308. *Chemistry of the Higher Fungi. Part VIII.** *A Series of Acetylenic Compounds from* Polyporus anthracophilus.

By J. D. Bu'Lock, E. R. H. Jones, and W. B. Turner.

The separation and characterisation of a series of acetylenic derivatives of *n*-decane and *n*-octane, metabolic products of the Basidiomycete *P. anthracophilus*, are described. The compounds are shown to be ethylenic-acetylenic alcohols, acids, hydroxy-acids and diacids, with oxygen functions at one or both ends of the molecule; the acids occur free, as methyl esters, and as esters with the hydroxy-compounds of the series.

With the exception of an acetylenic thiophen derivative, junipal, which was detected as an odoriferous metabolite of *Daedalea juniperina*,[1] the fungal polyacetylenes so far described in the literature are all produced by organisms which first attracted interest because of their antibiotic-producing activity. For comparative purposes, and with a view to biochemical studies, we wished to add further examples to the list of natural acetylenes, and a more selective approach seemed desirable. The need was in part met by our observation that some polyacetylene derivatives could be detected spectroscopically in crude extracts from malt-agar slope cultures of certain fungi. Several hundred species of wood-rotting Basidiomycetes (supplied through the kindness of the Director of the Forest Products Research Laboratory, Princes Risborough) were examined in this way and a number selected for further study; some of the results obtained with one of these are reported here.

The fungus is an Australian species, *Polyporus anthracophilus*, which grows well on the semi-synthetic medium used earlier.[2]

The yield of acetylenic compounds is very variable, but in general up to about 1% of the carbon supplied to the cultures as glucose has been accounted for as acetylenic compounds. For comparison it may be observed that our cultures appeared to convert

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* Part VII, *J.*, 1957, 1097.

[1] Birkinshaw and Chaplen, *Biochem. J.*, 1955, **60**, 255.

[2] Bu'Lock and Leadbeater, *ibid.*, 1956, **62**, 476.

about 0·5% of the carbon supplied as glucose into triterpenoids of the eburicoic acid type,[3] whilst with another Basidiomycete yields of polyacetylene derivatives of over 3% (based on carbon *utilised* from glucose) have been obtained.[2] The acetylenic metabolites of *P. anthracophilus* constitute a complex and variable mixture of at least seventeen compounds; these are closely related and so form an interesting series. The thirteen metabolites which we have characterised fully are represented by formulæ (I)—(VIII). The acids of this series occur free, and also combined as methyl esters and the more complex esters (VII) and (VIII). Some other components of the mixture are discussed on p. 1611.

(I) $CH_3\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2R$ $CH_3\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CH_2\cdot OH$ (II)

(III) $HO\cdot CH_2\cdot CH_2\cdot CH_2\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2R$ $RO_2C\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2R$ (IV)

(V) $RO_2C\cdot CH{=}CH\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2R$ $RO_2C\cdot CH_2\cdot CH_2\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2R$ (VI)

$CH_3\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2\cdot CH_2\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CH_3$ (VII)

$CH_3\cdot CH{=}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}CH\cdot CO_2Me$ (VIII)

(R = H or Me; all ethylenic bonds *trans*.)

The extraction of *P. anthracophilus* cultures and fractionation of the extracts are described in the Experimental section. It should be noted that the composition of extracts from the cultures was very variable, depending in rather a complex way upon the duration of fermentation. The general procedure in working-up was to separate neutral and acidic fractions and to methylate the latter. Fractions were then further resolved by repeated chromatography on alumina. The order of elution from the columns was a useful guide to the constitution of particular fractions, since the compounds always appeared in order of increasing polarity, *i.e.*, esters < diesters < alcohols < hydroxy-esters.

The ester (I; R = Me) is the most important acetylene derivative produced by *P. anthracophilus*; it is present mainly in the mycelium but occurs to the limit of its solubility in the aqueous medium. The free acid (I; R = H) occurs in comparatively small amounts. The ester crystallises readily; it was shown to contain the $\cdot CH{=}CH\cdot[C{\equiv}C]_2\cdot CH{=}CH\cdot CO_2R$ chromophore by its ultraviolet absorption spectrum and to be a derivative of methyl decanoate by hydrogenation and conversion of the product into methyl 3 : 5-dinitrobenzoate and decananilide. The infrared spectrum of the ester shows peaks at 945 and 955 cm.$^{-1}$ (*trans*-CH=CH–) and 1720 cm.$^{-1}$ (αβ-unsaturated ester). All these data identify it as methyl deca-*trans*-2 : *trans*-8-diene-4 : 6-diynoate (I; R = Me), *i.e.*, the *trans* : *trans*-matricaria ester synthesised by Sörensen *et al.*[4] The ultraviolet absorption spectra of (I; R = Me) (Fig. 1) and of its *cis*-2 : *trans*-8- and *cis*-2 : *cis*-8-isomer[4] are anomalous in that in them the characteristic acetylenic fine-structure spacing (*ca.* 2200 cm.$^{-1}$) is not sharp. The symmetrical diester (IV; R = Me) shows almost the same absorption maxima as the ester (I; R = Me) but with typical sharp fine-structure (Fig. 1); reasons for this are obscure.

Unlike the above ester (I; R = Me), the corresponding alcohol (II) is found mainly in the aqueous medium, and is the principal acetylenic constituent in rather old (150-day) cultures. It has the ultraviolet absorption spectrum of the $\cdot CH{=}CH\cdot[C{\equiv}C]_2\cdot CH{=}CH\cdot$ chromophore and its infrared spectrum indicates the presence of *trans*-CH=CH· and a hydroxyl group. Hydrogenation gave decanol (3 : 5-dinitrobenzoate), and identification of the alcohol as deca-*trans*-2 : *trans*-8-diene-4 : 6-diyn-1-ol (II) was confirmed by direct comparison with a synthetic[4] specimen of *trans* : *trans*-matricarianol kindly supplied by Professor Sörensen, and also with material obtained by reduction of the ester (I; R = Me) with lithium aluminium hydride at –50°.

The hydroxy-ester (III; R = Me) also accumulates preferentially in the aqueous medium, together with the corresponding acid. Hydrolysis of the low-melting ester

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[3] Gascoigne, Holker, Ralph, and Robertson, *J.*, 1951, 2346.

[4] Bruun, Christensen, Haug, Stene, and Sörensen, *Acta Chem. Scand.*, 1951, **5**, 1244.

gave the crystalline acid. Both show the ultraviolet absorption spectrum of the diyne-ene-carbonyloxy-chromophore; comparison of the infrared spectra of the ester and of synthetic [5] methyl dec-*trans*-2-ene-4 : 6-diynoate (*trans*-lachnophyllum ester) showed many similarities but that of the natural ester contained extra bands attributable to a primary hydroxyl group. Hydrogenation of the acid, followed by chromic acid oxidation, gave sebacic acid, which suggested the structure methyl 10-hydroxydec-*trans*-2-ene-4 : 6-diynoate (III; R = Me) for the natural ester. This was therefore synthesised by the oxidative coupling of pent-4-yn-1-ol and methyl pent-*trans*-2-en-4-ynoate. The low-melting product was spectroscopically identical with the natural ester and on hydrolysis gave the acid (III; R = H).

From the mixture of acids produced by *P. anthracophilus* the diacid (IV; R = H) could be isolated by virtue of its relative insolubility in organic solvents; both the free acid and its dimethyl ester occur in the cultures. As already noted, both show ultraviolet absorption with maxima quite close to those of matricaria esters but differing in having sharp fine-structure (Fig. 1). The chromophore could not be immediately identified but clearly it involved at least nine carbon atoms, and since the equivalent weight of the acid was *ca.* 100 it was presumed to be dibasic. Hydrogenation gave sebacic acid, so that the natural acid is deca-*trans*-2 : *trans*-8-diene-4 : 6-diyne-1 : 10-dioic acid (IV; R = H), which had been previously synthesised together with the dimethyl ester by Heilbron *et al.*[6] Repetition of the synthesis confirmed this identification.

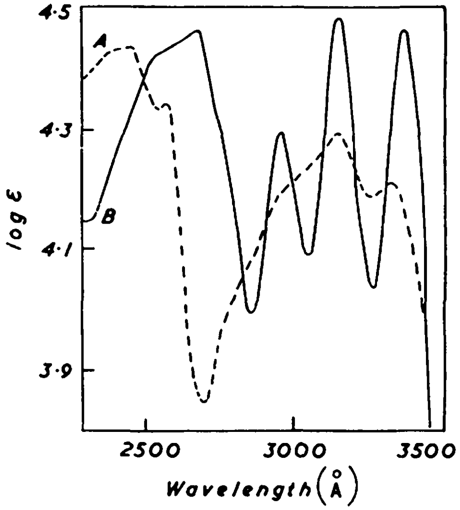

FIG. 1. *Ultraviolet absorption spectra (in ethanol) of* (I; R = Me) *(curve* A) *and* (IV; R = Me) *(curve* B) *showing fine-structure differences.*

The acids from *P. anthracophilus* having been converted into the methyl esters, the separation of another dimethyl ester $C_6H_4(CO_2Me)_2$ (V; R = Me) from the above diester (IV; R = Me) was only achieved with some difficulty. The ultraviolet absorption spectrum of this compound shows no acetylenic fine-structure, but instead resembles that of a polyene ester. Its infrared absorption spectrum shows only one carbonyloxy-peak (αβ-unsaturated), and the acetylenic band (*ca.* 2200 cm.$^{-1}$) is absent. However, the intensity of the ultraviolet absorption is much lower than would be expected for a diethyl octa-2 : 4 : 6-triene-1 : 8-dioate and comparable to that recorded [7] for dimethyl octa-2 : 4-dien-6-yne-1 : 8-dioate; such a lowering of the extinction coefficient is the characteristic effect of replacing a double by a triple bond. The infrared spectrum of the diester differs from that of dimethyl octa-2 : 4-dien-6-yne-1 : 8-dioate since the latter shows a strong band at 2200 cm.$^{-1}$ due to the triple bond,[7] but its properties are consistent with the structure dimethyl octa-*trans*-2 : *trans*-6-dien-4-yne-1 : 8-dioate (V; R = Me) (in this the triple bond is symmetrically placed so that its stretching vibration is inactive in infrared absorption). Accordingly, we found that the diester would not react with maleic anhydride despite the *trans*-configuration of its double bonds (deduced from the infrared spectrum); this observation renders structures with adjacent double bonds unlikely.

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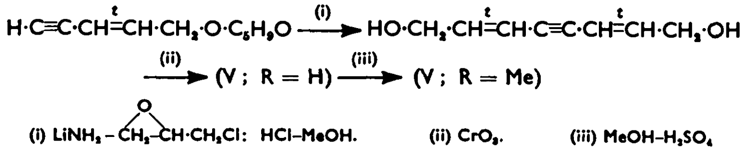
[5] Sample prepared by Mr. Ian Bell; cf. Bruun, Haug, and Sörensen, *Acta Chem. Scand.*, 1950, **4**, 850.

[6] Heilbron, Jones, and Sondheimer, *J.*, 1947, 1586.

[7] Jones, Shaw, and Whiting, *J.*, 1954, 3212.

Neither the acid (V; R = H) nor its ester (V; R = Me) had been described previously, and therefore the ester was synthesised from *trans*-pent-2-en-4-yn-1-yl tetrahydropyranyl ether by the route outlined below. The product was identical with the naturally-derived compound.



$$H{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}O{\cdot}C_5H_9O \xrightarrow{(i)} HO{\cdot}CH_2{\cdot}CH\overset{t}{=}CH{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH \xrightarrow{(ii)} (V;\ R = H) \xrightarrow{(iii)} (V;\ R = Me)$$

(i) LiNH$_2$ – CH$_2$–CH·CH$_2$Cl: HCl–MeOH. (ii) CrO$_3$. (iii) MeOH–H$_2$SO$_4$

A third diacid (VI; R = H) shows an ultraviolet absorption spectrum of the diyn-ene-carbonyloxy-type already encountered in the hydroxy-ester (III). However, comparison of the infra-red spectra showed that the corresponding ester contains no hydroxyl group; on the other hand it contains two dissimilar ester groups, revealed by bands at 1740 cm.$^{-1}$ and 1720 cm.$^{-1}$ (αβ-unsaturated). Formulation of this diester as dimethyl dec-*trans*-2-ene-4 : 6-diyne-1 : 10-dioate (VI; R = Me) was confirmed by synthesis of this compound by chromic acid oxidation of the hydroxy-ester (III; R = Me) and methylation of the product.

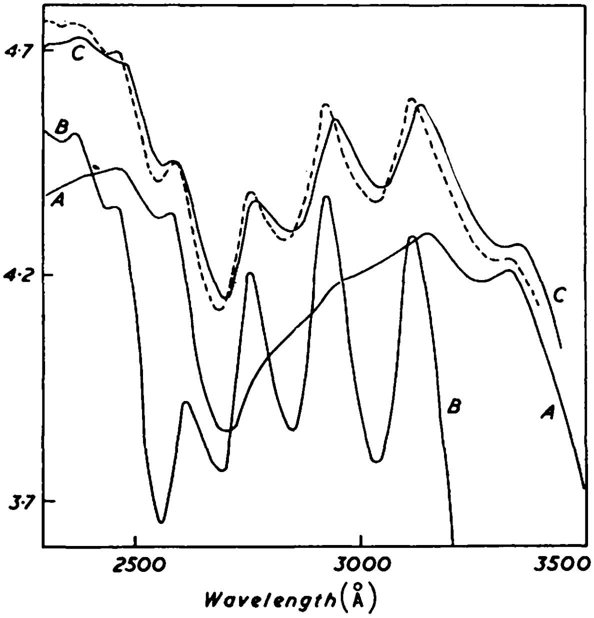
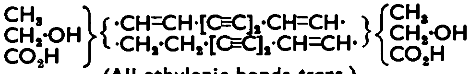


FIG. 2. *Ultraviolet absorption spectra (in ethanol) of* (I; R = Me) *(curve* A), (II) *(curve* B), *and* (VII) *(curve* C). *The broken line indicates the summation of curves* A *and* B.

The nature of the "mixed esters" (VII) and (VIII) was first deduced from their absorption spectra. The ester (VII) gave an ultraviolet spectrum almost identical with that obtained by summation of the curves for the methyl ester (I; R = Me) and the alcohol (II) (cf. Fig. 2), whilst in its infrared spectrum the intensity of the carbonyl band was approximately one-half of that in the spectrum of (I; R = Me). Similarly, the ultraviolet absorption spectrum of the diester (VIII) could be duplicated by summation of the curves for the ester (I; R = Me) and the hydroxy-ester (III; R = Me) (Fig. 3). Both structures were fully confirmed by the synthesis of (VII) and (VIII) from the chloride of the acid (I; R = H) and the hydroxy-compounds (II) and (III; R = Me), respectively. Having regard to the circumstances of their isolation, we consider the esters (VII) and (VIII) to be genuine metabolites of *P. anthracophilus* and not artefacts formed during the working-up process.

With the exception of the C_8-diester (V; R = Me) and of the "mixed esters" (VII) and (VIII) the formulæ of the compounds so far described can be summarised in (IX). Of the fifteen possibilities represented by this general formula (IX) we have described the characterisation of five compounds, *viz.*, (I), (II), (III), (IV), and (VI). Of the remaining possibilities, two are hydrocarbons and two are glycols, and we have had no indication of the production of either class of compound by *P. anthracophilus.* Some of the possibilities, if produced only in trace amounts, might well have escaped detection, but there are indications that at least four further members of the series are produced by the fungus.



$$\left.\begin{matrix}\mathrm{CH_3}\\ \mathrm{CH_2{\cdot}OH}\\ \mathrm{CO_2H}\end{matrix}\right\}\left\{\begin{matrix}\mathrm{{\cdot}CH{=}CH{\cdot}[C{\equiv}C]_2{\cdot}CH{=}CH{\cdot}}\\ \mathrm{{\cdot}CH_2{\cdot}CH_2{\cdot}[C{\equiv}C]_2{\cdot}CH{=}CH{\cdot}}\end{matrix}\right\}\left\{\begin{matrix}\mathrm{CH_3}\\ \mathrm{CH_2{\cdot}OH}\\ \mathrm{CO_2H}\end{matrix}\right. \quad \text{(IX)}$$

(All ethylenic bonds *trans.*)

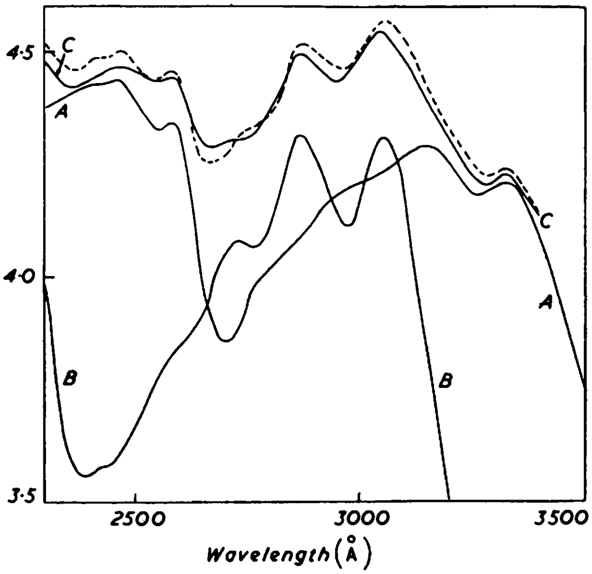


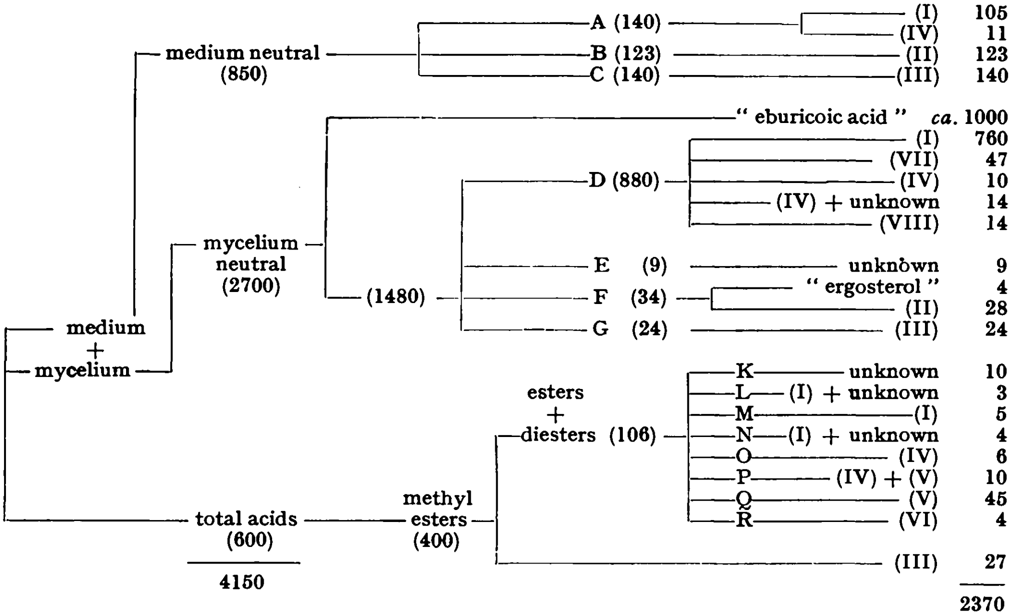
FIG. 3. *Ultraviolet absorption spectra (in ethanol) of* (I; R = Me) (*curve* A), (III; R = Me) (*curve* B), *and* (VIII) (*curve* C). *The broken line indicates the summation of curves* A *and* B.

A neutral compound which behaves on alumina as an alcohol, and having the absorption spectrum of an ene-diyne, might well be one of the two possible decenediynols represented by the above formula. Similarly a compound with a characteristic ultraviolet absorption spectrum, similar to that of (I; R = Me), but which behaves on alumina as a hydroxy-ester, is probably the hydroxy-ester (X). A third compound, possibly the methyl ester of the conjugated decenediynoic acid, has the ultraviolet absorption spectrum of the hydroxy-ester (III; R = Me) but behaves on alumina like the non-hydroxylic ester (I; R = Me), whilst a fourth is of similar polarity but has the ultraviolet absorption of an ene-diyne, and thus might be an ester of the isomeric decenediynoic acid with the carbonyloxy-group not in conjugation. These compounds occur in minute amounts, but may occur in larger quantities in other strains of *P. anthracophilus* or in some apparently related species now being studied. In addition to these acetylenic compounds, our *P. anthracophilus* cultures contained an appreciable quantity of, presumably, eburicoic and dehydroeburicoic acid [3] and smaller amounts of ergosterol-like material (cf. Experimental).

$$\mathrm{HO{\cdot}CH_2{\cdot}CH{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}CH{\cdot}CO_2Me} \quad \text{(X)}$$

The acetylenic compounds from *P. anthracophilus* may be compared with the series of C_{10} polyacetylene derivatives from Compositae extensively studied by the Sörensens

Fractionation of extracts from P. anthracophilus.*



* Weights noted in this flow-sheet, including those in the final column, are the total weights (in mg.) of fractions after evaporation. Yields of recrystallised material are given in the text.

and their co-workers.[8] Indeed our isolation of the alcohol (II) (*trans* : *trans*-matricarianol) provides the first example of the occurrence of the same polyacetylene in fungi and in higher plants.[9] However, despite common features there are three important differences between the two series. First, all the compounds from *P. anthracophilus* are *trans*- or *trans* : *trans*-isomers; this contrasts sharply with the Compositae series where the *cis*-configuration is more common. Secondly, the occurrence in *P. anthracophilus* of the eight-carbon

Ultraviolet absorption spectra in ethanol: $\lambda_{max.}$ (Å) *and* log ε (*in parentheses*).

I; R = Me †	—	—	—	2340 * (4·38)	2460 (4·41)	2580 (4·32)	2960 * (4·20)	3140 (4·28)	3330 (4·20)	—
I; R = H †	—	—	—	—	2450 (4·37)	2560 (4·27)	—	3100 (4·23)	3290 (4·16)	—
II	— —	2175 (4·51)	2315 (4·53)	2370 (4·52)	2470 (4·35)	2610 (3·94)	2760 (4·23)	2930 (4·39)	3120 (4·30)	—
III; R = Me	—	2150 (4·45)	2230 (4·53)	—	2430 * (3·58)	2580 * (3·83)	2730 (4·08)	2870 (4·31)	3050 (4·30)	—
III; R = H	—	2150 (4·48)	2220 (4·56)	—	2430 * (3·66)	2550 * (3·81)	2700 (4·10)	2850 (4·32)	3030 (4·31)	—
IV; R = Me	—	2160 (4·39)	—	—	—	—	2690 (4·45)	2980 (4·29)	3170 (4·48)	3390 (4·46)
IV; R = H	—	2160 (4·38)	—	—	—	2580 * (4·41)	2670 (4·26)	2960 (4·26)	3150 (4·44)	3380 (4·42)
V; R = Me	2050 * (4·04)	2140 (4·06)	—	—	2400 * (4·00)	—	2780 * (4·27)	2920 (4·44)	3070 (4·40)	—
VI; R = Me	—	2145 (4·42)	2230 (4·54)	—	2430 * (3·28)	2550 * (3·69)	2700 (4·08)	2850 (4·32)	3030 (4·31)	—
VII		2130 (4·66)	2330 * (4·71)	2385 (4·72)	2460 * (4·68)	2590 (4·45)	2775 (4·36)	2950 (4·55)	3140 (4·58)	3350 (4·28)
VIII	—	—	2230 (4·72)	—	2465 (4·45)	2590 (4·42)	—	2870 (4·52)	3050 (4·57)	3340 (4·22)

* Inflexion. † Compare Sörensen *et al.*[4]

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[8] Sörensen, *Chem. and Ind.*, 1953, 240.

[9] Holme and Sörensen, *Acta Chem. Scand.*, 1954, **8**, 34.

system (V) is not paralleled in the Compositae though this chain length is known in other fungal acetylenes, *e.g.*, junipal,[1] diatretyne I, and agrocybin.[10] Thirdly, the compounds with oxygen functions at both ends of the carbon chain are without parallel in the Compositae, though such structures are typical of certain other fungal acetylenes.[10]. The biogenetic implications of these and other observations with *P. anthracophilus* are to be discussed more fully elsewhere.

EXPERIMENTAL

Ultraviolet absorption spectra, cf. Table, were measured in ethanol (chromatographic fractions were examined directly) on Unicam SP-500 instruments, infrared absorption spectra on a Perkin-Elmer Model 21 spectrophotometer, and m.p.s (corrected) on a Kofler block. Alumina for chromatography was Peter Spence grade " H," deactivated by treatment with 5% of 10% acetic acid. Light petroleum refers to the fraction with b. p. 60—80°. Evaporations were carried out under reduced pressure in nitrogen.

Characterisation and Synthesis of the Components.

Methyl Deca-trans-2 : trans-8-*diene*-4 : 6-*diynoate* (trans : trans-*Matricaria Ester*) (I; R = Me).—This ester crystallised from aqueous ethanol or light petroleum as needles, m. p. 62—63° (lit., m. p. 60—61°) (Found: C, 75·3; H, 5·6. Calc. for $C_{11}H_{10}O_2$: C, 75·8; H, 5·8%), with an ultraviolet absorption spectrum (cf. Table) agreeing closely with that described by Sörensen *et al.*[4] The compound (300 mg.), hydrogenated in light petroleum over 2% palladium on calcium carbonate (500 mg.), gave methyl decanoate, which was converted directly into methyl 3 : 5-dinitrobenzoate, m. p. and mixed m. p. 106·5—109·5°, and decananilide, m. p. and mixed m. p. 63—65°. Reaction of the ester (I; R = Me) (100 mg.) in methanol (8 c.c.) with aqueous potassium hydroxide (200 mg. in 9 c.c.; 8 hr. at 0° under nitrogen) and isolation of the acidic product gave, after recrystallisation from acetone–light petroleum, deca-*trans*-2 : *trans*-8-diene-4 : 6-diynoic acid (*trans* : *trans*-matricaria acid) (I; R = H), plates, m. p. 175—177° (decomp.) [lit., m. p. 173° (decomp.)] with an ultraviolet absorption spectrum (cf. Table) in agreement with the data of Sörensen *et al.*[4] Reduction of the ester (I; R = Me) (19 mg.) in dry ether (25 c.c.), cooled in solid carbon dioxide–acetone, with a solution of lithium aluminium hydride in ether (*ca.* 50% excess), washing the solution with dilute sulphuric acid and water, evaporation of the solvent, and crystallisation of the residue from aqueous ethanol afforded *trans* : *trans*-matricarianol (II), m. p. and mixed m. p. 101—105° (see below).

Deca-trans-2 : trans-8-*diene*-4 : 6-*diyn*-1-*ol* (trans : trans-*Matricarianol*) (II).—This alcohol crystallised from aqueous ethanol or light petroleum as needles, m. p. 105·5—106·5° undepressed on admixture with synthetic material[4] or with the reduction product from the ester (I; R = Me). It had ultraviolet (see Table) and infrared absorption spectra identical with those of synthetic material (Found: C, 82·5; H, 7·0. Calc. for $C_{10}H_{10}O$: C, 82·2; H, 6·8%). Hydrogenation of the alcohol (II) (37 mg.) in ethanol over 2% palladium on calcium carbonate (50 mg.) gave an oil, converted directly into decyl 3 : 5-dinitrobenzoate, m. p. and mixed m. p. 55—57°.

Methyl 10-*Hydroxydec*-trans-2-*ene*-4 : 6-*diynoate* (III; R = Me).—(*a*) Isolated as an almost colourless oil after low-temperature crystallisation from ether–light petroleum, the *ester* was characterised by its ultraviolet (cf. Table) and infrared absorption spectra. Hydrolysis of the ester (80 mg.) by the method used for (I; R = Me) gave, on crystallisation of the product from aqueous ethanol, 10-*hydroxydec*-trans-2-*ene*-4 : 6-*diynoic acid* (III; R = H) as plates, m. p. 154·5—156°; ultraviolet absorption spectrum, see Table (Found: C, 67·6; H, 6·0. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·7%). Hydrogenation of the acid (12 mg.) in ethanol over 2% palladium on carbon (20 mg.) gave a crystalline solid (11 mg.), which was oxidised by excess of 8N-chromic acid in acetone to sebacic acid, m. p. and mixed m. p. 128·5—132·5°.

(*b*) To a solution of cuprous chloride (17 g.) and ammonium chloride (28 g.) in water (85 c.c.) containing a little hydrochloric acid (pH *ca.* 3·5) were added methyl pent-*trans*-2-en-4-ynoate (1·6 g.) and pent-4-ynol (4·7 g.), followed by hydrogen peroxide (100 vol.; 30 c.c.) added dropwise during 1 hr. at 10°. The mixture was then acidified, and isolation with ether gave a semi-crystalline mixture (4·6 g.). Adsorption on alumina (400 g.) from benzene and elution with the same solvent gave a fraction containing the diester (IV; R = Me), and then one containing the desired hydroxy-ester (III; R = Me), purified by low-temperature crystallisation to constant

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[10] Bu'Lock, Jones, Mansfield, Thompson, and Whiting, *Chem. and Ind.*, 1954, 990.

extinction coefficient; for ultraviolet absorption spectrum, see Table. The ultraviolet and infrared absorption spectra were identical with those of the naturally-derived compound. The hydroxy-ester was hydrolysed as described above to give the hydroxy-acid (III; R = H) which when recrystallised from aqueous ethanol had m. p. and mixed m. p. with naturally-derived material, 153—154° (ultraviolet absorption spectrum, see Table).

Deca-trans-2 : trans-8-*diene*-4 : 6-*diyne*-1 : 10-*dioic acid* (IV; R = H).—Precipitation from aqueous ethanol gave the acid as an amorphous powder, decomp. *ca.* 200°, characterised by its ultraviolet (see Table) and infrared absorption spectra and equivalent weight (potentiometric titration) [Found: equiv., 95. Calc. for $C_8H_4(CO_2H)_2$: equiv., 95]. Hydrogenation of the diacid (58 mg.) over platinum oxide (16 mg.) in ethanol, removal of catalyst and solvent, and recrystallisation of the product from water yielded sebacic acid, m. p. and mixed m. p. 130·5—133°. The dimethyl ester was isolated by chromatography of the mixed esters (cf. below) and also by methylation of (IV; R = H); the diacid (78 mg.) was dissolved in dry methanol containing 3% concentrated sulphuric acid and the solution set aside at 15° for *ca.* 40 hr., pale yellow plates separating. The mixture was diluted with water and extracted with ether; isolation of the neutral product and recrystallisation from ethanol yielded the diester (IV; R = Me) as plates, m. p. 104·5—107·5° (Found: C, 66·3; H, 4·6. Calc. for $C_{12}H_{10}O_4$: C, 66·1; H, 4·6%). The dimethyl ester, prepared by the method of Heilbron, Jones, and Sondheimer,[6] had m. p. 105·5—108·5° and was identical with the naturally derived material.

Dimethyl Octa-trans-2 : trans-6-*dien*-4-*yne*-1 : 8-*dioate* (C_8 *Diester*) (V; R = Me).—(*a*) Isolated from the esterified " acid fraction " (see below), this *diester* crystallised from light petroleum as plates, m. p. 117—119·5° (Found: C, 61·8; H, 5·5. $C_{10}H_{10}O_4$ requires C, 61·8; H, 5·2%); ultraviolet absorption spectrum, see Table. Hydrogenation of (V; R = Me) (14 mg.) in ethanol over 2% palladium on calcium carbonate (40 mg.), removal of catalyst and solvent, hydrolysis of the residual oil with potassium hydroxide in methanol, and isolation and crystallisation of the acidic product gave suberic acid, m. p. and mixed m. p. 140—142°.

(*b*) To a mixture of pent-*trans*-2-en-4-ynol (5·5 g.) and dihydropyran (6·0 g.), concentrated hydrochloric acid (0·05 c.c.) was added with cooling. The mixture was set aside at 20° for 16 hr., potassium hydroxide (*ca.* 0·1 g.) was added and the filtered mixture distilled to give 2-pent-2′-en-4′-yn-1′-yloxytetrahydropyran (9·8 g.), b. p. 45—47°/0·15 mm., n_D^{18} 1·4889. This (9·2 g.) in dry ether (10 ml.) was added to a stirred suspension of lithium amide (from 0·4 g. of lithium) in liquid ammonia through which a stream of nitrogen was passing. Epichlorohydrin (5·2 g.) was added dropwise and the mixture stirred under nitrogen for 7 hr. Ammonium chloride (3 g.) was added, the ammonia evaporated, and the residue extracted with ether. The ether-insoluble material was taken up in water, and the solution filtered to remove traces of gum and extracted with ether. The combined ether extracts were washed successively with dilute sulphuric acid, aqueous sodium carbonate, and water, dried (Na_2SO_4) and evaporated leaving a yellow oil (10 g.). This was dissolved in light petroleum–benzene (6 : 1) and adsorbed on alumina; elution with the same solvent yielded unchanged materials (6·4 g.) and elution with benzene yielded an oil (2·9 g.) which was hydrolysed in methanol (50 c.c.) containing sulphuric acid (0·2 c.c.). The solution was left overnight, sodium carbonate was added, and the filtered solution was evaporated. Low-temperature crystallisation from ether and recrystallisation from ether–light petroleum gave octa-*trans*-2 : *trans*-6-dien-4-yne-1 : 8-diol (0·4 g.), m. p. 78—81°, raised by further recrystallisation to 80—81·5°. The crude diol had been obtained previously by Heilbron *et al.*,[11] and has since been obtained crystalline by Bohlmann and Mannhardt,[12] who report m. p. 80—80·5°.

Oxidation of the diol (0·3 g.) with manganese dioxide in methylene chloride was incomplete and the partially oxidised material in acetone was treated with an excess of 8N-chromic acid in acetone. The mixture was worked to give an acid fraction containing a pale yellow solid (215 mg.). This was dissolved in methanol (50 c.c.) containing 3% by volume of concentrated sulphuric acid, and the mixture set aside at 20° for 44 hr. A neutral fraction (230 mg.) was obtained which, after chromatography on alumina, afforded the diester, m. p. and mixed m. p. 118—119°; ultraviolet spectrum, see Table; infrared spectrum identical with that of the naturally-derived material.

Dimethyl Dec-trans-2-*ene*-4 : 6-*diyne*-1 : 10-*dioate* (VI; R = Me).—(*a*) Isolated from the esterified acid fraction (see below) this diester had m. p. 56·5—58° (from light petroleum).

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[11] Heilbron, Jones, Lacey, McCombie, and Raphael, *J.*, 1945, 77.

[12] Bohlmann and Mannhardt, *Chem. Ber.*, 1956, **89**, 1307.

(*b*) To a solution of the hydroxy-ester (III; R = Me) (94 mg.) in acetone (8 c.c.), 8N-chromic acid (0·25 c.c.) was added dropwise. After 5 min. the mixture was diluted with water and extracted repeatedly with ether. The combined ether extracts were extracted with aqueous sodium hydrogen carbonate, and this extract acidified and extracted with ether. Evaporation of the ether solution gave a solid (43 mg.), which was methylated to give the *diester* (VI; R = Me) (37 mg.), silky needles (from light petroleum), identical in m. p. (56·5—58°), mixed m. p., and ultraviolet (see Table) and infrared spectra with the naturally-derived compound (Found: C, 65·5; H, 5·6. $C_{12}H_{12}O_4$ requires C, 65·4; H, 5·5%).

Deca-trans-2 : trans-8-*diene*-4 : 6-*diynyl Deca*-trans-2 : trans-8-*diene*-4 : 6-*diynoate* (VII).—(*a*) This *ester* was isolated as described below, forming needles, m. p. 124—126° (Found : C, 83·6; H, 5·9. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6%); for ultraviolet absorption spectrum, see Table and Fig. 2.

(*b*) Deca-*trans*-2 : *trans*-8-diene-4 : 6-diynoic acid (I; R = H) (35 mg.) was treated with thionyl chloride (*ca.* 0·3 c.c.) with slight warming until the acid dissolved; after 30 min. at 20°, excess of thionyl chloride was distilled off under reduced pressure. To the yellow residue was added a solution of deca-*trans*-2 : *trans*-8-diene-4 : 6-diyn-1-ol (II) (34 mg.) in dry pyridine (0·4 c.c.); the mixture was left overnight at 0°, then diluted with water and extracted with ether. The combined ether extracts were washed successively with aqueous sodium hydrogen carbonate, water, dilute hydrochloric acid, and water, dried ($MgSO_4$), and evaporated. The residue (50 mg.) was dissolved in light petroleum and purified by chromatography on a short column of alumina, followed by recrystallisation from the same solvent, to give needles, m. p. and mixed m. p. 124·5—126°, with ultraviolet and infrared absorption spectra identical with those of the natural ester.

Methyl 10-(*Deca*-trans-2 : trans-8-*diene*-4 : 6-*diyn*-1-*oyloxy*)-*dec*-trans-2-*ene*-4 : 6-*diynoate* (VIII).—(*a*) This diester, isolated as described below, crystallised from light petroleum as plates, m. p. 91—93° (Found : C, 75·5; H, 5·7. $C_{21}H_{18}O_4$ requires C, 75·4; H, 5·4%); for ultraviolet absorption spectrum see Table and Fig. 3.

(*b*) Reaction of the acid chloride from deca-*trans*-2 : *trans*-8-diene-4 : 6-diynoic acid (93 mg.) with the hydroxy-ester (III; R = Me) (72 mg.) and purification of the product as described for (VII) gave the required diester (VIII) as plates, m. p. and mixed m. p. 91—94°, having ultraviolet and infrared absorption spectra identical with those of the natural material.

Extraction and Separation of the Components.

Because of the varying composition of extracts it is impossible to describe a generally applicable procedure; moreover, crude fractions and mother liquors from several runs were often combined and worked up together. The following account, summarised in the flow-sheet, describes the extraction and working-up of a batch of 16 cultures taken 68 days after inoculation.

The culture medium (*ca.* 10 l.) was extracted with ether (5 × 1 l.) and the mycelium extracted continuously (48 hr.) with ether. The extracts were each divided into an acidic and a neutral fraction by extraction with saturated aqueous sodium hydrogen carbonate; the neutral fractions were kept separate whilst the bicarbonate extracts were combined, acidified, and extracted with ether to give a single acid fraction.

Neutral Fraction from the Medium.—Evaporation yielded yellow oil and solid (850 mg.) which was adsorbed on alumina (150 g.) from 1 : 4 light petroleum–benzene. Elution with the same solvent gave successively three main fractions with longest-wave absorption maxima respectively at *ca.* 3350 (" A "), 3130 (" B "), and 3050 Å (" C ").

Fraction " A " gave an oil (140 mg.) which was separated by chromatography on alumina (25 g.) and elution with light petroleum, into two fractions having the ultraviolet spectra of esters (I) and (IV), respectively. Evaporation of the first fraction gave a yellow oil (105 mg.) which, when treated with a few drops of ethanol and set aside at 0°, yielded *trans* : *trans*-matricaria ester (I; R = Me) (12 mg.) as needles, m. p. 60—63° (after two recrystallisations from aqueous ethanol). The second fraction gave crystals (11 mg.) from which ester (IV; R = Me), m. p. 106·5—110°, was obtained after two recrystallisations from alcohol.

Removal of solvent from fraction " B " and three recrystallisations from ethanol gave *trans* : *trans*-matricarianol (II), as needles (64 mg.), m. p. 105—107°. Similarly, fraction " C " afforded the hydroxy-ester (III) as a yellow oil, low-temperature crystallisation of which from ether–light petroleum gave an almost colourless oil (52 mg.) with $\varepsilon = 20,200$ at 3050 Å.

Neutral Fraction from the Mycelium.—Evaporation of the ether yielded a yellow oily solid

(2·7 g.). Trituration with benzene left a white residue (*ca.* 1 g.) which gave a positive Liebermann–Burkhardt test and an ultraviolet spectrum suggesting that it contained eburicoic and dehydroeburicoic acids.[3]

Evaporation of the benzene afforded a mixture (1·48 g.) of oil and crystals which was dissolved in light petroleum–benzene (1 : 2) and adsorbed on alumina (200 g.). Elution with the same solvent gave three successive fractions with longest-wavelength ultraviolet absorption maxima respectively at 3350 (" D "), 3470 (" E "), and 3130 Å (" F "). Further elution with benzene gave a fourth fraction (" G ") with longest-wavelength ultraviolet absorption maximum at 3050 Å.

Removal of solvent from " D " yielded pale yellow crystals (880 mg.), which were adsorbed from light petroleum on to alumina (200 g.). Elution with light petroleum gave, first, a fraction containing *trans* : *trans*-matricaria ester (I; R = Me), m. p. 63—64° (460 mg.; after three recrystallisations from light petroleum). Immediately thereafter was eluted a fraction having ultraviolet absorption (Fig. 2) corresponding to a mixture of (I) and *trans* : *trans*-matricarianol (II), evaporation of which yielded the ester (VII) as needles, m. p. 125—127·5° (8 mg., after three recrystallisations from ethanol). Elution with benzene–light petroleum (1 : 1) gave a fraction affording the diester (IV), which formed plates, m. p. 107—110° (3 mg., after three recrystallisations from light petroleum). Continued elution with the same solvent then gave a fraction containing (IV) mixed with other diesters (longest-wavelength ultraviolet absorption maximum at 3050 Å; 10 mg. of yellow gum and crystals), which was not worked up (cf. working up of the acid fraction below), followed by a fraction with ultraviolet absorption (Fig. 3) corresponding to a mixture of esters (I; R = Me) and (III; R = Me). Evaporation gave a gum which crystallised on trituration with light petroleum affording ester (VIII) as prisms, m. p. 92—94° (4 mg., after three recrystallisations from light petroleum).

Evaporation of " E " gave a yellow gum (9 mg.); the ultraviolet absorption of this material showed a single broad maximum at *ca.* 3470 Å; its infrared spectrum contained a strong band at 1660 cm.$^{-1}$ and no band attributable to $C\equiv C$, $CH=C=CH$, or *trans*-$CH=CH$ groups.

Evaporation of " F " yielded pale yellow crystals (34 mg.). Repeated recrystallisation of this material from aqueous ethanol gave plates (4 mg.), m. p. 138—147°, of a compound showing ultraviolet and infrared absorption spectra similar to those of ergosterol. From the mother-liquors was isolated *trans* : *trans*-matricarianol (II) (13 mg.), m. p. 103—107° (from light petroleum).

Evaporation of " G " gave a yellow gum (24 mg.) containing (by spectroscopic assay) *ca.* 14 mg. of the hydroxy-ester (III).

Total Acid Fraction.—Evaporation of the combined acid fractions gave a yellow-brown gum (600 mg.). This was esterified with methanolic sulphuric acid, and the neutral ester fraction (400 mg.) was adsorbed on alumina (100 g.) from benzene–light petroleum (1 : 1); elution with the same solvent and evaporation gave a crystalline " ester + diester " fraction (see below). Elution with benzene gave a fraction containing the hydroxy-ester (III; R = Me) (*ca.* 20 mg., assayed spectroscopically).

The " ester + diester " fraction (106 mg.) was adsorbed on alumina (30 g.) from light petroleum; elution with the same solvent yielded eight successive fractions " K—R." Fraction " K," a gum (10 mg.), showed no distinctive ultraviolet absorption. " L " contained an oil (3·5 mg.) showing the ultraviolet absorption spectrum of matricaria ester modified by that of a component with absorption maxima at 2850 and 3050 Å, probably therefore a conjugated diynene ester. " M " contained crude *trans* : *trans*-matricaria ester (I; R = Me) (5 mg.), identified by its infrared absorption spectrum; " N " gave a gum (4 mg.) containing the ester (I; R = Me) together with material having the ultraviolet absorption spectrum of an ene-diyne; " O " gave crystalline diester (IV), m. p. 90—107° (2 mg., after two recrystallisations from light petroleum). " P " gave a crystalline mixture (10·5 mg.) of (IV) with dimethyl octa-*trans*-2 : *trans*-6-dien-4-ynedioate (V); " Q " consisted mainly of the latter compound, m. p. 117—119° (15 mg., after two recrystallisations). Fraction " R " (4 mg.) consisted mainly of the diester (VI; R = Me).

Some different procedures used with other batches may be noted. When the acid fraction was rich in the dibasic acid (IV; R = H) some of this could be isolated by deposition from ethanol before the esterification. In the fractionation of the " esters + diesters " mixture, the fractions containing the diester (IV; R = Me) and the diester (V; R = Me) were efficiently resolved by crystallisation from carbon disulphide, in which (V) is less soluble. When larger fractions composed mainly of the hydroxy-ester (III; R = Me) were purified by re-chromatography, a small fraction was obtained with ultraviolet absorption corresponding to that of (*III*; R = Me) together with a compound with a spectrum of matricaria ester type; this is the component presumed to be the as-yet-unknown hydroxy-ester (IX).

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The University, Manchester, 13.
The Dyson Perrins Laboratory, Oxford.

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