

Natural Acetylenes. Part XLV.¹ Polyacetylenes from Cultures of the Fungus *Collybia peronata* (Bolt. ex Fr.) Kummer ²

By Cathryn A. Higham, Sir Ewart R. H. Jones,* John W. Keeping, and Viktor Thaller, Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

The new polyacetylenes $\text{HO}_2\text{C}\cdot[\text{C}\equiv\text{C}]_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$, *cis*- and *trans*- $\text{Me}[\text{C}\equiv\text{C}]_2\cdot\text{CH}=\text{CH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$, $[\text{Me}[\text{C}\equiv\text{C}]_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}]$, and $(-)\text{-Me}[\text{C}\equiv\text{C}]_3\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{OH}$, as well as the known diol $\text{HO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot[\text{C}\equiv\text{C}]_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$, also new as a fungal metabolite, have been detected in extracts from the culture fluids of *C. peronata* and also synthesised.

THE constituents of mycelial cultures of *Marasmius* species were investigated by Bendz³ and *M. ramealis* (Bull. ex Fr.) Fr. and *M. peronatus* (Bolt. ex Fr.) Fr. [synonym *M. urens* (Bull. ex Fr.) Fr.] were found to

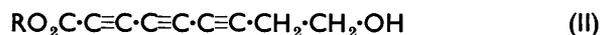
produce antibiotically active polyacetylenes. The presence of the antibiotic marasin (I) and several unidentified polyacetylenes was established in the former and that of

¹ Part XLIV, R. K. Bentley, C. A. Higham, J. K. Jenkins, Sir Ewart R. H. Jones, and V. Thaller, preceding paper.

² A more detailed account of the work described in this paper is in the D.Phil. Thesis of C. A. Higham, Oxford, 1973.

³ G. Bendz, *Arkiv Kemi*, 1959, **15**, 131.

an unidentified diynene in the latter. In a recent attempt to classify the order Agaricales,⁴ the two species have been reallocated within the family Tricholomataceae to different genera of the *Collybiaeae* tribe: they are now called *Marasmiellus ramealis* (Bull. ex Fr.) Singer and *Collybia peronata* (Bolt. ex Fr.) Kummer. The latter has now been found to be a good producer of polyacetylenes. Those present in the culture fluid in sufficient quantities for characterisation have been identified as the compounds (II; R = H)—(VI).



All six are new as metabolites and, with the exception of the C₈ diol (VI),⁵ are new compounds; their identity has been confirmed by synthesis. The five C₉ metabolites show obvious structural similarities and are most probably derived in the fungus from common C₁₀ precursors; their biogenetic relationship to the C₈ diol (VI) is more difficult to visualise.

The esterified acid fraction yielded, in addition to the hydroxy-ester (II; R = Me), a considerable amount of dimethyl fumarate and traces of material with enediene and triene chromophores.

The least polar homogeneous fraction obtained by liquid chromatography of the neutral metabolites consisted of the *cis*- and *trans*-diynenols (III). The two isomers were separated by g.l.c., which established their ratio as 3 : 1, and from the relative intensities of the i.r. bands the *cis*-isomer appeared to be the major component. The synthetic mixture of *cis*- and *trans*-diynenols (III) (*cis* : *trans* ratio ca. 1 : 1) showed retention times identical with those of the natural compounds.

The triynol (IV) came next in polarity and was followed by the laevorotatory triynediol (V). In the mass spectrum this gave in addition to a molecular ion (*m/e* 148) a base peak at *m/e* 117 (*M*⁺ - CH₂OH), due to the periodate fission-like fragmentation already observed with another acetylenic α-glycol.⁶

Two more polyacetylene-containing fractions were obtained during the diol (V) purification; traces of more polar material with λ_{max.} 300, 282.5, 267, 252, and 244 nm, possibly two or more chromophoric systems, and a less polar crystalline triyne. The latter gave an n.m.r. spectrum which indicated the presence of the aldehyde hydrate (VII). Failure to obtain this material from

extracts of subsequently grown cultures prevented proper identification. The most polar polyacetylene was the diynediol (VI).⁵



The new compounds were synthesised by standard reactions. Methyl 5-bromopentadiynoate and but-3-yn-1-ol were coupled to the hydroxy-ester (II; R = Me), hexa-2,4-diyne and Ph₃P=CH·CH₂·CH₂·OH were condensed to give the *cis*- and *trans*-diynenols (III), penta-1,3-diyne and 4-bromobut-3-yn-1-ol yielded the triynol (IV), and 1-bromopenta-1,3-diyne and but-3-yne-1,2-diol gave the racemic diol (V) (details are given in the Experimental section).

EXPERIMENTAL

Instruments used: u.v., Unicam SP 800; i.r., Unicam SP 200 and SP 1000; n.m.r., Perkin-Elmer R10 and R14; mass spectra (direct insertion), Varian-MAT CH7 and A.E.I. MS9; specific rotations, Perkin-Elmer 141; m.p.s (corr.), Kofler hot-stage apparatus.

Solution chromatography: SiO₂ H.B.L. M60 in columns and Merck HF_{254 + 366} in 0.3 mm (t.l.c.) and PF_{254 + 366} in 1 mm (p.l.c.) layers, respectively.

Gas-liquid chromatography (g.l.c.): poly(ethylene glycol succinate) (10%) on Embacel (1500 × 4 mm) with N₂ (45 ml min⁻¹).

Petrol refers to light petroleum of b.p. 30–40°.

Growth of Collybia peronata.—The fungus * was grown as static culture (no structural changes, or improvement in the quantities of metabolites produced occurred in a shaken culture) on a medium consisting of glucose (40 g), casein hydrolysate (1.0 g), KH₂PO₃ (1.0 g), KCl (0.5 g), MgSO₄ (hydrated) (0.5 g), FeSO₄ (0.01 g), and yeast extract (2.0 g) (quantities per litre of water). After 28 days' growth, citrate (25 ml containing 2.2 g Na citrate adjusted to pH 5.5 with citric acid) was added to each flask. The medium was withdrawn when maximum polyacetylene concentration (estimated by u.v.) was reached (56 days) and replaced by a 4% glucose solution. In this, maximum polyacetylene concentration occurred 28 days after reflooding. Better yields of polyacetylenes were obtained from refloods; three consecutive refloods could be used.

Isolation of Metabolites.—The culture medium (45 l; 60 flasks) was continuously extracted with Et₂O for 48 h. The extract was concentrated to 300 ml and separated with saturated NaHCO₃ solution into acidic and neutral fractions, and each fraction was analysed separately.

Acidic Fraction.—The concentrated fraction was esterified with 4% H₂SO₄ in MeOH and the resulting oil (400 mg) was separated by p.l.c. (Et₂O) into four bands (A–D in order of increasing polarity). Band C (*R_F* 0.5) yielded *methyl 9-hydroxynona-2,4,6-triynoate* (II; R = Me) (60 mg) (Found: *M*⁺, 176.0473. C₁₀H₈O₃ requires *M*, 176.0473), λ_{max.} (EtOH) 324 (ε 9700), 308 (15,100), 289 (12,600), 272.5 (8000), 258 (4800), 227 (86,000), and 218 (71,000) nm, ν_{max.} (CCl₄) 3645, 3500 (OH), 2210 (C≡C), and 1725 (C=O)

⁴ R. Singer, 'The Agaricales in Modern Taxonomy,' J. Cramer, Weinheim, 1962.

⁵ K. Bowden, I. M. Heilbron, E. R. H. Jones, and K. H. Sargent, *J. Chem. Soc.*, 1947, 1579.

⁶ R. K. Bentley, Sir Ewart R. H. Jones, R. A. M. Ross, and V. Thaller, *J.C.S. Perkin I*, 1973, 140.

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

cm^{-1} , τ (CCl_4) 8.0br (OH), 7.46 (t, J 7 Hz, $\text{C}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), 6.27 (t, J 7 Hz, $\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), and 6.24 (s, $\text{CO}_2\cdot\text{CH}_3$), m/e 176 (M^+ , 100%), 146 (95), 145 (48), 115 (53), 114 (75), and 86 (59). The least polar band A (R_F 0.8) gave dimethyl fumarate (174.5 mg), plates (from Et_2O), m.p. and mixed m.p. 101–102°. Band B showed weak absorption at λ_{max} (Et_2O) 280, 265, and 250 nm, and band D showed absorption at λ_{max} (Et_2O) 210 nm.

Neutral Fraction.—The concentrated fraction (orange oil) was chromatographed on a SiO_2 column (150 g) with Et_2O , and nine 100 ml fractions were collected. Fractions 2 and 3 were combined, concentrated, and purified by p.l.c. (Et_2O); the major zone (R_F 0.7; an enediyne contaminated by a trace of a triyne) gave on further p.l.c. purification (petrol– Et_2O , 1:1; continuous elution for 2 h) a band which yielded *non-cis* and *trans-3-ene-5,7-diyn-1-ol* (III) (6.5 mg) (Found: M^+ , 134.0729. $\text{C}_9\text{H}_{10}\text{O}$ requires M , 134.0732), λ_{max} (EtOH) 281.5 (ϵ 10,800), 265.5 (13,400), 251.5 (9100), 238.5 (4300), 227 (2400), and 212 (34,200) nm, ν_{max} (CCl_4) 3630, 3400 (OH), 3030 ($\text{CH}=\text{CH}$), 2230 ($\text{C}\equiv\text{C}$), and 955 (*trans*- $\text{CH}=\text{CH}$) cm^{-1} , ν_{max} (CS_2) 3625, 3480 (OH), 3018 ($\text{CH}=\text{CH}$), 2235 ($\text{C}\equiv\text{C}$), 950 (*trans*- $\text{CH}=\text{CH}$), and 788 (*cis*- $\text{CH}=\text{CH}$) cm^{-1} , τ (CCl_4) 8.18br (OH), 8.02 (s, $\text{CH}_3\cdot\text{C}\equiv\text{C}$), 7.50 (m, $\text{CH}=\text{CH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), 6.36 (t, J 7 Hz, $\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), 4.46 (m, $\text{CH}=\text{CH}\cdot\text{CH}_2$), and 3.87 (m, $\text{C}\equiv\text{C}\cdot\text{CH}=\text{CH}$), m/e 134 (M^+ , 90%), 103 (80), 102 (64), 78 (75), 77 (100), 75 (63), and 63 (59). G.l.c. analysis (72.5°) showed *non-cis-3-ene-5,7-diyn-1-ol* (t_R 3.2 min) and *non-trans-3-ene-5,7-diyn-1-ol* (3.8 min) to be present in a 3:1 ratio (the peak allocation of the two isomers was made tentatively on the basis of the relative intensities of the 788 and 950 cm^{-1} peaks in the i.r. spectrum of the *cis-trans*-mixture, which suggested a predominance of the *cis*-isomer).

Fractions 4–7 were combined, concentrated, and separated by p.l.c. (Et_2O): two polyacetylene bands (A, R_F 0.6, and B, 0.5) were obtained. Band A yielded on further p.l.c. (petrol– Me_2CO , 9:1) *nona-3,5,7-triyn-1-ol* (IV) (79 mg), plates (from CCl_4 -petrol), which rapidly turned purple on exposure to light, m.p. 63.5–64° (decomp.) (Found: C, 80.5; H, 5.6. $\text{C}_9\text{H}_8\text{O}$ requires C, 81.8; H, 6.1%), λ_{max} (EtOH) 307.5 (ϵ 100), 290inf (165), 282.5 (275), 267 (330), 252.5 (235), 240 (125), and 209.5 (100,000) nm, ν_{max} (CCl_4) 3628, 3620, 3495 (OH), and 2226 ($\text{C}\equiv\text{C}$), τ (CCl_4) 8.10br (OH), 8.04 (s, $\text{CH}_3\cdot\text{C}\equiv\text{C}$), 7.50 (t, J 7 Hz, $\text{C}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), and 6.31 (t, J 7 Hz, $\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), m/e 132 (M^+ , 100%), 102 (97), 76 (64), 75 (97), 74 (86), and 59 (95). Band B was further separated by p.l.c. (petrol– Et_2O , 1:1; 3 elutions) into one major (R_F 0.6) and two minor bands, one running just in front and the other at the tail of the main fraction. This yielded (–)-*nona-3,5,7-triyn-1,2-diol* (V) (25 mg), plates (from CH_2Cl_2 at –40°), m.p. 127.5–128.5° (Found: M^+ , 148.053. $\text{C}_9\text{H}_8\text{O}_2$ requires M , 148.052), $[\alpha]_D^{20}$ –40 (589 nm), –41 (578), –47 (546), –83 (436), and –149° (365) (c 0.347 in Et_2O), λ_{max} (EtOH) 308.5 (ϵ 205), 305.5 (200), 295.5 (190), 289 (320), 286 (340), 278 (280), 272inf (310), 267.5 (350), 262.5inf (255), 252.5 (270), 239.5 (200), and 208.5 (127,000) nm, ν_{max} (CHCl_3) 3480, 3600 (OH), and 2242 ($\text{C}\equiv\text{C}$) cm^{-1} , τ (CDCl_3) 8.46 (s, $\text{C}\equiv\text{C}\cdot\text{CH}\cdot\text{OH}$; disappears on addition of D_2O), 8.03 (s, $\text{CH}_3\cdot\text{C}\equiv\text{C}$), 7.61 (d, J 6 Hz, $\text{CH}_2\cdot\text{OH}$; disappears on addition of D_2O), 6.30 (dd, J 6 and 6 Hz, $\text{HO}\cdot\text{CH}\cdot\text{CH}_2\cdot\text{OH}$; collapses on addition of D_2O to a

doublet, J 6 Hz), and 5.48 [t, J 6 Hz, $\text{C}\equiv\text{C}\cdot\text{CH}(\text{OH})\cdot\text{CH}_2$], m/e 148 (M^+ , 30%), 130 (36), 117 (100), 102 (20), 89 (25), 63 (78), and 62 (25). The less polar band yielded brown plates (CH_2Cl_2 - CCl_4) which decomposed without melting above 70°, λ_{max} 210 nm, ν_{max} (CHCl_3) 3625 and 3455 (OH) and 2265 ($\text{C}\equiv\text{C}$) cm^{-1} , τ (CDCl_3) 7.50 (2H, dt, J 7 and 2 Hz), 6.86br (3H, s, disappeared on D_2O addition), 6.24 (2H, t, J 7 Hz), and 4.85 (1H, t, J 2 Hz); the triplet signals at τ 7.50 and 4.85 sharpened considerably on D_2O addition. The extract from the more polar band had λ_{max} (Et_2O) 300, 282.5, 267, 252, and 244 nm.

Fraction 8 gave on p.l.c. (Et_2O) two polyacetylene bands with R_F 0.3 and 0.2, of which the R_F 0.2 band yielded on further p.l.c. (CHCl_3 ; continuous elution for 5 h) octa-3,5-diyne-1,8-diol (VI) (13 mg), plates (from Et_2O), which rapidly turned red in light, m.p. and mixed m.p. 41.5–43° (lit.,⁵ 42–43°), τ (CCl_4) 8.30br (OH), 7.44 (t, J 7 Hz, $\text{C}\equiv\text{C}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), and 6.23 (t, J 7 Hz, $\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$), m/e 138 (M^+ , 18%), 107 (55), 79 (50), 78 (46), 77 (77), and 74 (100).

Methyl 9-Hydroxynona-2,4,6-triynoate (II; $R = \text{Me}$).—Penta-2,4-diyn-1-ol⁷ (1.6 g, 20 mmol) and NaOBr [20 ml; prepared from Br_2 (11 ml), water (50 ml), ice (100 g), and NaOH (20 g)] were shaken for 2 h. Addition of saturated NH_4Cl solution, extraction with Et_2O (3×20 ml), and drying (MgSO_4) and concentration of the extract yielded 5-bromopenta-2,4-diyn-1-ol (3.0 g, 94%), a white solid which quickly turned pink in light, m.p. 45–46°. This (1.5 g, 9.5 mmol) in $\text{AnalaR Me}_2\text{CO}$ (15 ml) was cooled in ice-water whilst chromic acid (8N; 5 ml) was added dropwise, with stirring, over 10 min. Stirring was continued at 20° (15 min), the mixture was poured into H_2O (50 ml) and extracted with Et_2O (3×50 ml), and the extracts were washed with NaHCO_3 solution (25 ml) and brine (25 ml), dried (MgSO_4), and concentrated to yield 5-bromopenta-2,4-diyne-1-ol⁸ (435 mg, 23%), λ_{max} (Et_2O) 268 (rel. E 0.68), 253.5 (1.0), 241 (0.98), 224.5 (0.68), and 218 (0.55) nm, ν_{max} (CCl_4) 3400 (OH), 2180 ($\text{C}\equiv\text{C}$), and 1733 ($\text{C}=\text{O}$) cm^{-1} . The bromo-acid (262 mg, 1.5 mmol) was methylated in 4% H_2SO_4 - MeOH for 60 h. Usual work-up yielded methyl 5-bromopenta-2,4-diyne-1-ol (164 mg, 58%), ν_{max} (CCl_4) 2220 ($\text{C}\equiv\text{C}$) and 1720 ($\text{C}=\text{O}$) cm^{-1} , τ (CCl_4) 6.26 (s, CO_2CH_3).

But-3-yn-1-ol (62 mg, 0.9 mmol) in MeOH (2 ml) was added to CuCl (6 mg), EtNH_2 (0.3 ml), and $\text{NH}_2\text{OH}\cdot\text{HCl}$ (70 mg) stirred in MeOH (2 ml) at 20° (pale yellow solution). After 10 min, methyl 5-bromopenta-2,4-diyne-1-ol in MeOH (2 ml) was added to the mixture and stirring was continued for 2 h. Usual work-up and p.l.c. (Et_2O) gave methyl 9-hydroxynona-2,4,6-triynoate (II; $R = \text{Me}$) (17 mg, 11%), identical with the natural sample.

Non-cis and trans-3-ene-5,7-diyn-1-ol (III).—To CuCl (60 mg), $\text{NH}_2\text{OH}\cdot\text{HCl}$ (2.1 g), and EtNH_2 (20 ml) stirred at 20°, 3,3-diethoxyprop-1-yne⁹ (1.92 g, 15 mmol) in MeOH (15 ml) was added, followed after 1 h by 1-bromoprop-1-yne¹⁰ (1.785 g, 15 mmol) in MeOH (40 ml); stirring was continued for 3 h. Usual work-up gave 1,1-diethoxyhexa-2,4-diyne (1.67 g, 67%), ν_{max} (CCl_4) 2260 and 2160 ($\text{C}\equiv\text{C}$) cm^{-1} , τ (CCl_4) 8.82 (t, J 7 Hz, $\text{CH}_3\cdot\text{CH}_2\cdot\text{O} \times 2$), 8.06 (s, $\text{CH}_3\cdot\text{C}\equiv\text{C}$), 6.44 (m, $\text{O}\cdot\text{CH}_2\cdot\text{CH}_3 \times 2$), and 4.89 (s, $\text{C}\equiv\text{C}\cdot\text{CH}$). This (500 mg, 3.0 mmol) and HCl (2N; 10 ml) were shaken for 15 min, Et_2O (10 ml) was added, the mixture was shaken again, and the organic layer was separated with more

⁷ E. R. H. Jones, J. M. Thompson, and M. C. Whiting, *J. Chem. Soc.*, 1957, 2012.

⁸ F. Bohlmann, W. Sucrow, and I. Queck, *Chem. Ber.*, 1964, **97**, 2586.

⁹ J. P. Ward and D. A. van Dorp, *Rec. Trav. chim.*, 1967, **86**, 545.

¹⁰ W. Chodkiewicz, *Ann. Chim. (France)*, 1957, **2**, 819.

Et₂O, washed with NaHCO₃ solution, dried (MgSO₄), and concentrated to 10 ml. The hexa-2,4-diynal solution, λ_{max} (EtOH) 285.5 (rel. *E* 0.8), 269.5 (1.0), 255.5 (0.75), and 243 (0.50) nm, was added directly (dropwise) to the deep orange phosphorane solution prepared from 1-hydroxypropyltriphenylphosphonium iodide¹¹ [this (2.02 g, 4.5 mmol) stirred at 20° under N₂ in dry Et₂O (35 ml) was treated dropwise with BuⁿLi (2.34N; 3.84 ml) in hexane and stirring was continued for 45 min]. After 45 min, dil. HCl was added and the organic layer was separated, dried (MgSO₄), and concentrated. P.l.c. (Et₂O; 1 h) gave non-*cis*- and -*trans*-3-ene-5,7-diyn-1-ols (III) (54 mg, 13%; 55% *trans* by g.l.c. analysis), identical with the natural isomer mixture except for the *cis* : *trans* ratio.

Nona-3,5,7-triyn-1-ol (IV).—A solution of hexa-2,4-diynal [from 125 mg of acetal (0.75 mmol) prepared as above] was evaporated almost to dryness, the residue was immediately dissolved in MeOH (4 ml), aqueous KOH [4 ml; prepared from KOH (1.6 g) and H₂O (10 ml)] was added, and the solution was stirred at 50° for 12 min. Et₂O (10 ml) was added to the cooled mixture, the organic layer was separated, and the aqueous layer was washed with Et₂O (5 ml). The combined Et₂O extracts were added to CuCl (7 mg), NH₂OH.HCl (75 mg), and EtNH₂ (1 ml) stirred in MeOH (2 ml) (yellow solution) and were followed after 5 min by 4-bromobut-3-yn-1-ol (109 mg, 0.75 mmol) in Et₂O (3 ml); stirring was continued for 1 h. Usual work-up and p.l.c. (petrol-Et₂O, 1 : 4) gave nona-3,5,7-triyn-1-ol (IV) (52 mg,

52%) m.p. and mixed m.p. 63–64°, identical with the natural product.

(±)-*Nona-3,5,7-triyn-1,2-diol* (V).—Penta-1,3-diyne [prepared as above from 1,1-diethoxyhexa-2,4-diyne (250 mg)] was shaken with an excess of NaOBr [3 ml; prepared from Br₂ (11 ml), H₂O (50 ml), ice (100 g), and NaOH (20 g)] for 2 h. Addition of saturated NH₄Cl solution, extraction with Et₂O (3 × 10 ml), drying (MgSO₄), and concentration to ca. 10 ml gave an Et₂O solution of 1-bromopenta-1,3-diyne.¹⁰ A sample was further concentrated and gave only a singlet at τ (CCl₄) 8.1 in the n.m.r. spectrum.

But-3-yne-1,2-diol¹² (13 mg, 0.15 mmol) in MeOH (1 ml) was added to CuCl (2 mg), NH₂OH.HCl (20 mg) and EtNH₂ (0.2 ml) stirred in MeOH (0.5 ml) at 20°. After 5 min, 1-bromopenta-1,3-diyne in Et₂O (1 ml, 0.15 mmol) was added and stirring was continued for 1 h. Usual work-up and p.l.c. gave needles (from CH₂Cl₂ at –40°) of (±)-nona-3,5,7-triyn-1,2-diol (V) (5 mg, 22.5%), m.p. 82–83°; the spectra were identical with those of the natural compound.

We thank the S.R.C. for a studentship (C. A. H.) and research grant support.

[4/660 Received, 1st April, 1974]

¹¹ D. Bhattacharjee, D.Phil. Thesis, Oxford, 1968.

¹² Sir Ewart R. H. Jones, G. Lowe, and B. E. Lowe, *J. Chem. Soc.*, 1964, 1476