Natural Acetylenes. Part XLI.[1] Polyacetylenes from Fungal Fruiting Bodies

By **Iain W. Farrell, John W. Keeping, Martin G. Pellatt,** and **Viktor Thaller,*** The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

Polyacetylenes have been detected in sporophores of five Basidiomycete species. The new hydroxy-acids, HO·CH_2·CH=^{c}CH·[C≡C]$_2$·CH_2·CH_2·CO_2H and HO·CH_2·CH=^{t}CH·[C≡C]$_2$·CH=^{t}CH·CO_2H, were found in *Fistulina hepatica* and isolated as the methyl esters.

POLYACETYLENES occur widely in mycelial cultures of Basidiomycetes but little is known about their occurrence in fungal fruiting bodies. Anchel mentioned [2] the detection of polyacetylenes in extracts of sporophores belonging to several genera of Basidiomycetes (no details were given), and Schulte *et al.*[3] isolated several polyacetylenes from pulverised dried sporophores of the Polyporaceae *Fomes officinalis*. Fungal fruiting bodies of seventy Basidiomycete species have now been screened for polyacetylenes. Most of the crude sporophore extracts contained considerable amounts of steroidal matter (ergosterol was isolated in several instances) which absorbed strongly in the 260—290 nm region, and a reliable detection of polyacetylenes could be made only after partial separation by chromatography. The extracts of eleven species, suspected to contain polyacetylenes, were thus further investigated, and the presence of polyacetylenes was eventually proved in five of them.

Mycelia and sporophores of *Serpula lacrymans* Pers. ex S. F. Gray † and sporophores of *Fistulina hepatica* (Hudson) Fr. were analysed. The diacids (I; R = H) and (II; R = H), already found in cultures,[4] have now also been detected as trace constituents of natural *S. lacrymans*.

$$RO_2C\cdot[C\equiv C]_3\cdot CH_2\cdot CH_2\cdot CO_2R \quad (I)$$

$$RO_2C\cdot CH=^{t}CH\cdot[C\equiv C]_2\cdot CH_2\cdot CH_2CO_2R \quad (II)$$

$$Me[C\equiv C]_4\cdot[CH(OH)]_3\cdot CH_2\cdot OH \quad (III)$$

$$MeO_2C\cdot CH=^{t}CH\cdot[C\equiv C]_2\cdot CH=^{t}CH\cdot CO_2Me \quad (IV)$$

$$HO\cdot H_2C\cdot CH=^{t}CH\cdot[C\equiv C]_2\cdot CH=^{t}CH\cdot CO_2Me \quad (V)$$

$$HO\cdot H_2C\cdot CH_2\cdot CH_2\cdot[C\equiv C]_2\cdot CH=^{t}CH\cdot CO_2Me \quad (VI)$$

$$HO\cdot H_2C\cdot CH=^{c}CH\cdot[C\equiv C]_2\cdot CH_2\cdot CH_2\cdot CO_2Me \quad (VII)$$

The polyacetylenes of *F. hepatica* sporophores, on the other hand, differed from those isolated from culture fluids in which only neutral polyacetylenes were present and in which the tetraynetetraol (III) was the main constituent.[5] The neutral fraction of the sporophore extract was a complex mixture of small amounts of polyacetylenes (the presence of a tetrayne chromophore was not obvious), none of which could be identified.

† This fungus has been previously classified as *Merulius lacrymans* Wulfen ex Fr.

[1] Part XL, A. G. Fallis, M. T. W. Hearn, Sir Ewart R. H. Jones, V. Thaller, and J. L. Turner, *J.C.S. Perkin I*, 1973, 743.

[2] M. Anchel in ' Antibiotics,' vol. 2, eds. D. Gottlieb and P. D. Shaw, Springer-Verlag, Berlin-Heidelberg-New York, 1967, p. 189.

[3] K. E. Schulte, G. Rücker, and H. Fachmann, *Arch. Pharm.*, 1969, **302**, 965.

[4] J. N. Gardner, E. R. H. Jones, P. R. Leeming, and J. S. Stephenson, *J. Chem. Soc.*, 1960, 691.

[5] Sir Ewart R. H. Jones, G. Lowe, and P. V. R. Shannon, *J. Chem. Soc.* (*C*), 1966, 139.

In the esterified acid fraction at least seven polyacetylenes were present and five were identified as the methyl esters (II),[4] (IV),[4] (V), (VI),[4] and (VII). The hydroxy-esters (V) and (VII) were new. The latter was the major constituent; its structure follows unequivocally from spectral data. The hydroxy-ester (V) was present in small amounts; it was identical with an authentic specimen synthesised for comparison.

Too little fungal material of the remaining three 'positive' species was available for an analysis of the polyacetylene content to be successful. *Hydnum repandum* L. ex Gr. contained both neutral and acidic polyacetylenes, *Stereum hirsutum* (Willd. ex Fr.) Fr. only neutral, and *Clitocybe flaccida* (Sow. ex Fr.) Kummer acidic polyacetylenes. Most of these polyacetylenes appeared to be diynenes. *H. repandum* and *S. hirsutum* were previously examined in culture and did not produce polyacetylenes under standard growth conditions. *C. flaccida* on the other hand did.

EXPERIMENTAL

Instruments: u.v., Unicam SP 800 (solutions in Et_2O); i.r., Unicam SP 1000; n.m.r., Perkin-Elmer R14; mass spectra (direct insertion), A.E.I. MS9.

Liquid chromatography: SiO_2 H.B.L. M60 in columns and Merck $HF_{254+366}$ and $PF_{254+366}$ in 0·3 (t.l.c.) and 1 mm (p.l.c.) layers, respectively. T.l.c. plates were freed from impurities by repeated elution with Et_2O when used for the purification of samples for mass spectral analysis.

Petrol refers to the light petroleum fraction b.p. 30—40°.

Screening of Fungal Fruiting Bodies and Isolation of Polyacetylenes.—The fungi were collected in autumn in and around Oxford, cut into small pieces, and extracted repeatedly, first with Et_2O and then with Me_2CO (for *F. hepatica* MeOH was used in addition) for 48 h at 20° with each solvent. The combined and concentrated extracts were continuously extracted with Et_2O, and the u.v. spectra of the Et_2O extracts were recorded. Extracts for which the presence of polyacetylenes was indicated were separated into neutral and acid parts ($NaHCO_3$), and the latter were esterified [MeOH–H_2SO_4 (97 : 3); 48 h)]. The neutral and acidified acid parts were chromatographed, separately, on layers or columns and the u.v. spectra of the fractions were recorded. For *S. lacrymans* and *F. hepatica* the fractions showing polyacetylene absorption were purified by repeated p.l.c. and t.l.c. in several solvent systems. The presence of known polyacetylenes was confirmed by direct comparison (u.v. and mass spectra, and t.l.c. in several solvent systems) with authentic specimens.

Polyacetylenes of Serpula lacrymans *Pers. ex S. F. Gray.*—Mycelia and sporophores (1 kg) yielded a mixture of methyl esters (2 g) from which *ca.* 0·1 mg of each of the dimethyl esters (I)[2] and (II)[2] (R = Me) was isolated. No polyacetylenes were detected in the neutral part.

Polyacetylenes of Fistulina hepatica (*Hudson*) *Fr.*—Sporophores (3 kg) yielded a neutral part (0·5 g) containing *ca.* 15 mg of polyacetylenes which were separated by p.l.c. (Et_2O) into six polyacetylene-containing bands (five bands had longest $\lambda_{max.}$ at 314 and one at 323 nm). Repeated t.l.c. of the individual bands failed to provide sufficient material to allow identification of the polyacetylenes present.

The esterified acid part which contained *ca.* 25 mg of polyacetylenes (estimated by u.v.) was separated into the dimethyl esters (II; R = Me)[4] and (IV),[4] the hydroxy-esters (V) (see below) and (VI),[4] and *methyl* 10-*hydroxydec*-cis-8-*ene*-4,6-*diynoate* (VII), R_F 0·49 (Et_2O) (Found: M^+, 192·0785. $C_{11}H_{12}O_3$ requires M, 192·0786), $\lambda_{max.}$ 283 (rel. E 4·1), 267 (4·8), 252·5 (3·45), 235 (1·7), and 228 (1·0) nm, $\nu_{max.}$ (CCl_4) 3620 (OH), 2220 (C≡C), 1745 (ester CO), 1440, 1360, 1170, and 1025 cm^{-1}, $\nu_{max.}$ (CS_2) 725 cm^{-1} (*cis*-CH=CH), τ (CCl_4) 8·1 (1H, s, OH; disappears on addition of D_2O), 7·43 (4H, AB system, $OC{\cdot}CH_2{\cdot}CH_2{\cdot}C{\equiv}C$), 6·32 (3H, s, CO_2Me), 5·68 (2H, d, J 6 Hz, $=CH{\cdot}CH_2{\cdot}OH$), 4·48 (1H, d, J 11 Hz, $CH{=}CH{\cdot}C{\equiv}C$), and 3·85 (1H, dt, J 11 and 6 Hz, $HO{\cdot}CH_2{\cdot}CH{=}CH$), *m/e* 192 ($M^+$, 25%), 177 (15), 161 (22), 149 (26), 133 (80), 119 (68), 105 (55), 103 (60), 91 (89), and 77 (100). Esters with R_F 0·68 (Et_2O), $\lambda_{max.}$ 303, 285, 268, 252, and 238 nm, and R_F 0·38 (Et_2O), $\lambda_{max.}$ 331·5, 310, 291, 273·5, 259, 245·5, and 233 nm, were not identified.

Methyl 10-*Hydroxydeca*-trans-2,trans-8-*diene*-4,6-*diynoate* (V) (with M. T. W. Hearn).—To CuCl (50 mg), $NH_2{\cdot}OH{,}HCl$ (1·0 g), $EtNH_2$–H_2O (40%; 1·5 ml), and MeOH (5 ml), stirred under N_2 at 0°, methyl pent-*trans*-2-en-4-ynoate[1] (275 mg, 2·5 mmol) in Et_2O (5 ml) and then after 5 min, while a faint precipitate persisted, 5-bromopent-*trans*-2-en-4-yn-1-ol (425 mg, 2·6 mmol) in MeOH (5 ml) were added dropwise. Stirring was continued for 30 min, ice (100 g)–HCl (5%; 50 ml) was added, and the mixture was extracted with Et_2O. The extract (700 mg) was separated by p.l.c. (petrol–Et_2O, 3 : 2; 3 elutions); the band with R_F 0·4 yielded on repeated crystallisation (Et_2O–petrol and CCl_4–petrol) pale yellow prisms (205 mg) of the *hydroxy-ester* (V), m.p. 80—81° (Found: C, 69·5; H, 5·4. $C_{11}H_{10}O_3$ requires C, 69·5; H, 5·3%), $\lambda_{max.}$ (EtOH) 333 (ε 17,500), 313 (21,000), 293infl (16,000), 260 (23,000), 248 (28,500), and 235infl (22,500) nm, $\nu_{max.}$ (CCl_4) 3610, 3400, 2200, 2105, 1730, 1613, and 952 cm^{-1}, $\nu_{max.}$ (CS_2) 952 cm^{-1}, τ (CCl_4) 8·85br (OH), 6·26 (s, CO_2CH_3), 5·78 (m, $HOCH_2$), 4·15 (dtd, J 16, 2·5, and 1 Hz $HOCH_2{\cdot}CH{=}CH$), 3·76 (d, J 16 Hz, $CH{=}CH{\cdot}CO_2Me$), 3·57 (dt, J 16 and 5 Hz, $HOCH_2{\cdot}CH{=}CH$), and 3·22 (dd, J 16 and 1 Hz, $CH{=}CH{\cdot}CO_2Me$), *m/e* 190 (M^+, 100%), 175 (32), 161 (40), 159 (38), 158 (26), 148 (26), 147 (70), 146 (28), 133 (25), 131 (34), 130 (34), 129 (24), 119 (60), 118 (30), 117 (24), 103 (51), 102 (42), 98 (24), 91 (56), 89 (30), 87 (48), 86 (34), 77 (72), 75 (48), 74 (36), and 65 (30).

Screening of Hydnum repandum *L. ex Fr.*—Sporophores (15 g) were used. Repeated t.l.c. of the neutral fraction gave two polyacetylene-containing bands, both with $\lambda_{max.}$ 283·5, 268, 254, and 235 nm. The methyl esters were separated into one band with $\lambda_{max.}$ 302, 283, 267, 254, and 238 nm and two bands with $\lambda_{max.}$ 283·5, 268, 253·5, and 240 nm.

Screening of Clitocybe flaccida (*Sow. ex Fr.*) *Kummer.*—Sporophores (20 g) gave a methyl ester band with $\lambda_{max.}$ 343, 320, 300, 280, and 254 nm. The chromophore deteriorated rapidly on t.l.c.

Screening of Stereum hirsutum (*Willd. ex Fr.*) *Fr.*—Sporophores (100 g) gave two neutral polyacetylene containing bands, both with $\lambda_{max.}$ 282, 267, and 251 nm.

We thank the S.R.C. for support.

[3/946 *Received*, 10*th May*, 1973]