Natural Acetylenes. Part 54.[1] Polyacetylenes from Fungal Cultures of Some Tricholomataceae and Corticiaceae Species [2]

By **Mahtabuddin Ahmed, John W. Keeping, Theodore A. Macrides,** and **Viktor Thaller,*** The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

Extracts from the culture fluids of *Lentinus lepideus*, *Leucopaxillus giganteus*, *Lyophyllum decastes*, and *Peniophora resinosa* were found to contain $Me[C{\equiv}C]_3{\cdot}CH{=}\overset{t}{C}H{\cdot}CH_2OH$ as the major polyacetylene. The first two contained also the corresponding methyl ether, which was synthesised, and the third contained the corresponding aldehyde. The new polyacetylene (+)-$Me[C{\equiv}C]_3{\cdot}CH{=}\overset{t}{C}H{\cdot}CH(OH)Et$ was isolated from the last culture.

As part of a continuing study of the polyacetylene content of Basidiomycete fungi we have examined the extracts of culture fluids of the Tricholomataceae species *Lentinus lepideus* (Fr.) Fr., *Leucopaxillus giganteus* (Sow. *ex* Fr.) Singer, and *Lyophyllum decastes* (Fr. *ex* Fr.) Singer and the Corticiaceae species *Peniophora resinosa* Jackson & Dearden. The new C_{12} polyacetylene (1) and the C_{10} polyacetylenes (2)—(4) have been isolated; additional polyacetylenes were present as mixtures in amounts too small for isolation and identification. No polyacetylenic acids were detected in the above extracts.

The previously analysed Corticiaceae cultures *Resinicium bicolor* (Alb. and Schw. *ex* Fr.) Parm. (described as *Odontia bicolor* [3]) and *Laeticorticium roseum* (Pers. *ex* Fr.) Donk (described as *Aleurodiscus roseus* [4]) were noted for their allene content, the latter also containing dehydromatricarianol (2) as the major and the aldehyde (4) as a

[1] Part 53, I. W. Farrell, M. T. W. Hearn, and V. Thaller, preceding paper.

[2] A more detailed account of the work described in this paper is in the D.Phil. Theses of (*a*) M. Ahmed, Oxford, 1976, and (*b*) T. A. Macrides, Oxford, 1977.

[3] R. E. Bew, J. R. Chapman, Sir Ewart R. H. Jones, B. E. Lowe, and G. Lowe, *J. Chem. Soc.* (*C*), 1966, 129; M. Ahmed, Sir Ewart R. H. Jones, M. T. W. Hearn, and V. Thaller, *J. Chem. Research*, 1977, (*S*) 125; (*M*) 1579.

[4] R. C. Cambie, A. Hirschberg, E. R. H. Jones, and G. Lowe, *J. Chem. Soc.*, 1963, 4120.

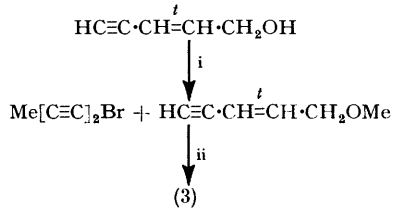
minor constituent. No allenes were detected in the *P. resinosa* extract: only the C_{12} alcohol (1) (*ca.* 0.2 mg l^{-1} culture fluid) and dehydromatricarianol (2) (*ca.* 1 mg l^{-1}

(+)-Me[C≡C]$_3$·CH=^{t}CH·CH(OH)Et (1)

Me[C≡C]$_3$·CH=^{t}CHR

(2) R = CH_2OH
(3) R = CH_2OMe
(4) R = CHO
(5) R = COEt

culture fluid) were identified. The new, optically active alcohol (1) had to be a triynenol: it and dehydromatricarianol (2) had identical u.v. absorption patterns which suffered the same bathochromic shift on oxidation by manganese dioxide. The ^1H n.m.r. and mass spectra of the alcohol (1) and its oxidation product (5) established the presence of the propynyl and ethyl groups and the molecular weights, and thus the complete structures of the two molecules. In the present investigation, *P. resinosa* was grown in a glucose medium with ammonium nitrate as nitrogen source. With mycological peptone as nitrogen source, polyenes appeared to be the main metabolites.



SCHEME

Reagents: i, NaH–HCONMe$_2$, MeI; ii, CuCl, NH_2OH·HCl, $EtNH_2$

The widely distributed dehydromatricarianol (2) was also the main polyacetylene in the extracts from the three Tricholomataceae cultures (0.1—0.7 mg l^{-1} culture fluid). *L. decastes* contained in addition dehydromatricarianal (4) (*ca.* 0.1 mg l^{-1} culture fluid) and the *L. giganteus* and *L. lepideus* cultures contained the dehydromatricarianol methyl ether (3) (*ca.* 0.15 and 0.02 mg l^{-1} culture fluid, respectively). This was reported to be present in low concentrations in *Clitocybe candida* cultures [5] [this organism is now considered [6] to be *Leucopaxillus candidus* (Bres.) Singer] in which dehydromatricarianol (2) was the major metabolite. Since methanol was used in the separation of these *L. candidus* polyacetylenes, formation of the methyl ether (3) during work-up could not be excluded. In the present investigation no methanol was used at any stage of fungal growth, work-up, or separation. The methyl ether (3) must therefore be a genuine metabolite in fungal cultures. It is interesting that two out of the three organisms known to produce it belong to the genus *Leucopaxillus*. The methyl ether (3) was synthesised for comparison by the route indicated in the Scheme.

EXPERIMENTAL

For general techniques see Part 43.[7] Crystallisations were carried out from Et_2O–petrol at −40 °C.

Growth and Extraction of Fungal Cultures and General Work-up of Extracts.—The fungi were grown on 3% malt extract in static cultures (for *P. resinosa* see special medium). When maximum polyacetylene concentrations (estimated by u.v.) were reached, the media were decanted and replaced by a 4% glucose solution. In this the fungi were kept until maximum polyacetylene concentrations were reached again. The media were decanted and the culture fluids and the glucose refloods were each continuously extracted with Et_2O for 48 h. The extracts were concentrated to *ca.* 200 ml and separated into neutral and acidic fractions ($NaHCO_3$); the latter were esterified with 4% H_2SO_4 in MeOH. The culture and reflood extracts appeared (u.v. and t.l.c.) to be identical; they were combined and analysed together.

Isolation of Polyacetylenes from Cultures of Peniophora resinosa *Jackson & Dearden.**—The fungus was grown in static culture on a medium of glucose (20 g), $CaCl_2$ and $MgSO_4$ (0.5 g each), $Na_2B_4O_7$, $ZnSO_4$, $FeCl_3$, and $CuSO_4$ (0.005 g each), yeast extract (2 g), and NH_4NO_3 (0.6 g) per l H_2O for 46 days in 76 flasks (53 l culture fluid). Only the neutral fraction showed polyacetylene absorption (u.v. and i.r.). It was concentrated and separated by p.l.c. (petrol–Et_2O, 1 : 1) into 6 bands. Those with R_F 0.5 (A), 0.45 (B), and 0.35 (C) contained polyacetylenes. Band A was further purified by t.l.c. (C_6H_6, cont. elution, 6 h) and yielded on crystallisation plates (11 mg) of *dodec*-trans-4-*ene*-6,8,10-*triyn*-3-*ol* (1), m.p. 37—38° (Found: M^+, 172.0886. $C_{12}H_{12}O$ requires M, 172.0888), $[\alpha]^{20}$ +12.5° (589 nm), +11.6° (578), +15.7° (546), and +35° (436) (*c* 0.702 in EtOH), $\lambda_{max.}$(Et_2O) 331 (ε 10 200), 309 (15 300), 290 (11 800), 274 (5 700), 259 (2 900), 244 (82 700), and 232 (51 000) nm, $\nu_{max.}$($CHCl_3$) 3 580 and 3 400 (free and bonded OH), 2 223 (C≡C), and 956 cm^{-1} (*trans*-CH=CH), τ($CDCl_3$) 9.08 (t, *J* 8 Hz, CH_2·*CH*$_3$), 8.48 (m, *CH*$_2$·CH_3), 8.44 (s, OH; disappears on addition of D_2O), 8.05 (s, CH_3·C≡C), 5.89 [m, −CH=CH·*CH*(OH)·CH_2], 4.28 (d, *J* 16 Hz, C≡C·*CH*=CH), and 3.55 (dd, *J* 6 and 16 Hz, CH=*CH*·CH), *m/e* 172 (M^+, 5%), 157 (9), 143 (17), 141 (14), 115 (43), 89 (26), 63 (26), 59 (37), 57 (46), 45 (29), and 31 (100). The alcohol (1) (8.5 mg) and MnO_2 (260 mg) were shaken in CH_2Cl_2 (2.5 ml) for 4 h in the dark under N_2 at 20 °C. Filtration (Celite) and separation by t.l.c. (petrol–Et_2O, 1 : 1) gave *dodec*-trans-4-*ene*-6,8,10-*triyn*-3-*one* (5) (5 mg, 60%), R_F 0.4 (Found: M^+, 170.0728. $C_{12}H_{10}O$ requires M, 170.0732), $\lambda_{max.}$(Et_2O) 348.5 (rel. *E* 2.4), 325 (3.1), 305 (1.95), 287 (1), 261 (7.1), and 250 (5.7) nm, $\nu_{max.}$(CCl_4) 2 224 and 2 180 (C≡C), 1 693 (CO), 1 677 (CH=CH), and 960 cm^{-1} (*trans*-CH=CH), *m/e* 170 (M^+, 58%), 141 (100), 113 (24), 111 (27), 109 (24), 97 (35), 95 (29), 87 (23), 83 (32), 81 (25), 71 (29), 69 (39), 57 (37), and 55 (37). The more polar band B gave on t.l.c. (C_6H_6–$CHCl_3$, 1 : 1, cont. elution, 2 h) and crystallisation dec-*trans*-2-ene-4,6,8-triyn-1-ol (2) (49 mg), m.p. 127—128° (decomp.) (lit.,[4] 128—129°), $\lambda_{max.}$(EtOH) 328 (ε 13 000), 307 (19 100), 288 (14 400), 272 (7 600), 257 (3 750), 242 (138 000), and 230 (82 500) nm,

* Supplied by The Central Experimental Farm, Ottawa, Canada.

[5] E. J. McWhorter and M. Anchel, *J. Org. Chem.*, 1965, **30**, 2359.

[6] R. Singer, 'The Agaricales in Modern Taxonomy,' Cramer, Weinheim, 1962.

[7] M. Ahmed, G. C. Barley, M. T. W. Hearn, Sir Ewart R. H. Jones, V. Thaller, and J. A. Yates, *J.C.S. Perkin I*, 1974, 1981.

$\nu_{max.}$($CHCl_3$) 3 580, 3 370, 2 222, and 948 cm^{-1}, τ($CDCl_3$) 8.42br (s, OH; disappears on D_2O addition), 8.02 (s, CH_3·C≡C), 5.75 (dd, *J* 6 and 1.5 Hz, CH=CH·CH_2O), 4.20 (d, *J* 16 Hz, C≡C·C*H*=CH–), and 3.53 (dt, *J* 16 and 6 Hz, CH=C*H*·CH_2), *m/e* 144 (M^+, 54%), 116 (34), 115 (100), and 74 (32). This (15 mg) and MnO_2 in CH_2Cl_2 yielded on crystallisation dec-*trans*-2-ene-4,6,8-triynal (**4**) (12 mg), m.p. 106–107° (lit.,[4] 108—109°), $\lambda_{max.}$(Et_2O) 348.5 (rel. *E* 2.7), 325.5 (3.4), 288 (1), 259 (9.1), 246.5 (5.7), and 235 nm (2.7), $\nu_{max.}$(CCl_4) 2 235, 2 170, 1 690, and 958 cm^{-1}, *m/e* 142 (M^+, 100%), 114 (57), 113 (24), 88 (73), 87 (37), 86 (21), 63 (16), and 62 (15). Band C contained traces of an apparent triynediene.

Isolation of Polyacetylenes from Cultures of Leucopaxillus giganteus (*Sow.* ex *Fr.*) *Singer.**—The fungus was grown for 46 days and in the replacement culture for 14 days (28 flasks, 20 l culture fluid). Only the neutral fraction showed polyacetylene absorption. It was concentrated and separated by t.l.c. (Et_2O) into several fractions. Those with R_F 0.5 and 0.3 showed polyacetylene absorption and were further purified. The R_F 0.5 band yielded on t.l.c. (CH_2Cl_2, cont. elution, 1 h) and crystallisation 1-methoxydec-*trans*-2-ene-4,6,8-triyne (**3**) (3 mg), identical with a synthetic specimen (see below). The more polar band (R_F 0.3) was separated by t.l.c. (CH_2Cl_2, cont. elution, 5.5 h) into a band with apparent R_F 0.7 which gave on crystallisation dehydromatricarianol (**2**) (10 mg) and a band with apparent R_F 0.2 which was rechromatographed (CH_2Cl_2, 16 h) and yielded an unidentified polyacetylene fraction (2 mg), $\lambda_{max.}$(Et_2O) 283.5 (rel. *E* 2.85), 267.5 (3.5), 253 (2.25), 240 (1.25), 228 (1), and 212 (11.5) nm, and possibly M^+ 178.

Isolation of Polyacetylenes from Cultures of Lyophyllum decastes (*Fr.* ex *Fr.*) *Singer.**—The fungus was grown for 45 days and in the replacement medium for 63 days (34 flasks, 24 l culture fluid). Only the neutral fraction showed polyacetylene absorption. It was concentrated and separated by t.l.c. (Et_2O) into several fractions. Bands at R_F 0.45 and 0.35 contained polyacetylenes. The less polar band was rechromatographed (CH_2Cl_2, cont. elution, 1.25 h) and gave on crystallisation dehydromatricarianal (**4**) (3 mg). The band with R_F 0.35 gave on t.l.c. (CH_2Cl_2, cont. elution, 4.5 h) bands with apparent R_F values 0.6 and 0.4. The former yielded dehydromatricarianol (**2**) (4 mg). The more polar band contained a polyacetylene (2 mg) which could not be identified, $\lambda_{max.}$(Et_2O) 282 (rel. *E* 3), 266 (4.2), 251.5 (2.7), 239 (1.6), 228 (1), and 212 nm (14.5), $\nu_{max.}$(CCl_4) 3 622, 3 020, 2 240, and 930 cm^{-1}, *m/e* 148 (41%), 133 (24), and 91 (100), together with a large number of minor fragments. This (2 mg) and MnO_2 (100 mg) were shaken in CH_2Cl_2 (2 ml) for 1.5 h. No change in the u.v. spectrum was observed.

Isolation of Polyacetylenes from Cultures of Lentinus lepideus (*Fr.*) *Fr.*†—The culture was grown for 65 days and in the replacement medium for 15 days. Only the neutral fraction showed polyacetylene absorption. It was concentrated (orange oil, 75 mg) and separated by p.l.c. (petrol–Et_2O, 1 : 1, cont. elution, 1 h) into bands A—E (in order of increasing polarities). Band A was rechromatographed (petrol–EtOAc, 49 : 1, cont. elution, 2 h) and yielded the methyl ether (**3**) (1 mg). The combined fractions B and C were rechromatographed (petrol–Et_2O, 3 : 1 cont. elution, 1.5 h) and gave on crystallisation dec-*trans*-2-ene-4,6,8-triyn-1-ol (**2**) (40 mg). Fractions D and E contained traces of polyacetylenes which were not identified.

Synthesis of 1-*Methoxydec*-trans-2-*ene*-4,6,8-*triyne* (**3**).—Pent-*trans*-2-en-4-yn-1-ol (492 mg, 6 mmol) and MeI (852 mg, 6 mmol) were added in quick succession to NaH (144 mg, 6 mmol) stirred in $HCONMe_2$ (5 ml) under N_2 at 0 °C. After an initial darkening of the mixture a yellow precipitate was formed. Stirring was continued for 48 h before H_2O (5 ml) was added and the mixture was extracted with petrol. The extract was dried ($MgSO_4$) and concentrated to give the crude 1-methoxypent-*trans*-2-en-4-yne (pale yellow liquid) (280 mg, 50%), $\nu_{max.}$(CCl_4) 3 300 (H–C≡C), 2 100 (C≡C), 1 110 (OMe), and 955 cm^{-1} (*trans*-CH=CH). To this (96 mg, 1 mmol), CuCl (2 mg), NH_2OH·HCl (69 mg), and $EtNH_2$ (40%; 1 ml) stirred in MeOH (5 ml) at 20 °C, 1-bromopenta-1,3-diyne (143 mg, 1 mmol) in MeOH (2 ml) was added dropwise and stirring was continued for 1 h. Work-up with KCN–petrol, treatment of the petrol extract with $AgNO_3$, filtration, concentration, and separation of the residue by p.l.c. (petrol–Et_2O, 49 : 1) gave 1-methoxydec-*trans*-2-ene-4,6,8-triyne (**3**) (95 mg, 60%), R_F 0.75, which crystallised, m.p. 38—39° (lit.,[5] 40—40.5° and sintering at 34°), $\lambda_{max.}$ (Et_2O) 329 (rel. *E* 3.7), 308 (5.7), 288.5 (4.35), 272 (2.3), 257.5 (1), 241.5 (37), and 230 nm (26), $\nu_{max.}$(CCl_4) 2 280, 2 240, 1 115, 1 070, and 960 cm^{-1}, τ($CDCl_3$) 8.01 (s, CH_3·C≡C), 6.62 (s, OCH_3), 6.01 (dd, *J* 6 and 1.5 Hz, CH=CH·CH_2O), 4.23 (d, *J* 16 Hz, C≡C·C*H*=CH), and 3.62 (dt, *J* 16 and 6 Hz, CH=C*H*·CH_2), *m/e* 158 (M^+, 97%), 143 (79), 127 (34), 115 (100), 100 (33), and 74 (43).

We thank the University of Dacca, Bangladesh, for a scholarship (to M. A.), Procter and Gamble (U.K.) for a research studentship (to T. A. M.), and the S.R.C. for research grant support.

[8/168 *Received*, 1*st February*, 1978]

* Supplied by the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

† Kindly supplied by Professor G. Linnemann, Institut für Forstbotanik und Forstgenetik der Förstlichen Fakultät, Göttingen University, Hann-Münden, Germany.