

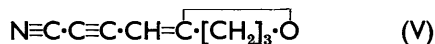
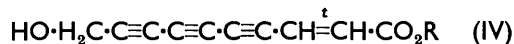
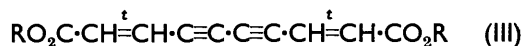
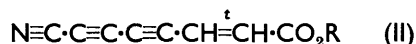
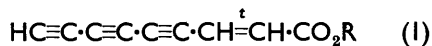
Natural Acetylenes. Part XLVI.¹ Polyacetylenes from Cultures of the Fungus *Lepista glaucocana* (Bres.) Singer

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The new acetylenic nitrile $\text{N}\equiv\text{C}\cdot\text{C}\equiv\text{C}\cdot\text{CH}=\overline{\text{C}}\cdot[\text{CH}_2]_3\cdot\text{O}$ has been isolated from the culture fluids of *L. glaucocana*. It is accompanied by four known acetylenic acids already found to be produced by *L. diemii*.

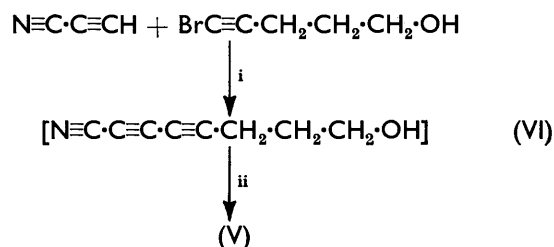
THE occurrence of the polyacetylenes (I)—(IV) ($\text{R} = \text{H}$) in the culture fluid of *Lepista diemii* Singer was reported recently.² Another member of the genus, *L. glaucocana* (Bres.) Singer, has now been investigated and found to produce the same four acetylenic acids, with the nitrile acid (II; $\text{R} = \text{H}$) and the hydroxy-acid (IV; $\text{R} = \text{H}$) again as the major constituents. In addition, the nitrile enol ether (V) was isolated from the neutral fraction of the shake-culture extract (it was not detected in the surface culture). The ethylenic proton signal at τ 5.58 indicates the *Z*-configuration for the enol ether double bond.³

A very polar aromatic aldehyde and some acids, probably terpenic, also detected in the culture extract, are being further investigated.



The enol ether (V) was synthesised by the route indicated [the coupling product (VI) was not isolated] in poor yield. The major reaction product was 5-aminoisoxazole.⁴ The formation of heterocycles under Chodkiewicz coupling conditions might account for the poor

yields often obtained with propiolic acid derivatives, e.g. the amide.⁵



Reagents: i, $\text{CuCl}\cdot\text{NH}_2\text{OH}\cdot\text{EtNH}_2$, ii, $\text{NaOH}(\text{H}_2\text{O})$

The enol ether (V) is the second acetylenic nitrile found in nature. It is most likely derived in the fungal culture from the nitrile acid (II; $\text{R} = \text{H}$) by reduction to, and cyclisation of the alcohol (VI). Biosynthetic experiments aimed at discovering the link between the two nitriles are under way.

EXPERIMENTAL

For general techniques see Part XLI.⁶

Growth of Lepista glaucocana.—The fungus was grown on 3% malt extract until maximum polyacetylene concentrations were reached (6–10 days in shaken cultures and 33–35 days in surface cultures).

Isolation of the Metabolites.—A variation in metabolite concentrations similar to that previously observed with *L. diemii*² was found under the different growth conditions. The isolation of metabolites from a shaken culture grown on malt extract illustrates the generally used procedure. No neutral polyacetylenes were detected in the surface cultures.

The culture medium (75 l; 96 flasks) was continuously extracted with Et_2O (48 h). The extract was concentrated

¹ Part XLV, C. A. Higham, Sir Ewart R. H. Jones, J. W. Keeping, and V. Thaller, *J.C.S. Perkin I*, 1974, 1991.

² V. Thaller and J. L. Turner, *J.C.S. Perkin I*, 1972, 2032.

³ F. Bohlmann, C. Arndt, and J. Starnick, *Tetrahedron Letters*, 1963, 1605.

⁴ I. Iwai and N. Nakamura, *Chem. and Pharm. Bull. (Japan)*, 1966, 14, 1277.

⁵ 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.

⁶ I. W. Farrell, J. W. Keeping, M. G. Pellatt, and V. Thaller, *J.C.S. Perkin I*, 1973, 2642.

to ca. 200 ml and separated into a neutral and an acidic fraction with sat. aqueous NaHCO_3 .

Acid Fraction.—The concentrated acid fraction was esterified ($\text{MeOH-H}_2\text{SO}_4$) and the methyl esters (1.95 g) were separated on an SiO_2 column (150 g) by stepwise elution (petrol to 30% $\text{MeOH-Et}_2\text{O}$). Fractions of similar composition were combined and subjected to repeated p.l.c. (petrol- Et_2O , 9:1; continuous elution). The following polyacetylenes, in order of increasing polarity, were isolated: methyl non-*trans*-2-ene-4,6,8-triynoate (I; R = Me) (10.2 mg), m.p. 55–60° (decomp.) [lit.,² 60° (decomp.)], methyl 7-cyanohept-*trans*-2-ene-4,6-diynoate (II; R = Me) (383 mg), m.p. 102–103° (lit.,² 102–103.5°), dimethyl deca-*trans*-2,*trans*-8-diene-4,6-diynedioate (III; R = Me) (13.7 mg), m.p. 104–106° (lit.,² 105–107°), and methyl 10-hydroxydec-*trans*-2-ene-4,6,8-triynoate (IV; R = Me) (540 mg), m.p. 114–117° (lit.,² 115–116°); u.v., i.r., n.m.r., and mass spectra were identical with those of authentic specimens. Intermediate between the cyano-ester (II; R = Me) and the diester (III; R = Me) fraction occurred a fraction (200 mg) possibly containing three terpene esters; these are being investigated.

Neutral Fraction.—This (yellow oil; 920 mg) absorbed strongly near 275 nm in the u.v. Repeated p.l.c. (petrol- Et_2O , 4:1; four elutions) yielded a band (R_F ca. 0.5) which gave prisms (Et_2O -petrol) of 4-(*tetrahydro-2-furylidene*)but-2-ynenitrile (V) (71 mg), m.p. 48–49° (M^+ , 133.0529. $\text{C}_8\text{H}_7\text{NO}$ requires M , 133.0528), λ_{max} (EtOH) 286sh (ϵ 26,000), 277.5 (32,500), and 266.5sh (24,500) nm, ν_{max} (CCl_4) 2250 and 2135 ($\text{C}\equiv\text{N}$ and $\text{C}\equiv\text{C}$) and 1650 ($\text{C}=\text{C}$) cm^{-1} , τ (CCl_4) 7.87 (2H, quintet, J 6 Hz, $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$), 7.27 (2H, t,

J 6 Hz, $\text{CH}=\text{C-CH}_2\text{-CH}_2$), 5.65 (2H, t, J 6 Hz, $\text{CH}_2\text{-CH}_2\text{-O}$), and 5.58 (1H, s, $\text{C}\equiv\text{C-CH}=\text{C}$), m/e 133 (95%), 104 (7), 91 (100), 76 (8), and 63 (38).

A more polar band (R_F 0.2) yielded a liquid, R_F 0.15 (petrol- Et_2O , 4:1), λ_{max} (Et_2O) 279 and 225sh nm, ν_{max} (CHCl_3) 3600 and 3350 (free and bonded OH), 1680 (arom. CO), 1520, and 1020 cm^{-1} , τ ($\text{CCl}_4\text{-CD}_3\text{OD}$) 5.80br (2H), 5.43 (2H, s), 3.55 (1H, d, J 3.5 Hz), 2.79 (1H, d, J 3.5 Hz), and 0.54 (1H, s, CHO).

(*Z*)-4-(*Tetrahydro-2-furylidene*)but-2-ynenitrile (V).—Propiolonitrile (510 mg, 10 mmol) in MeOH (5 ml) was added dropwise to CuCl (100 mg)-aq. EtNH_2 (2.5 ml; 30%)– $\text{H}_2\text{NOH.HCl}$ (1.0 g) stirred in MeOH (5 ml) under N_2 . After 5 min, 5-bromopent-4-yn-1-ol⁷ (1.52 g, 10 mmol) in MeOH (5 ml) was added over 5 min. The mixture was stirred at 0° for 90 min, KCN (1.0 g)-ice (25 g)- H_2O (50 ml) was added and the products were extracted with Et_2O (4 × 50 ml). The residue (1.2 g) was stirred with NaOH (0.1N; 50 ml) for 4 h. Acidification and Et_2O extraction gave a yellow oil (1.1 g) which on p.l.c. yielded the enol nitrile (V) (120 mg, 9%), m.p. and mixed m.p. 47–49°; spectra identical with those of the natural product. A more polar major fraction (510 mg) yielded 5-aminoisoxazole, m.p. 74–76° (Et_2O -petrol) (lit.,⁴ 75–77°) (Found: C, 42.7; H, 5.1; N, 33.45. Calc. for $\text{C}_3\text{H}_4\text{N}_2\text{O}$: C, 42.85; H, 4.8; N, 33.3%); u.v., i.r., and n.m.r. spectra identical with those reported; m/e 84 (M^+ , 100%), 68 (49), 44 (76), 41 (89), 40 (70), 30 (71), and 29 (89).

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