Natural Acetylenes. Part 52.[1] Polyacetylenic Acids and Aromatic Aldehydes from Cultures of the Fungus *Camarophyllus virgineus* (Wulfen ex Fr.) Kummer

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The new polyacetylene $HO{\cdot}H_2C{\cdot}[C{\equiv}C]_2{\cdot}CH{=}CH{\cdot}CO_2H$ (*t*) and *p*-(methylamino)benzaldehyde, not found previously as a fungal metabolite, have been detected together with known natural polyacetylenes in extracts from culture fluids of *C. virgineus*. Hygrophoraceae species have not been reported previously as polyacetylene producers. The synthesis of some of the polyacetylenes is described.

HYGROPHORACEAE species have not been reported previously as polyacetylene producers. *Camarophyllus virgineus* has now been found to contain the polyacetylenic acids (1)—(5) (R = H) and the benzenoid aldehydes (6) and (7). Of these the C_8 hydroxy-acid (2; R = H) is a new compound, and both it and the aldehyde (7) have not been found before as fungal metabolites.

The C_8 nitrile acid (1; R = H) and the C_{10} hydroxy-acid (3; R = H), conventionally called diatretyne 2 and 3, respectively, appear to be restricted to Tricholomataceae species (as delimited by Singer), but there is one instance of diatretyne 3 occurring in one Coprinaceae species.[2] The appearance of the two diatretynes (1) and (3) (R = H) in Hygrophoraceae species, especially if this turns out to be widespread, might be taken as yet another indication of a close phylogenetic relationship between the Hygrophoraceae and the Tricholomataceae (*cf.* ref. 3). The co-occurrence of the acids (1), (3), and (4) (R = H) has already been reported in *Lepista diemii* (Tricholomataceae, tribus *Clytocybeae*) cultures.[4]

The esterified acid fraction was separated by chromatography and the esters were identified by their spectra and comparison with authentic specimens, some synthesised for this purpose. Other polyacetylenes were undoubtedly present but were not identified.

$$NC{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (1)$$

$$HO{\cdot}H_2C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (2)$$

$$HO{\cdot}H_2C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (3)$$

$$RO_2C{\cdot}CH{=}^{t}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CO_2R \quad (4)$$

$$HO{\cdot}H_2C{\cdot}CH{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}CH{\cdot}CO_2R \quad (5)$$

$$p\text{-}MeO{\cdot}C_6H_4{\cdot}CHO \quad (6)$$

$$p\text{-}MeNH{\cdot}C_6H_4{\cdot}CHO \quad (7)$$

The ester (2; R = Me) was amongst the most polar esters present. It was synthesised by two routes (Scheme 1). The *cis,trans*-isomer mixture formed in the Wittig reaction (*cis* : *trans* 1 : 2.5) was separated by t.l.c., the combined yield was 70%. The yield of the *trans*-isomer by the alternative route was 62%.

The acid (2; R = H) is one of the comparatively

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[3] R. Singer, 'The Agaricales in Modern Taxonomy,' J. Cramer, Weinheim, 1962, p. 204.

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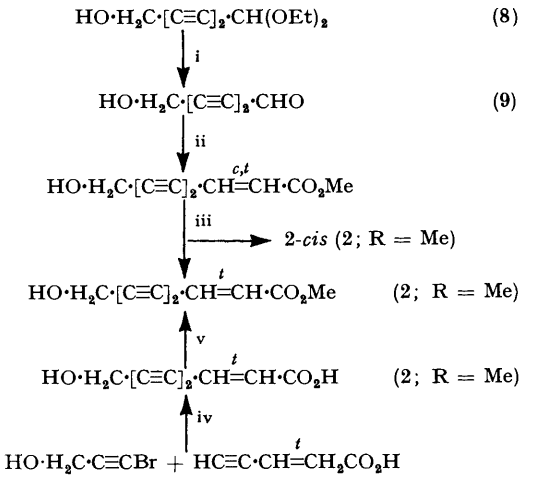
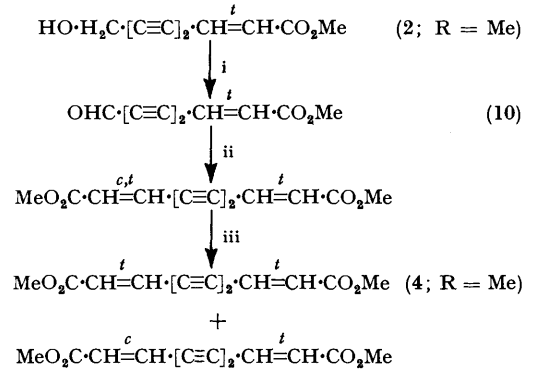
small group of C_8 polyacetylenes. Their biogenesis has been the subject of recent investigations and two pathways seem to be operative: in one [5] carbon atoms 11—18 and in the other [6] carbon atoms 9—16 of the C_{18} precursor chain form the polyacetylene carbon skeletons.



SCHEME 1 Reagents: i, 2N-HCl; ii, $Ph_3P{=}CH{\cdot}CO_2Me$; iii, t.l.c.; iv, CuCl, NH_2OH,HCl, $EtNH_2$; v, MeOH–H_2SO_4

The co-occurrence of the hydroxy-acid (2; R = H) and the nitrile-acid (1; R = H) in *C. virgineus* cultures, and their considerable structural similarity, makes it tempting to postulate for both metabolites analogous carbon skeleton derivations, *i.e.* retention of C(9)—(16) as demonstrated for diatretyne 2 (1; R = H).[6]

The diester (4; R = Me) was synthesised by a route (Scheme 2) different from that described [7] earlier. Both



SCHEME 2 Reagents: i, MnO_2; ii, $Ph_3P{=}CH{\cdot}CO_2Me$; iii, t.l.c.

the *trans,trans*- and *cis,trans*-isomers, the latter not described before were obtained, in a 2 : 1 ratio, the combined yield being 74%.

The hydroxy-ester (5; R = Me) was not obtained pure, but u.v. and mass spectra leave no doubt about its structure. Its isomerisation, as demonstrated by chromatography on silica gel, suggests that it is a mixture of the three possible isomers containing α *cis*-double bonds; the pure synthetic *trans,trans*-isomer was in our experience stable when subjected to the same treatment.[8]

Repeated chromatography of the neutral fraction gave anisaldehyde (6) and the *N*-methylamino-aldehyde (7). The latter was synthesised for comparison from *N*-methylformanilide [9] and converted into the *N*-nitroso-derivative (11), a known fungal metabolite [from *Clitocybsuaveoleus* (Schum. ex Fr., Quel. 404) [10]]. The *N*-nitroso-compound (11) was subjected to the extraction and separation procedure used to isolate the *N*-methylamino-aldehyde (7) from *C. virgineus* cultures. This treatment failed to produce detectable amounts of the aldehyde (7), which cannot therefore be an artefact formed from the nitrosamine (11) during isolation.

$$p\text{-Me(NO)N}{\cdot}C_6H_4{\cdot}CHO \qquad (11)$$

EXPERIMENTAL

For general techniques see Part 41.[8]

Growth of Camarophyllus virgineus *and Isolation of the Metabolites.*—The fungus was grown as a surface culture on 3% malt extract for 45 days, at which point maximum polyacetylene concentration was reached.

The culture medium (18 l; 24 flasks) was continuously extracted with Et_2O (48 h). The extract was concentrated to *ca.* 200 ml and separated into a neutral and acidic fraction with saturated aqueous $NaHCO_3$.

Acid Fraction.—The concentrated acid fraction was esterified (MeOH–H_2SO_4, 24 : 1) and the methyl esters (530 mg) were separated on 1 mm layers (p.l.c.) (petrol–Et_2O, 1 : 1; continuous elution for 2 h) into eight fractions (A—H). The least polar fraction A (140 mg) was rechromatographed (petrol–Et_2O, 3 : 1) and yielded a sweet-smelling oil which solidified at −40 °C (it showed no u.v. absorption and no n.m.r. bands below τ 8) and the nitrile ester (1; R = Me).

Fraction B was a mixture (80 mg) of the cyano-ester (1; R = Me)[4] and the diester (4; R = Me).[4]

Rechromatography of fraction C (125 mg) gave an aromatic oil (24 mg), $\lambda_{max.}$ 305 nm, and an unidentified liquid enediyne ester (30 mg), $\lambda_{max.}$ (Et_2O) 303, 285, 269, 255, 241, and 223 nm, $\nu_{max.}$ ($CHCl_3$) 3 520, 2 215, 1 725, and 910 cm^{-1}.

Fraction D (50 mg) yielded the hydroxy-ester (3; R = Me)[4] and what appeared to be a trace of its *cis*-isomer, $\lambda_{max.}$ (Et_2O) 346, 323, 303, 285, 257, and 240 nm.

Fraction E (5 mg) yielded methyl 8-hydroxyoct-*trans*-2-ene-4,6-diynoate (2; R = Me), identical with a synthetic specimen (see below).

Fraction F (10 mg) contained the hydroxy-ester (5; R = Me) (*cf.* ref. 8); repeated chromatography gave three bands which in order of increasing polarity, had $\lambda_{max.}$ (Et_2O) 332.5 (rel.*E* 1.0), 311.5 (1.15), 290infl (0.85), 258.5 (1.50), and 246 nm (1.60), 333 (rel.*E* 1.0), 312 (1.5), 293infl (0.86), 258.5 (1.41), and 246 nm (1.57), and 334 (rel.*E* 1.0), 312.5 (1.21), 295infl (0.96), 259 (1.86), and 247 nm (2.0), *m/e* (all three

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fractions combined) 190 (M^+, 42%), 175 (10), 159 (13), 147 (18), 97 (37), 95 (45), and 43 (100).

Fraction G (20 mg), R_F 0.35 (Et_2O), $\lambda_{max.}$ (Et_2O) 305, 286, 270, 255, 223, and 215 nm, $\nu_{max.}$ (CCl_4) 1 725 and 955 cm^{-1}, and fraction H (10 mg), $\lambda_{max.}$ (Et_2O) 303.5, 285, 269, 250, 223, and 215 nm, $\nu_{max.}$ (CS_2), 1 725 and 955 cm^{-1}, R_F 0.30 (Et_2O), could not be purified sufficiently for further investigation.

Neutral Fraction.—The concentrated neutral fraction, a reddish-brown liquid (275 mg) was separated by p.l.c. into two major components and each was further purified. The less polar one yielded anisaldehyde (6) (10 mg). The more polar component gave *p*-(methylamino)benzaldehyde (7) (27 mg), identical with a synthetic specimen.

Synthesis of Methyl 8-*Hydroxyoct*-trans-2-*ene*-4,6-*diynoate* (2; R = Me) *and its* cis-*isomer*.—6,6-Diethoxyhexa-2,4-diyn-1-ol (8) [11] (190 mg, 1.04 mmol) and HCl (2N; 25 ml) were shaken for 15 min. Isolation with Et_2O gave the crude aldehyde (9) (102 mg, 0.94 mmol, 90%), an unstable oil, $\lambda_{max.}$ (Et_2O) 285 (rel.*E* 2.22), 269.5 (2.81), 255 (1.77), and 243 (1.0) nm. This was immediately dissolved in CH_2Cl_2 (10 ml) and added dropwise to $Ph_3P{=}CH{\cdot}CO_2Me$ [12] (270 mg, 0.8 mmol) stirred in CH_2Cl_2 (15 ml) at −15 °C. Stirring was continued for 0.5 h at −15 °C and 0.5 h at 20 °C. Concentration of the mixture and p.l.c. of the residue (petrol–Et_2O, 1 : 1; 2 elutions) gave two fractions: the less polar (R_F 0.55) gave on crystallisation (CCl_4–petrol) the trans-*hydroxy-ester* (2; R = Me) (66 mg, 45%), m.p. 80—82° (Found: C, 66.2; H, 5.1. $C_9H_8O_3$ requires C, 65.85; H, 4.9%), $\lambda_{max.}$ (EtOH) 301 (ε 19 600), 283 (20 600), 268 (11 800), 255 (5 150), 243 (2 500), 223 (33 400), and 215 (27 500) nm, $\nu_{max.}$ (CCl_4) 3 618, 3 500, 2 240, 2 150, 1 730, 1 617, and 960 cm^{-1}, τ ($CDCl_3$) 7.9br (OH), 6.21 (s, $CO_2{\cdot}CH_3$), 5.58 (d, *J* 1 Hz, $CH_2{\cdot}OH$), 3.64 (d, *J* 16 Hz, *trans*-CH=C*H*·CO_2Me), and 3.16 (dt, *J* 16 and 1 Hz, *trans*-C*H*=CH·CO_2Me), *m/e* 164 (M^+, 51%), 149 (34), 135 (28), 133 (26), 121 (56), 105 (30), 77 (66), and 65 (100). Crystallisation (CS_2) of the more polar fraction (R_F 0.4) gave the *cis-isomer* (26 mg, 17.5%), m.p. 49—50° (Found: C, 65.9; H, 4.8%), $\lambda_{max.}$ (EtOH) 304 (ε 12 400), 286 (13,600), 270.5 (8 150), 257infl (4 050), 241 (1 950), 224.5 (25 000), and 216.5 nm (21 900), $\nu_{max.}$ (CCl_4) 3 620, 3 500, 2 240, 2 180, 2 150, 1 730, 1 720, and 1 610 cm^{-1}, $\nu_{max.}$ (CS_2) 815 cm^{-1}, τ ($CDCl_3$) 7.74br (OH), 6.20 (s, $CO_2{\cdot}CH_3$), 5.55 (d, *J* 1 Hz, $CH_2{\cdot}OH$), 3.80 (dd, *J* 11 and 1 Hz, *cis*-C*H*=CH·CO_2Me), and 3.68 (d, *J* 11 Hz, *cis*-CH=C*H*·CO_2Me), *m/e* 164 (M^+, 100%), 149 (30), 135 (39), 133 (40), 121 (61), 105 (21), 75 (35), and 65 (69).

Pent-*trans*-2-en-4-ynoic acid (500 mg, 5 mmol) was coupled with 3-bromoprop-2-yn-1-ol (635 mg, 5 mmol); the resulting acid yielded on esterification the *trans*-hydroxy-ester (2; R = Me) (573 mg, 62%).

Synthesis of Dimethyl Deca-trans-2,trans-8-*diene*-4,6-*diyne*-1,10-*dioate* (4; R = Me) *and Dimethyl Deca*-cis-2,-trans-8-*diene*-4,6-*diyne*-1,10-*dioate* [2-cis- (4; R = Me)].—The hydroxy-ester (2; R = Me) (72 mg, 0.44 mmol) and MnO_2 (720 mg) were shaken in CH_2Cl_2 (20 ml) for 3 h. The resulting crude aldehyde ester (10) was an unstable liquid (62 mg, 0.38 mmol, 86%), R_F 0.43 (petrol–Et_2O, 2 : 1), $\lambda_{max.}$ (Et_2O) 318, 299, 282, 267, 245, and 236 nm; it was immediately dissolved in CH_2Cl_2 (2 ml) and treated with $Ph_3P{=}CH{\cdot}CO_2Me$ (128 mg, 0.38 mmol) in CH_2Cl_2 (2 ml for 0.5 h). The mixture was concentrated and the residue was purified by p.l.c. (petrol–Et_2O, 4 : 1; 3 elutions). The band with R_F 0.35 gave, on crystallisation (petrol), plates of the *trans,trans*-diester (4; R = Me) (41 mg), m.p. 105—107° (lit.,[7] 106°; lit.,[13] 103—106°). The band with R_F 0.25 gave on crystallisation (petrol) plates of the 2-cis-*isomer* (20 mg), m.p. 76—78° (Found: C, 66.3; H, 4.7. $C_{12}H_{10}O_4$ requires C, 66.05; H, 4.6%), $\lambda_{max.}$ (EtOH) 342 (ε 20 700), 319 (22 300), 299 (15 600), 272.5 (24 800), 265 (23 300), 218.5 (22 000), and 211.5 nm (21 300), $\nu_{max.}$ (CCl_4) 2 160, 1 732, 1 615, and 960 cm^{-1}, $\nu_{max.}$ (CS_2) 790 cm^{-1}, τ (CCl_4) 6.23 (s, $CO_2{\cdot}CH_3$), 3.81 (s, *cis*-CH=C*H*·CO_2Me), 3.65 (d, *J* 16 Hz, *trans*-CH=C*H*·CO_2Me), and 3.15 (d, *J* 16 Hz, *trans*-C*H*=CH·CO_2Me) *m/e* 218 (M^+, 58%), 203 (68), 189 (20), 187 (50), 175 (32), 159 (42), 147 (100), 99 (51), and 74 (60).

Synthesis of p-(*Methylamino*)*benzaldehyde* (7) *and* p-[*Methyl*(*nitroso*)*amino*]*benzaldehyde* (11).—*N*-Methylformanilide [14] was converted by successive treatment [9] with $POCl_3$, PCl_5, and NaOH) into the crude aldehyde (7); this was purified by p.l.c. (petrol–Et_2O, 1 : 1; continuous elution) and crystallisation (Et_2O–petrol); m.p. 56—57° (lit.,[9] 56—57°), $\lambda_{max.}$ (EtOH) 335 (ε 23 000) and 239 nm (6 100), $\nu_{max.}$ ($CHCl_3$) 3 450sh, 3 400br, 1 670, 1 600, and 1 160 cm^{-1}, τ (CCl_4) 7.15 (3 H, s, NH·C*H*$_3$), 5.0br (1 H, s, N*H*CH$_3$; disappears on D_2O addition), 3.55 and 2.50 (2 H each, 2 d, *J* 9 Hz, C_6H_4), and 0.44 (1 H, s, CHO), *m/e* 135 (M^+, 95%), 134 (100), 106 (22), 79 (16), 77 (23), and 65 (10). The aldehyde (7) (300 mg, 2.22 mmol) in HCl (2N; 5 ml) was stirred and treated at −7 °C with $NaNO_2$ (168 mg, 2.44 mmol) in H_2O (5 ml). A yellow precipitate appeared. Stirring was continued for 1 h at 20 °C and the solid was crystallised from EtOH–H_2O, yielding the *N*-nitroso-aldehyde (11), m.p. 81—81.5° (lit.,[10] 81—82°), $\lambda_{max.}$ (EtOH) 297 (ε 15 100) and 218 nm (11 500), $\lambda_{min.}$ (EtOH) 240 nm (2 200), $\nu_{max.}$ ($CHCl_3$) 1 690 and 1 605 cm^{-1}, $\nu_{max.}$ (CS_2) 820 cm^{-1}, τ ($CDCl_3$) 6.55 (3 H, 2, NCH_3), 2.30 and 2.05 (2 H each, 2 d, *J* 9 Hz, C_6H_4), and 0.05 (1 H, s, CHO), *m/e* 164 (M^+, 15%), 134 (100), 79 (24), and 77 (62). This (5 mg) was refluxed in Et_2O for 42 h and left over saturated $NaHCO_3$–H_2O for 24 h. No change in the u.v. spectrum of the solution was detected.

We thank the S.R.C. for studentships (to I. W. F. and J. L. T.) and research grant support and Mr. J. W. Keeping of the mycological work.

[7/422 *Received*, 9*th March*, 1977]

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