methylxanthone (lichexanthone) [1].

3,6,8-Trihydroxy-1-methylxanthone occurs naturally as a metabolite of the lichen *Lecanora reuteri* and was given the trivial name norlichexanthone, [3a] and has been synthesized [3b].

Biosynthetically norlichexanthone would appear to be related to griseofulvin, the griseoxanthones and the griseophenones but it is the first such product in which all of the oxygen substituents occur as free phenolic hydroxyl groups. There are two ways to conceive of its formation.