Natural Acetylenes. Part XIX.[1] Metabolites from Some *Poria* Species

By R. E. Bew, R. C. Cambie, Sir Ewart R. H. Jones, and G. Lowe

Nemotinic acid (I; R=H) and nemotin (II) have been isolated from the culture media of *Poria subacida*, *P. colorea*, and *P. mutans*. *P. subacida* also yields methyl nemotinate (I; R=Me).

The ene-diyne hydroxy-acid (V; R=H) has been obtained from *P. selecta*, and the all-*trans*-C_{12}-tetraene diol (VI) from *P. pearsonii*. The latter compound is also elaborated by the fungus *Coprinus flocculosus*. *Daedalea juniperina* produces several polyenes, one of which has been identified as all-*trans*-decatetraenol (VII).

Of the three *Poria* species hitherto investigated, two, *P. corticola*[2] and *P. tenuis*,[2] have been shown[3] to elaborate the C_{11} metabolites nemotinic acid (I; R = H) and nemotin (II). The third, *P. sinuosa*, did not produce either of these but instead a complex mixture of at least

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

(II) $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}\overline{CH{\cdot}CH_2{\cdot}CH_2{\cdot}COO}$

(III) $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{t}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$

(IV) $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Me$

(V) $HO{\cdot}CH_2{\cdot}CH{=}^{t}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CO_2R$

(VI) $HOCH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH_2OH$

(VII) $CH_3{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH{=}^{t}CH{\cdot}CH_2{\cdot}OH$

ten polyacetylenes, seven of which had not previously been isolated.[4] During our screening of the Basidiomycetes, a further four *Poria* species have been found to produce polyacetylenic and polyenic metabolites.

The first three species examined, *P. subacida*, *P. colorea*, and *P. mutans*, all yielded nemotinic acid and nemotin but *P. subacida* produced, in addition, methyl nemotinate (I; R = Me). This ester, previously prepared from the acid (I; R = H),[3] had not been isolated from a fungus. Although it was never obtained completely free of nemotin, its presence was proved by alkali isomerisation to a mixture of nemotin A (III) and the triyne, methyl isonemotinate (IV).[5] The principal polyacetylenic metabolite of *P. selecta* was acidic, and after isolation as its methyl ester (0·7 mg./l. of culture fluid) was identified as either *trans*-9-hydroxynon-7-ene-3,5-diynoic acid (V; R = H) or *trans*-9-hydroxynon-3-ene-5,7-diynoic acid, by its ultraviolet and infrared spectra and by comparative gas–liquid chromatography of a perhydro-derivative. Oxidation of the hydroxy-ester with manganese dioxide gave a diyne-ene aldehyde with an ultraviolet spectrum very similar to that of *trans*-9-hydroxynon-2-ene-4,6-diyn-1-al obtained during the investigation of a metabolite of the fungus, *P. sinuosa*.[4] The alternative ene-diyne aldehyde would be expected to exhibit somewhat different ultraviolet absorption. When the hydroxy-ester was treated briefly with lithium aluminium hydride an allene ($\nu_{max.}$ 1942 cm.$^{-1}$) was formed, as expected from (V; R = Me),[6] together with

[1] Part XVIII, preceding Paper.

[2] M. Anchel, J. Polatnick, and F. Kavanagh, *Arch. Biochem.*, 1950, **25**, 208; F. Kavanagh, A. Hervey, and W. J. Robbins, *Proc. Nat. Acad. Sci. U.S.A.*, 1950, **36**, 1.

[3] J. D. Bu'Lock, E. R. H. Jones, and P. R. Leeming, *J. Chem. Soc.*, 1955, 4270.

[4] R. C. Cambie, J. N. Gardner, E. R. H. Jones, G. Lowe, and G. Read, *J. Chem. Soc.*, 1963, 2056.

[5] J. D. Bu'Lock, E. R. H. Jones, P. R. Leeming, and J. M. Thompson, *J. Chem. Soc.*, 1956, 3767.

[6] E. B. Bates, E. R. H. Jones, and M. C. Whiting, *J. Chem. Soc.*, 1954, 1854.

a small amount of an ene-diyne diol. The latter, separated by chromatography, proved to be *trans*-non-2-ene-4,6-diyne-1,9-diol.[4]

The hydroxy-acid (V; R = H) was synthesised from but-3-yn-1-oic acid[7] and *trans*-5-bromopent-2-en-4-yn-1-ol. In previous couplings[8] where *trans*-pent-2-en-4-1-ol was one of the components, it has been more usual to carry out the reaction using the α-bromoacetylenic derivative of the second component rather than that of the pentenynol. In the present case, however, preparation of 4-bromobut-3-yn-1-oic acid was not possible using a hypohalous acid salt[9] since the parent acid is isomerised even by potassium carbonate.[10] The synthetic acid was converted to its methyl ester (V; R = Me), which had properties identical to those of the ester of the natural product. This metabolite is a further exception[11] to the generalisation[12] that fungal polyacetylenes containing an odd number of directly-linked carbon atoms possess a terminal ethynyl group.

During attempts to supplement the yields of polyacetylenes from *P. selecta* the fungus was grown on a 4% glucose solution as reflood medium. Spectroscopic estimations revealed only traces of ene-diyne compounds (0·03 mg./l.) and ethereal extracts contained only oxalic acid. Oxalic acid was also isolated when *P. carbonica* was grown on a malt medium and, as in the above case, only traces of polyacetylenes were produced.

In contrast to other *Poria* species, the culture medium of *P. pearsonii* was found to contain polyenic rather than polyacetylenic material. The polyenic material (0·2 mg./l.) was neutral, and consisted of a single compound which was readily purified. The same compound was obtained as the only isolated product from the culture medium of the fungus, *Coprinus flocculosus*. Here it was produced to the extent of *ca.* 2 mg./l. in both initial and reflood cultures and was readily isolated by chromatography. Analytical and spectroscopic data indicated that the compound ($C_{12}H_{18}O_2$) was an "all *trans*" tetraene diol (cf. ref. 13). Oxidation with manganese dioxide produced a chromophore typical of a tetraene aldehyde.[14] Hydrogenation with the uptake of 4 mols./mol. of hydrogen gave dodecane-1,12-diol, identical with an authentic sample. The structure was thus unequivocally established as "all-*trans*"-dodeca-2,4,6,8-tetraene-1,12-diol (VI). Excluding carotenoids and macrolide polyenes this represents only the second isolation of straight-chain aliphatic polyenic material from a fungus, the C_{14}-hexa-ene dicarboxylic acid, corticrocin having been isolated previously from *Corticium croceum* Bres. by Erdtman.[15] However, several polyenes have recently been isolated[16] from the fungus *Daedalea juniperina* grown on Birkinshaw's synthetic medium containing 0·5% mycological peptone, one of which has been shown to be "*all-trans*"-deca-2,4,6,8-tetraenol (VII), m. p. 110—111°, $\lambda_{max.}$ 3110 (ε 63,500), 2970 (ε 74,000), 2845 (ε 45,000), and 2745 Å (ε 21,500), $\nu_{max.}^{t}$ 3515 (OH), 995 cm.$^{-1}$ (C=C), which on catalytic hydrogenation gave n-decanol, on oxidation with manganese dioxide gave decatetraenal ($\lambda_{max.}$ 3495, 3335, 3200 Å), and was unchanged after treatment with iodine.

EXPERIMENTAL

For general experimental methods and conditions of culture growth see Part X[17] and later Parts of this Series.* Shake cultures were grown in penicillin flasks. The medium (750 c.c.) was inoculated with finely macerated mycelium and shaken at 80 r.p.m. Infrared spectra, unless otherwise stated, were measured in CS_2 on a Perkin-Elmer 21 instrument. The conditions for ultraviolet spectra, m.p.s, optical rotations and alumina chromatography were as described in the previous Paper unless otherwise stated. Unless otherwise stated, light petroleum refers to the fraction with b. p. 60—80°. Conditions for gas–liquid chromatography (g.l.c.) of perhydro-derivatives were those described in Part XIV.[4]

Poria subacida *Pk.*—The fungus was grown as a surface culture on a 3% malt medium, the polyacetylene content (10 mg./l.) being maximal after 27 days. Replacement of the medium with 0·2M-sodium acetate solution gave a maximum poly-yne concentration (70 mg./l.) after a further 14 days. The culture fluids (22 l.) were continuously extracted with ether for 24 hr. and the concentrated extract was distributed for 80 transfers in a 50 tube Craig-type counter-current apparatus between benzene and water. The addition of 2N-hydrochloric acid (0·1 c.c.) to each tube alleviated emulsion formation. The most polar fraction contained nemotinic acid (I; R = H) (94 mg.), $[\alpha]_D^{20}$ +300° (*c* 0·15 in CH_2Cl_2), which gave spectral data identical to those recorded.[3] Identification was confirmed by isomerisation with alkali to isonemotinic acid and by treatment with acid to give nemotin (II), each of which showed spectral characteristics identical to those recorded.[3,5] Isomerisation of the nemotin with alkali followed by hydrogenation and esterification gave methyl undecanoate, not differentiated from authentic material by g.l.c.

Methyl Nemotinate (I; R = Me).—Initial fractions from the above distribution contained neutral material (47 mg.) which was redistributed between cyclohexane and 10% aqueous ethanol for 50 transfers. This gave nemotin (II: 15 mg.), $[\alpha]_D^{20}$ +350° (*c* 0·2 in CH_2Cl_2) (correct spectroscopic data), and a less polar mixture (30 mg.) of nemotin and methyl nemotinate. The nemotin was identified as above.

[7] Sir Ian Heilbron, E. R. H. Jones, and F. Sondheimer, *J. Chem. Soc.*, 1949, 604.
[8] W. Chodkiewicz, *Ann. Chim.* (*France*), 1957, **2**, 819; J. Meier, W. Chodkiewicz, P. Cadiot, and A. Willemart, *Compt. rend.*, 1957, **245**, 1634.
[9] F. Strauss, L. Kollek, and W. Heyn, *Ber.*, 1930, **63**, 1868.
[10] G. Eglinton, E. R. H. Jones, G. H. Mansfield, and M. C. Whiting, *J. Chem. Soc.*, 1954, 3197.
[11] For other examples see E. R. H. Jones, P. R. Leeming, and W. Remers, *J. Chem. Soc.*, 1960, 2257, and ref. 4.
[12] E. R. H. Jones and J. S. Stephenson, *J. Chem. Soc.*, 1959, 2197.
[13] P. Nayler and M. C. Whiting, *J. Chem. Soc.*, 1954, 4006.
[14] E. R. Blout and M. Fields, *J. Amer. Chem. Soc.*, 1948, **70**, 189.
[15] H. Erdtman, *Acta Chem. Scand.*, 1948, **2**, 209; B. L. Shaw and M. C. Whiting, *J. Chem. Soc.*, 1954, 3217; B. C. L. Weedon, 1954, 4168.
[16] J. R. Chapman, D.Phil. Thesis, Oxford, 1963.
[17] J. N. Gardner, E. R. H. Jones, P. R. Leeming, and J. S. Stephenson, *J. Chem. Soc.*, 1960, 691.

The presence of methyl nemotinate was inferred from the following data for the purest fraction (estimated spectroscopically as 60% pure), $\lambda_{max.}$ (relative ε in brackets) 2780 (1·0), 2630 (1·2), 2495 (0·83), 2375 (0·46), 2090 Å (4·0); $\nu_{max.}$ 3590 (OH), 3290 (HC≡C), 1950 (CH=C=CH), 1795 (γ-lactone, weak), 1735 (ester C=O), 853 cm.$^{-1}$ (CH=C=CH). Isomerisation with 0·1N-sodium hydroxide for 15 min. at 20° gave a mixture of nemotin A (III) and methyl isonemotinate (IV), $\lambda_{max.}$ (relative ε in brackets), 3280 (1·0), 3070 (1·4), 2890 (1·1), 2720 (0·57), 2580 (0·27), 2410 (7·9), 2300 (5·6), 2080 Å (10·4); $\nu_{max.}$ 3460 (OH), 3420 (OH of CO_2H), 3290 (HC≡C), 1735 (ester C=O), 1710 cm.$^{-1}$ (CO_2H).

Poria colorea *Overh. et Englerth.*—The culture fluid (20 l.) of the fungus, grown on a 3% malt medium, produced a maximum concentration of polyacetylenes (1 mg./l.) after 33 days. Extraction with ether and separation with sodium hydrogen carbonate solution gave nemotinic acid (I; R = H; 16 mg.) and nemotin (II; 7 mg.), each identified as above.

Poria mutans *Pk.* (*with Dr. A. Hirschberg*).—The fungus was grown on a 3% malt medium and produced maximum polyacetylene concentration (12 mg./l.) after 35 days. The culture fluid (25 l.) was extracted and the extracts separated into acidic (211 mg.) and neutral (35 mg.) polyacetylenes in the usual manner. The acidic fraction had spectral data indicative of nemotinic acid but contaminated with some nemotin (from acid-catalysed cyclisation during acidification of alkaline solutions). Treatment of the fraction with 0·1N-sodium hydroxide gave a 96% conversion to isonemotinic acid (correct spectroscopic data).

The neutral fraction contained nemotin, identified as above and by co-distribution with an equal amount of authentic material between cyclohexane–30% aqueous ethanol for 50 transfers, whence a single smooth distribution curve was obtained.

Poria selecta *Karst. ex Rom.*—The concentrated ether extracts (2 × 1 l.) of two batches of culture fluid (each of 20 l.) of the fungus, grown on a 3% malt medium for 40 and 61 days, respectively, were extracted with saturated sodium hydrogen carbonate solution (3 × 25 c.c.). Spectroscopic examination showed that the neutral fraction contained compounds (8 mg.) with ene-diyne chromophores, but attempts to isolate pure compounds by alumina chromatography were unsuccessful. The fraction had $\lambda_{max.}$ (ether) 2810, 2660, 2520, 2420 Å; $\nu_{max.}$ 3704 (OH), 3497 (broad, OH), 1779 (γ-lactone), 1754 (non-conjugated ester C=O), 1733 (conjugated ester C=O), 1718, 1114 cm.$^{-1}$ (conjugated CH=O). The chromophore was unchanged when an aliquot was treated with 0·1N-sodium hydroxide for 2 hr. at 20°, and the compounds were not extracted into water from a benzene solution. Hydrogenation and g.l.c. examination showed the presence of n-nonanol and at least three other minor components.

Methyl trans-9-*Hydroxynon*-7-*ene*-3,5-*diynoate* (V; R = Me).—The acid fraction (29 mg.) (ene-diyne chromophore) was esterified with 5% sulphuric acid in methanol at 20° for 3 days and the neutral product (22 mg.) chromatographed on alumina. Fractions eluted with benzene crystallised from dichloromethane–hexane (charcoal) at −70° to yield methyl *trans*-9-hydroxynon-7-ene-3,5-diynoate as low melting plates (cf. synthetic material below). Infrared and ultraviolet absorption data were identical with those of the synthetic compound described below.

The ester (2 mg.) in methanol (10 c.c.) was hydrogenated over 10% palladium–charcoal (20 mg.) for 1 hr. at 20°. Removal of catalyst and solvent gave an oil which was dissolved in dry ether (2 c.c.) and treated with lithium aluminium hydride (5 mg.) at 20° for 5 min. The reaction was completed by brief warming and then excess of reagent was decomposed by addition of water. Acidification followed by extraction with ether (4 × 2 c.c.) gave an oil which was heated under reflux with methyl iodide (1 c.c.) and excess of silver oxide for 8 hr. The cooled mixture was filtered, the silver oxide washed with hot ether, and the solvent evaporated. Percolation of the residue in light petroleum solution (b. p. 30—40°) through alumina (2 g.) gave nonane-1,9-diol dimethyl ether (g.l.c. comparison).

The hydroxy-ester (1 mg.) in dichloromethane (5 c.c.) was shaken with activated manganese dioxide (10 mg.) at 20° for 2 hr. The recovered product, methyl *trans*-9-oxonon-7-ene-3,5-diynoate, had $\lambda_{max.}$ (relative ε in brackets) 3110 (1·0), 2930 (0·98), 2830 (0·97), 2670 (0·77), 2520 (0·66), 2220 Å (2·5).

The hydroxy-ester (16 mg.) in dry ether (5 c.c.) was treated with lithium aluminium hydride (10 mg.) at 20° for 15 min. Work-up in the usual manner and isolation *via* ether gave an oil, $\nu_{max.}$ (liquid film) 3636 (OH), 1942 (CH=C=CH), 948 cm.$^{-1}$ (*trans* CH=CH), which was chromatographed from benzene on alumina. Fractions eluted with benzene–ether (2 : 1) crystallised from ether–hexane (charcoal) to yield *trans*-non-2-ene-4,6-diyne-1,9-diol (4 mg.) as needles, m. p. 56—57° (decomp.), undepressed by a synthetic sample prepared as described previously,[4] (correct spectroscopic data).

Synthesis of trans-9-*Hydroxynon*-7-*ene*-3,5-*diynoic Acid.*—*But*-3-*yn*-1-*oic acid.* The following modification of the method of Heilbron, Jones, and Sondheimer [7] gave a higher yield. A chromic acid solution [from chromium trioxide (100 g.) and concentrated sulphuric acid (160 g.) made up to 500 c.c. with water] was added during 30 min. to a stirred and cooled 40% aqueous solution of but-3-yn-1-ol (38 g.) in acetone (150 c.c.) so that the temperature did not rise above 20°. Stirring was continued for 30 min., water added, and the solution saturated with sodium chloride and then extracted with ether (3 × 100 c.c.). The ether extracts were concentrated and extracted with saturated sodium hydrogen carbonate solution (3 × 100 c.c.) from which the product was obtained as a discoloured crystalline mass. Repeated extraction with light petroleum (b. p. 40—60°; 20 × 250 c.c.) gave solutions from which but-3-yn-1-oic acid rapidly crystallised as large rectangular plates (9 g., 40% yield), m. p. 84° (lit.,[7] m. p. 83—83·5°, 28% yield), $\nu_{max.}$ (Nujol) 3268 (HC≡C), 2618 (OH of CO_2H), 1725—1695 cm.$^{-1}$ (broad, CO_2H).

trans-5-*Bromopent*-2-*en*-4-*yn*-1-*ol.*—Freshly distilled and stirred *trans*-pent-2-en-4-yn-1-ol (1·0 g.) was cooled to 0° and treated dropwise with sodium hypobromite solution [10 c.c., prepared from a mixture of ice (50 g.), 10N-sodium hydroxide (20 c.c.), bromine (5 c.c.), and water (10 c.c.)]. Stirring was continued for 5 min., the crystalline precipitate collected and washed with ammonium chloride solution, and then with water. The dried solid was recrystallised from hexane with ice-cooling to yield needles of trans-5-*bromopent*-2-*en*-4-*yn*-1-*ol* (1·85 g., 94% yield), m. p. 37—38° (decomp.) (Found: C, 36·8; H, 2·8. C_5H_5BrO requires C, 37·3; H, 3·1%), $\nu_{max.}$ 3584 (OH), 2212 (disubstituted C≡C), 1370 and 1008 (C–O), 955 (sh.), and 948 cm.$^{-1}$ (*trans* CH=CH). The compound darkened and became oily on storage at −10° in the dark.

Synthetic Methyl trans-9-*Hydroxynon*-7-*ene*-3,5-*diynoate* (V; R = Me).—But-3-yn-1-oic acid (740 mg.) in methanol (1 c.c.) was added to a mixture of cuprous chloride (20 mg.) and 30% aqueous ethylamine (3 c.c.) (decolourised by the prior addition of a few crystals of hydroxylamine hydrochloride) while a rapid stream of nitrogen was passed through the stirred solution at 0°. *trans*-5-Bromopent-2-en-4-yn-1-ol (1·8 g.) in methanol (1 c.c.) was added, and the mixture kept at 0° for 5 min. and then at 20° for 10 min. with the further addition of hydroxylamine hydrochloride as required. The mixture was acidified with hydrochloric acid, extracted with ether (3 × 50 c.c.), and the acids separated *via* aqueous sodium hydrogen carbonate and ether extractions to yield a mixture of crystals and a pale yellow oil. The crystalline but-3-yn-1-oic acid, m. p. (and mixed) 81—83°, was removed by extraction with warm hexane and finally by washing an ethereal solution of the oil with aqueous silver nitrate solution.

The hydroxy-acid was esterified with 5% sulphuric acid *in* methanol (20 c.c.) for 3 days at 20° and the neutral fraction chromatographed from light petroleum–benzene (1 : 1) on alumina (50 g.). Fractions eluted with benzene gave *methyl* trans-9-*hydroxynon*-7-*ene*-3,5-*diynoate* (50 mg. 32% yield) which formed plates, m. p. 56° (decomp.), from dichloromethane–hexane (charcoal) at −70° (Found: C, 67·2; H, 5·8. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·7%), $\lambda_{max.}$ 2820 (ε 14,400), 2660 (ε 17,300), 2520 (ε 13,100), 2390 (ε 8600), 2260 (infl., ε 6700), 2140 Å (ε 13,400); $\nu_{max.}$ 3570 (OH), 1735 (non-conjugated ester C=O), 1330, 1250, 1040 (C–O), 952 (sh.) and 948 cm.$^{-1}$ (*trans* CH=CH).

trans-9-*Hydroxynon*-7-*ene*-3,5-*diynoic acid* (V). The above ester (20 mg.) in methanol (1 c.c.) was treated with 2N-sodium hydroxide and the solution kept at 20° for two days. The acid fraction, isolated *via* saturated sodium hydrogen carbonate solution, gave trans-9-*hydroxynon*-7-*ene*-3,5-*diynoic acid* (8 mg.) as an oil which could not be crystallised (Found: C, 65·3; H, 5·3. $C_9H_8O_3$ requires C, 65·85; H, 4·9%), $\lambda_{max.}$ 2820 (ε 14,500), 2660 (ε 18,500), 2520 (ε 14,500), 2390 (ε 10,000), 2270 (infl. ε 10,500), and 2130 Å (ε 50,500); $\nu_{max.}$ (Nujol) 3425 (OH), 2700—2500 (OH of CO_2H), 1712 (non-conjugated CO_2H), and 961 cm.$^{-1}$ (*trans* CH=CH).

Oxalic Acid.—The culture fluid (45 l.) of *P. selecta*, grown on a 4% glucose medium (29 days), was continuously extracted with ether to yield an extract which contained only traces of polyacetylenes (0·03 mg./l. of culture fluid) which exhibited ene-diyne chromophores. Evaporation and crystallisation from ethyl acetate yielded oxalic acid (12·6 g.), m. p. 189° (*p*-bromophenacylester, m. p. and mixed m. p. 241—242° decomp.).

Poria pearsonii *Pilat*.—The culture fluid (40 l.) of the fungus, grown on a 3% malt medium for 28 days, was continuously extracted with ether (2 × 1 l.) for 16 hr. The combined extracts, concentrated to suitable volume, were separated into neutral and acidic fractions. The latter exhibited no light absorption typical of polyacetylenic or polyenic material and gave no solid products after esterification with 5% sulphuric acid in methanol followed by chromatography on alumina. The neutral fraction showed typical tetraene spectral characteristics and was chromatographed on alumina.

All-trans-*Dodeca*-2,4,6,8-*tetraene*-1,12-*diol* (VI). Fractions eluted with light petroleum–ether (1 : 1) gave the *diol* (13 mg.) as plates, m. p. 125—128° (Found: C, 74·7; H, 9·0. $C_{12}H_{18}O_2$ requires C, 74·2; H, 9·3%), $\lambda_{max.}$ 3120 (ε 69,500), 2980 (ε 76,000), 2860 (ε 51,700), 2740 Å (infl., ε 25,800); $\nu_{max.}$ (Nujol) 3226 and 3145 (OH), 1374, 1081 (C–O), 998 cm.$^{-1}$ (conj. $\overset{t}{\text{CH}}$=CH). Oxidation of the compound (0·5 mg.) in dichloromethane solution (3 c.c.) with activated manganese dioxide for 48 hr. gave a product whose ethanolic solution had $\lambda_{max.}$ 3525 and 3440—3400 Å (infl.). The tetraene-diol (6·21 mg.) in methanol (5 c.c.) was hydrogenated with 10% palladium–charcoal (50 mg.) for 4 hr. at 12°. The uptake of hydrogen was 3·1 c.c. at N.T.P. corresponding to 4·3 mols./mol. Removal of catalyst and solvent, and crystallisation from hexane yielded dodecane-1,12-diol (6 mg.) as rods, m. p. (and mixed) 80—81° (Found: C, 71·4; H, 12·6. Calc. for $C_{12}H_{26}O_2$: C, 71·2; H, 12·9%). The infrared spectrum $\nu_{max.}$ (Nujol) 3320 (OH), 1362, 1350, 1057, 1040, 992 cm.$^{-1}$ (C–O), was identical with that of an authentic sample.

Coprinus flocculosus *D. C. sensu Romagn.*—The culture fluids (40 l.) of the fungus, grown on a 3% malt medium and reflooded with 4% glucose solution, for 35 and 20 days respectively, were worked-up in the usual manner. Chromatography of the neutral fraction on alumina gave all-*trans*-dodeca-2,4,6,8-tetraene-1,12-diol (20 mg.) in fractions eluted with ether–light petroleum (1 : 1). The compound had m. p. (and mixed) 124—125°, and spectroscopic data identical with those of the compound from *P. pearsonii*.

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The Dyson Perrins Laboratory,
Oxford University.

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