Further Polyacetylenes from *Polyporus anthracophilus*: Specific Incorporation of [1-^{14}C]Matricaria Esters into Polyacetylenic Metabolites of this Fungus

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A further eight C_{10} polyacetylenes have been isolated from cultures of *Polyporus anthracophilus*. Several of these, notably *Z,Z*- and 2*E*,8*Z*-matricaria ester and *Z,Z*-matricarianol, have not previously been isolated from fungi. When a mixture of *E,E*-[1-^{14}C]matricaria ester and 2*E*,8*Z*-[1-^{14}C]matricaria ester was fed to cultures of *P. anthracophilus*, one or both of these esters were specifically incorporated into *E,E*-matricarianol, *Z,Z*-matricarianol, and dimethyl *E,E*-deca-2,8-diene-4,6-diyne-1,10-dioate. This provides strong evidence that in this organism, matricaria esters are intermediates in the production of many of the polyacetylenic metabolites.

THE C_8, C_9, and C_{10} fungal polyacetylenes are generally derived from C(9)–C(18) of oleic acid.[1-4] Tracer experiments indicate that in some fungi *E*-dehydromatricaria ester (1), or a close relative, is an intermediate in the formation of several C_8, C_9, and C_{10} polyacetylenes [2,3,5] and that in one fungus *E*-lachnophyllum ester (2), or a close relative, is an intermediate in the formation of a C_9 polyacetylene.[4] Structural comparisons suggest that many of the polyacetylenes produced by *Polyporus anthracophilus* [6] are derived from *E,E*-matricaria ester (3), itself a major metabolite of this organism. We report here evidence for the presence in this fungus of eight polyacetylenes [compound (2) and compounds (4)—(10)] not previously shown to be present, and tracer experiments which indicate that polyacetylenes (6), (11), and (12) are derived from ester (3) and/or ester (4).

$^{10}CH_3{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH^{1}{\cdot}CO_2CH_3$ (1)

$CH_3{\cdot}CH_2CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (2)

$CH_3{\cdot}CH^{E}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (3)

$CH_3{\cdot}CH^{Z}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (4)

$CH_3CH^{Z}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{Z}{=}CH{\cdot}CO_2CH_3$ (5)

$CH_3{\cdot}CH^{Z}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}CCH^{Z}{=}CH{\cdot}CH_2OH$ (6)

$HOCH_2{\cdot}CH^{E}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (7)

$HOCH_2{\cdot}CH^{Z}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (8)

$HOCH_2CH^{E}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CH_2OH$ (9)

$HOCH_2CH_2CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CH_2OH$ (10)

$CH_3CH^{E}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CH_2OH$ (11)

$CH_3O_2C{\cdot}CH^{E}{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (12)

$CH_3CH_2CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CH_2OH$ (13)

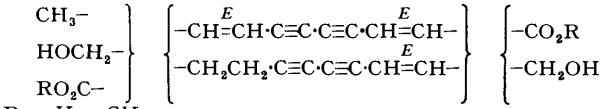
$HOCH_2CH_2CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH^{E}{=}CH{\cdot}CO_2CH_3$ (14)

Isolation of Further Polyacetylenes.—As the proportions of the various metabolites produced by *P. anthracophilus* vary considerably not only with the age of the culture but also from one culture to another,[6,7] we first showed that both the present cultures (grown in Oxford and in Lancaster) were producing sufficient of the major metabolites for tracer experiments. These were isolated in the usual way by extracting the culture fluid with ether and subjecting the neutral extracts to column chromatography. In the course of this, we isolated from the Oxford cultures, by means of preparative thin-layer chromatography (p.t.l.c.) on appropriate column fractions, small amounts of five polyacetylenes not previously shown to be metabolites of this fungus.[6] Three of these were suspected from their polarities and u.v. spectra to be ester (2), diol (9), and diol (10). This was confirmed by direct comparison with authentic samples. A sample of ester (2) was already to hand and the diols (9) and (10) were synthesised by oxidative coupling of *E*-pent-2-en-4-yn-1-ol and by Chodkiewicz coupling of pent-4-yn-1-ol with *E*-5-bromopent-2-en-4-yn-1-ol respectively. Attempts to separate the other two metabolites failed. The mixture was, nevertheless, easily shown by spectral measurements to consist of the isomeric hydroxymatricaria esters (7) and (8) (3 : 2). The ^{1}H n.m.r. spectrum was particularly helpful. The chemical shifts and coupling constants for the olefinic protons indicated that of the four carbon–carbon double

[1] J. D. Bu'Lock in 'Comparative Phytochemistry,' ed. T. Swain, Academic Press, London, 1966, p. 79; J. D. Bu'Lock and G. N. Smith, *J. Chem. Soc.* (*C*), 1967, 332.
[2] Sir Ewart R. H. Jones, V. Thaller, and J. L. Turner, *J.C.S. Perkin I*, 1975, 424.
[3] Sir Ewart R. H. Jones, C. M. Piggin, V. Thaller, and J. L. Turner, *J. Chem. Research*, 1977, (*S*) 68; (*M*) 0744.
[4] G. C. Barley, A. C. Day, U. Graf, Sir Ewart R. H. Jones, I. O'Neill, R. Tachikawa, V. Thaller, and R. A. Vere Hodge, *J. Chem. Soc.* (*C*), 1971, 3308.
[5] P. Hodge, Sir Ewart R. H. Jones, and G. Lowe, *J. Chem. Soc.* (*C*), 1966, 1216.
[6] J. D. Bu'Lock, E. R. H. Jones, and W. B. Turner, *J. Chem. Soc.*, 1957, 1607.
[7] J. D. Bu'Lock, D. C. Allport, and W. B. Turner, *J. Chem. Soc.*, 1961, 1654.

bonds present, three were *E* and one was *Z*. The presence of two sets of doublets at δ 4.22 and 4.46 due to methylene groups indicated that the *Z*-double bond was next to a methylene group. The chemical shifts attributed to the *E,E*-hydroxy-ester (7) agree well with those reported for a synthetic sample.[8] The five metabolites discussed above were also detected in the cultures grown in Lancaster.

The ester (2) and *E,E*-hydroxy-ester (7) were previously [6] suspected to be present in *P. anthracophilus*, but diols (9) and (10) have not previously been detected and the *Z,E*-hydroxy-ester (8) is a new compound. Including the previously published results, *P. anthracophilus* is now definitely known to produce all except one of the eleven possible combinations summarised in Scheme 1. The exception is *E*-lachnophyllol (13), but an



SCHEME 1

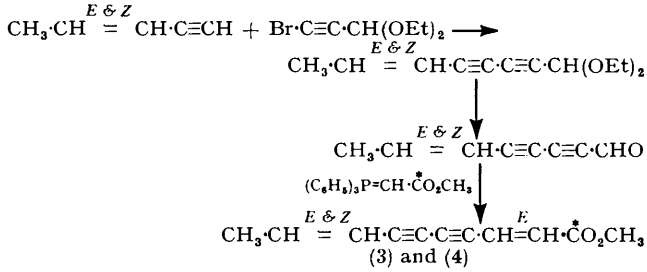
unidentified metabolite with the polarity and u.v. spectrum expected for this alcohol has been detected in *P. anthracophilus* cultures both by ourselves and previous workers.[6]

Following the tracer experiments (see below) it was suspected that the cultures grown in Lancaster produced not only *E,E*-matricaria ester (3) and *E,E*-matricarianol (11) but also their geometrical isomers. This proved to be so. The cultures were extracted and the extracts chromatographed as before. G.l.c. analysis of the matricaria ester fraction revealed the presence of three components in the ratio 5 : 4 : 1. Crystallisation of the fraction from pentane gave, as expected,[6] the *E,E*-ester (3). This was the largest of the three components. P.t.l.c. of the mother liquor permitted isolation of the smallest component. The spectroscopic data indicated the compound was *Z,Z*-matricaria ester (5) and the ^{1}H n.m.r. and u.v. spectra agreed well with those reported.[9,10] The p.t.l.c. also afforded an oil consisting of the third component and the *E,E*-ester (3) (4 : 1). The spectral data indicated that the new metabolite was the *Z,E*-ester (4). The ^{1}H n.m.r. and u.v. spectra were again in good agreement with those reported,[9,11] and the g.l.c. retention time was the same as that of the synthetic *Z,E*-ester (4) prepared as in Scheme 2. G.l.c. analysis of the extracts of the Oxford cultures showed the three esters (3), (4), and (5) were present in the ratio 3 : 1 : 1.

Crystallisation of the matricarianol fraction from pentane gave, as expected,[6] *E,E*-matricarianol (11). ^{1}H N.m.r. analysis of the mother liquors showed it contained not only the alcohol (11) but an equal amount of *Z,Z*-matricarianol (6). The presence of the latter was confirmed by comparison of the ^{1}H n.m.r. and other spectral data with that previously reported.[12] Analysis of the original matricarianol fractions showed the *E,E*- and *Z,Z*-isomers were present in the ratio 4 : 1. In extracts from Oxford cultures, the ratio was 1 : 2.

It should be noted that metabolites (4), (5), and (6) occur in relatively large amounts. They are of interest because almost all the previously isolated fungal polyacetylenes with 10 or less carbon atoms which contain an olefin bond, have the bond *E* rather than *Z*.[13] The latter is much more common in polyacetylenes from higher plants.[13] The *Z,Z*-ester (5) is, in fact, one of the commonest polyacetylenes in higher plants but it has not previously been found in fungi.[14]

Tracer Experiments.—The *E,E*-[1-^{14}C]matricaria ester (3) required for the incorporation experiments was synthesised as outlined in Scheme 2. This gave a mixture of labelled esters (3) and (4), each with the same specific activity, in the ratio 36 : 64. Attempts to isomerise ester (4) to ester (3) failed. At the time, the presence of the various metabolites with a *Z*-double bond was not suspected, so the mixture of esters (3) and (4) was used in the incorporation experiments.



SCHEME 2

The mixture of labelled esters was fed to cultures of *P. anthracophilus* when the rate of production of alcohol (11) was relatively high. Seven days later, the major metabolites were isolated using the usual procedures. The matricaria ester fraction contained only 16% of the activity fed to the fungus so both labelled esters were metabolised to a considerable extent.

The fraction containing the diester (12) contained 1.5% of the activity fed to the organism. The pure active diester (12) was converted into sebacic acid and this was decarboxylated. The carbon dioxide had a molar activity of 48% that of the sebacic acid. Hence the diester (12) was specifically labelled. This result indicates that *P. anthracophilus* can convert one or both of the matricaria esters (3) and (4) into the diester (12). One or both of the hydroxymatricaria esters (7) and (8) are probably intermediates in the oxidation of the terminal methyl group to a carboxy-group. Tracer

[9] I. W. Farrell, J. W. Keeping, M. G. Pellatt, and V. Thaller, *J.C.S. Perkin I*, 1973, 2642.
[9] F. Bohlmann, T. Burkhardt, and C. Zdero, 'Naturally Occurring Acetylenes,' Academic Press, London and New York, 1973, p. 300.
[10] Ref. 9, p. 13.
[11] T. Bruun, P. K. Christensen, C. M. Haug, J. Stene, and W. A. Sorensen, *Acta Chem. Scand.*, 1951, **5**, 1244.
[12] F. Bohlmann and C. Zdero, *Chem. Ber.*, 1969, **102**, 1679.
[13] Ref. 9, chapters 2, 3, and 6.
[14] R. E. Bew, D.Phil. Thesis, Oxford, 1961.

experiments in other fungi indicate that the *E*-dehydromatricaria ester (1) can be oxidised similarly.[2,5]

The matricarianol fraction contained 45% of the activity fed to the organism. Crystallisation gave the *E,E*-alcohol (11) and this was recrystallised to constant activity. In doing so the apparent specific activity of the *E,E*-alcohol (11) fell sharply, indicating the presence of a second metabolite of very high specific activity. This was shown (see above) to be *Z,Z*-matricarianol (6). The ratio of *E,E*- to *Z,Z*-alcohol in the extract was 3.5 : 1. Of the activity fed to the fungus, 15% was incorporated into *E,E*-matricarianol (11) and 30% into *Z,Z*-matricarianol (6). To locate the site of the activity in the *E,E*-alcohol (11), the material of constant activity was transformed through n-decanol to n-decanoic acid which was then subjected to a Schmidt decarboxylation. The carbon dioxide had the same molar activity as the alcohol (11); the n-nonylamine hydrochloride was inactive. The mixture of alcohols (11) and (6) in the mother liquors obtained from the crystallisation of alcohol (11) was degraded similarly with the same result. Hence one or both of the labelled esters (3) and (4) are specifically incorporated into *E,E*- and *Z,Z*-matricarianol (11) and (6) respectively. Both results show that *P. anthracophilus* has the ability to reduce the methoxycarbonyl group to a hydroxymethyl group. The latter result also shows the fungus can convert an *E*-2,3-olefinic bond to a *Z*-double bond. The different incorporations of activity into the two alcohols suggest they are not rapidly interconverted in the fungus and it may well be that the only rapid isomerisation that occurs in that of the C(2)–C(3) double bond when it is next to a methoxycarbonyl or closely related group. If this is so, the *E,E*-ester (3) would be the main precursor of *E,E*-alcohol (11) and the *Z,E*-ester (4) would, in the tracer experiments, be the main precursor of *Z,Z*-alcohol (6).

The fraction containing the hydroxy-ester (14) contained 0.6% of the activity fed to the organism but it was not sufficiently pure to justify degradation.

The above tracer results support the view that one or both of the matricaria esters (3) and (4), or close relatives, are intermediates in the biosynthesis of many of the C_{10} polyacetylenes in *P. anthracophilus*.

EXPERIMENTAL

Ultraviolet spectra were measured for solutions in ether. Unless indicated otherwise ^{1}H n.m.r. spectra were measured at 100 MHz for solutions in carbon tetrachloride. P.t.l.c. was carried out with 0.5-mm layers of Kieselgel HF_{254} nach Stahl and light petroleum (b.p. 40—60 °C)–ether mixtures as eluants. G.l.c. was carried out using a Pye 104 machine (flame ionisation detector) with a 5 ft column containing Apiezon L as the stationary phase. Counting methods were as described previously,[5] activities determined by scintillation counting being expressed in disintegration per second (d.p.s.) and activities determined by counting ' infinitely thick ' films being expressed as μCi/mmol.

Growth of P. anthracophilus *and General Extraction Procedures.*—The fungus was grown in 2-l penicillin flasks (usually 20 flasks per batch) as surface cultures supported on glass wool. In Oxford, the aqueous medium (750 ml) contained malt extract (30 g/l). In Lancaster the aqueous medium contained malt extract (17 g/l) supplemented with peptone (3 g/l). The fungus was incubated at 25 °C for from 40 to 120 days, polyacetylene production being monitored by u.v. spectroscopy. When the yield of polyacetylenes was judged to be optimal the neutral metabolites were extracted from the culture fluid and the extracts chromatographed on silica or alumina essentially as described before.[6] With mixtures of light petroleum (b.p. 40—60 °C) and ether, the order of elution of the major metabolites was as follows: a mixture of matricaria esters (3)—(5), lachnophyllum ester (2), diester (12), a mixture of matricarianols (6) and (11), probably lachnophyllum alcohol (13), a mixture of hydroxymatricaria esters (7) and (8), and hydroxylachnophyllum ester (14). Elution with methanol afforded a mixture of diols (9) and (10). The proportions of the metabolites varied considerably from growth to growth and in some growths certain metabolites were not detected.

Isolation of E,E-, E,Z-, *and* Z,Z-*Matricaria Esters* (3), (4), *and* (5) *respectively.*—These metabolites were isolated from Lancaster cultures 120 days after inoculation. The matricaria ester fraction from the column chromatography was crystallised from pentane at −78 °C and the crystallate was recrystallised several times to give *E,E*-matricaria ester (3) as a white solid, m.p. 62—63 °C (lit.,[6] m.p. 60—61 °C); $\lambda_{max.}$ 312 and 333 nm (log ε 4.25 and 4.20), $\lambda_{infl.}$ 295 nm (log ε 4.20); δ 1.86 (dd, *J* 1.5 and 7 Hz, CH_3), 3.71 (s, OCH_3), 5.56br (d, *J* 16 Hz, 8-H), 6.23 (d, *J* 16 Hz, 2-H), 6.33 (dq, *J* 7 and 16 Hz, 9-H), and 6.75 (d, *J* 16 Hz, 3-H); *m/e* 174 (M^+). The ester was pure by g.l.c. analysis.

P.t.l.c. of the mother liquors from the crystallisation of the *E,E*-ester afforded two fractions. The more polar fraction, a colourless oil pure by g.l.c. analysis, was identified as the *Z,Z*-matricaria ester (5). It had $\lambda_{max.}$ 312 and 333 nm (log ε 4.17 and 4.08), $\lambda_{infl.}$ 295 nm (log ε 4.00); *m/e* 174 (M^+); δ 1.95 (dd, *J* 1.5 and 7 Hz, CH_3), 3.70 (s, OCH_3), 5.55br (d, *J* 12 Hz, 8-H), 6.10 (s, 2-H and 3-H), and 6.21 (dq, *J* 7 and 12 Hz, 9-H). This spectral data agrees well with that reported for this ester.[9,10]

The less polar fraction, a colourless oil, was identified as a mixture of 2*E*,8*Z*- and *E,E*-matricaria esters (ratio 4 : 1 by g.l.c. analysis). The mixture had $\lambda_{max.}$ 312 and 333 nm (log ε 4.15 and 4.10), $\lambda_{infl.}$ 295 nm (log ε 4.08); *m/e* 174 (M^+). The ^{1}H n.m.r. spectrum had signals due to the *E,Z*-isomer (4) at δ 1.92 (dd, *J* 1.7 and 7 Hz, CH_3), 3.71 (s, OCH_3), 5.52br (d, *J* 12 Hz, 8-H), 6.19 (dq, *J* 7 and 12 Hz, 9-H), 6.22 (d, *J* 16 Hz, 2-H), and 6.75 (d, *J* 16 Hz, 3-H). This spectroscopic data agrees well with that reported in the literature.[9,11] By g.l.c. analysis, the ester had the same retention time as an authentic sample prepared as in Scheme 2.

G.l.c. analysis of the original matricaria ester fractions from Lancaster and Oxford cultures gave the results described in the text.

Isolation of the Lachnophyllum Ester (2).—This metabolite was isolated from Oxford cultures 40 days after inoculation. Column fractions rich in ester (2) were subjected to p.t.l.c. This gave an ethereal solution having $\lambda_{max.}$ 214, 223, 256, 271, 287, and 305 nm; *m/e* 176 (M^+, 100%); δ (60 MHz) 1.00 (t, *J* 8 Hz, CH_2), 1.4—1.7 (m, 9-H), 2.37 (t, *J* 6 Hz, 8-H), 3.78 (s, OCH_3), 6.25 (d, *J* 16 Hz, 3-H), and 6.77 (d, 16 Hz, 2-H). The spectra and t.l.c. R_F values in two solvent systems were identical with those

of synthetic ester (2) previously prepared in Oxford by G. Reid.

Isolation of E,E- *and* Z,Z-*Matricarianol* (11) *and* (6).—These metabolites were isolated from Lancaster cultures 120 days after inoculation but they were also detected in Oxford cultures 45 days after inoculation. The matricarianol fraction from the column chromatography was crystallised from pentane at −78 °C and the crystallate was recrystallised several times to give *E,E*-matricarianol (11) as a white solid, m.p. 103—104 °C (lit.,[6] 105.5—106.5°); $\lambda_{max.}$ 275, 293, and 312 nm (log ε 4.20, 4.42, and 4.32); δ 1.38 (s, OH), 1.82 (dd, *J* 1.5 and 7 Hz, CH_3), 4.22 (dd, *J* 1.8 and 5 Hz, CH_2), 5.58br (d, *J* 16 Hz, 8-H), 5.83br (d, *J* 16 Hz, 3-H), and 6.13—6.58 (m, 2-H and 9-H); *m/e* 146 (M^+). Evaporation of the mother liquors gave a clear oil which had $\lambda_{max.}$ 275, 293, and 312 nm (log ε 4.18, 4.45, and 4.28); *m/e* 146 (M^+). The ^{1}H n.m.r. spectrum indicated the presence of *E,E*-matricarianol (11) and *Z,Z*-matricarianol (6) (ratio 1 : 1). The peaks due to latter were at δ 1.23 (s, OH), 1.94 (d, *J* 1.7 Hz, CH_3), 4.40 (dd, 1.3 and 6.5 Hz, CH_2), 5.50br (d, *J* 11 Hz, 8-H), 5.61br (d, *J* 11 Hz, 3-H), and 6.0—6.5 (m, 2-H and 9-H). This spectral data agrees well with that previously reported.[12]

Isolation of E,E- *and* E,Z-10-*Hydroxymatricaria Esters* (7) *and* (8).—A mixture of these metabolites was isolated from Oxford cultures 45 days after inoculation. The column fractions containing the hydroxy-esters were subjected to p.t.l.c. in an unsuccessful attempt to resolve the mixture. The mixture was obtained as a colourless oil, $\nu_{max.}$(CCl_4) 3 540, 3 400, 2 200, 1 730, and 955 cm^{-1}; $\nu_{max.}$(CS_2) 3 520, 3 400, 1 730, 955, and 735 cm^{-1}; $\lambda_{max.}$ 246, 259, 310, and 332 nm (log ε 4.45, 4.40, 4.33, and 4.27), $\lambda_{infl.}$ 235 and 295 nm (log ε 4.41 and 4.25); *m/e* 190 (M^+, 100%) (Found: C, 69.2; H, 5.5. $C_{11}H_{10}O_3$ requires C, 69.5; H, 5.3%). The u.v. spectrum and the mass spectrum (including the detailed breakdown pattern) are similar to those reported for a synthetic sample of *E,E*-10-hydroxymatricaria ester (7).[8]

The ^{1}H n.m.r. spectrum indicated that the *E,E*- and *E,Z*-isomers were present in the ratio of 3 : 2. The peaks that were due to both isomers were at δ 1.19 (s, OH), 3.74 (s, OCH_3), 6.27 (dd, *J* 1 and 16 Hz, 2-H), and 6.79 (d, *J* 16 Hz, 3-H). The peaks that were due to the *E,E*-isomer were at δ 4.22 (dd, *J* 2 and 5.5 Hz, CH_2), 5.82br (d, *J* 16 Hz, 8-H), and 6.32br (d, *J* 16 Hz, 9-H). These shift values agree well with those reported for a synthetic sample.[8] The peaks due to the 2*E*,8*Z*-isomer were at δ 4.40 (dd, *J* 1.7 and 6.5 Hz, CH_2), 5.60br (d, *J* 11 Hz, 8-H), and 6.21br (d, *J* 11 Hz, 9-H).

Isolation of E,E-*Deca*-2,8-*diene*-4,6-*diyne*-1,10-*diol* (9) *and* E-*Dec*-2-*en*-4,6-*diyne*-1,10-*diol* (10).—These metabolites were isolated from Oxford cultures 60 days after inoculation. The diol fractions from the column chromatography were applied to a p.t.l.c. plate. Elution with ethyl acetate–dichloromethane (1 : 1) separated the mixture into two components. The less polar (9 mg) was a white solid, m.p. 156—158 °C, $\lambda_{max.}$ 218, 232, 237, 247, 262, 277, 294, and 313 nm; *m/e* 162 (M^+ corresponding to $C_{10}H_{10}O_2$). When this diol (2 mg) in ether was treated with active manganese dioxide for 6 h the u.v. spectrum changed to $\lambda_{max.}$ 223, 279, 305, 326, and 350 nm, indicating that both hydroxy-groups were allylic. The metabolite was identical (m.p., u.v., u.v. after oxidation, mass spectral fragmentation pattern) with a synthetic sample of *E,E*-deca-2,8-diene-4,6-diyne-1,10-diol (9), m.p. 157—158 °C (lit.,[14] 156—157 °C), prepared by oxidative dimerisation of *E*-pent-2-en-4-yn-1-ol.

The more polar diol (4 mg) was an oil with $\lambda_{max.}$ 231, 241, 253, 269, and 285 nm; *m/e* 164 (M^+ corresponding to $C_{10}H_{12}O_2$). When this diol was oxidised in the above manner, the u.v. spectrum changed to $\lambda_{max.}$ 224, 276, 293, and 313 nm indicating that only one hydroxy-group was allylic. The metabolite was identified (u.v., u.v. after oxidation, mass spectral fragmentation pattern) with a synthetic sample of *E*-dec-2-ene-4,6-diyne-1,10-diol (10) prepared by Chodkiewicz coupling of *E*-5-bromopent-2-en-4-yn-1-ol and pent-4-yn-1-ol.

Synthesis of E,E- *and* 2E,8Z-[1-^{14}C]*Matricaria Esters* (3) *and* (4).—The reaction scheme is outlined in Scheme 2. Chodkiewicz coupling of a mixture of *E*- and *Z*-pent-2-en-4-yne [15] and 3-bromoprop-2-ynal diethyl acetal (prepared by treating propiolic aldehyde diethyl acetal [16] with sodium hypobromite) gave a mixture of *E*- and *Z*-oct-6-ene-2,4-diynal diethyl acetal (87% yield) as an oil. The acetal grouping was hydrolysed (22%) by treatment with a mixture of conc. sulphuric acid, acetic acid, and water (3 : 1 : 3 v/v) at 20 °C for 2 h and the unstable aldehyde so formed allowed to react for 12 h at 20 °C with [1-^{14}C]methoxycarbonylmethylenetriphenylphosphorane [17] in ether. Column chromatography of the product gave a mixture of labelled *E,E*- and 2*E*,8*Z*-matricaria esters (55% yield). By g.l.c. analysis the ratio of isomers was 36 : 64. By radio-g.l.c. the ratio of the activities of the esters was 35 : 65. The mixture of esters had an activity of 2.9 × 10^7 d.p.s./mmol.

Incorporation of the Mixture of [1-^{14}C]*Matricaria Esters* (3) *and* (4) *into Metabolites of* P. anthracophilus.—The mixture of labelled esters (6 mg, 1.01 × 10^6 d.p.s.) in ethanol (18 ml) was distributed equally between 6 flasks of cultures of *P. anthracophilus* 94 days after inoculation. At this time, the concentration of hydroxy-ester (14) was falling slowly but the concentration of matricarianol (6) and (11) was increasing rapidly. Seven days later, the metabolites were extracted and separated by column chromatography using the usual procedure. This afforded the following fractions. (i) A mixture of matricaria esters (12.5 mg) with total activity 1.61 × 10^5 d.p.s. (16% of activity fed). By radio-g.l.c. analysis *E,E*- and 2*E*,8*Z*-matricaria esters (3) and (4) were present in the ratio 1.1 : 1 with activities in the ratio 1.4 : 1. (ii) Diester (12) (12.0 mg) with total activity 1.5 × 10^4 d.p.s. (1.5% incorporation). (iii) A mixture of matricarianols (129 mg) with total activity 4.6 × 10^5 d.p.s. (46% incorporation). (iv) Hydroxy-ester (14) (3.1 mg) with total activity 3.4 × 10^3 d.p.s. (0.3% incorporation).

Degradation of the Diester (12).—The diester (12) isolated from the incorporation experiment was recrystallised twice from pentane to give a white solid (8.4 mg), m.p. 97—100 °C. This was diluted with pure inactive diester (140 mg) and the mixture recrystallised to constant activity (76.1 d.p.s./mg). The pure diester was hydrogenated, the dimethyl sebacate hydrolysed, and the acid decarboxylated using the procedure described previously.[5] The sebacic acid had an activity of 0.451 μCi/mmol; the barium carbonate had an activity of 0.216 μCi/mmol.

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Degradation of E,E- *and* Z,Z-*Matricarianols* (11) *and* (6).—The mixture of matricarianols (129 mg) isolated from the incorporation experiment was analysed by ^{1}H n.m.r. spectroscopy. It contained the *E,E*-isomer (101 mg) and the *Z,Z*-isomer (28 mg).

To estimate the specific activity of the *E,E*-isomer the above material was recrystallised several times from pentane. The mixture obtained consisted of the *E,E*-isomer (82 mg) and the *Z,Z*-isomer (6 mg). Pure inactive *E,E*-isomer (82 mg) was added and the *E,E*-isomer recrystallised 5 times. The specific activity fell from 1 120 d.p.s./mg to a constant value of 766 d.p.s./mg. The latter corresponds to an activity for the undiluted *E,E*-isomer of 1.53×10^5 d.p.s./mg and an incorporation of 15%. The pure *E,E*-isomer (3.02 μCi/mmol) was hydrogenated, the decanol oxidised and the decanoic acid decarboxylated using the procedure previously described.[5] The barium carbonate had an activity of 3.03 μCi/mmol, and the n-nonylamine hydrochloride an activity of 0.01 μCi/mmol.

The mother liquors from the initial recrystallisation contained, by ^{1}H n.m.r. analysis, the *E,E*-isomer (19 mg) and the *Z,Z*-isomer (22 mg). The mixture had a specific activity of 6 120 d.p.s./mg. Hence the *Z,Z*-isomer had an activity of 10 920 d.p.s./mg, corresponding to an incorporation of 30%. Degradation of the mixture as above gave barium carbonate with an activity of 26.3 μCi/mmol and n-nonylamine hydrochloride with an activity of 0.17 μCi/mmol.

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