380. *Chemistry of the Higher Fungi. Part XIV.** *Polyacetylenic Metabolites of* Poria sinuosa *Fr.*

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The isolation and characterisation of ten polyacetylenic compounds from *Poria sinuosa* are described. Seven are new and include the C_{14} acid (VII; R = H), which has the longest carbon chain of any metabolite of this type yet found, and the first polyacetylenic *N*-acyl derivative (VI; R = H) of an α-amino-acid.

The Basidiomycete fungus *Poria sinuosa* Fr. produces a variety of polyacetylenic metabolites, most of which have not been encountered hitherto. This study has taken several years to complete; conditions of growth are particularly difficult to reproduce and the various metabolites cannot be regularly isolated in constant proportions.

The extraction and fractionation of the extracts are described in the Experimental section and summarised in the Table. The ten metabolites isolated and characterised are represented by formulæ (I—X; R = H). Of these compounds, all but (I), (II), and (IV) are new.

$$\text{(I)}\quad CH_3{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$$

$$\text{(II)}\quad HC{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$$

$$\text{(III)}\quad HO{\cdot}CH_2{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$$

$$\text{(IV)}\quad HO{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$$

$$\text{(V)}\quad HC{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$$

$$\text{(VI)}\quad HC{\equiv}C{\cdot}C{\equiv}C\overset{t}{\cdot}C{\equiv}C{\cdot}CH{=}CH{\cdot}CO{\cdot}NH{\cdot}CH(CO_2R){\cdot}CHMe_2$$

$$\text{(VII)}\quad RO_2C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CH\overset{c}{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2R$$

$$\text{(VIII)}\quad RO_2C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CO_2R$$

$$\text{(IX)}\quad HO{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CH(OH){\cdot}CO_2R$$

$$\text{(X)}\quad HO{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}CH(OH){\cdot}CO_2R$$

The alcohol (I) has previously been obtained from *Pleurotus ulmarius*,[1] and also from *Tricholoma grammopodium*, *Clitocybe obbata* and *C. candida*;[2] alcohols (II) and (IV) have been isolated from *Coprinus quadrifidus*.[3] Before its isolation from *P. sinuosa*, the acid (V; R = H) had also been obtained[4] from *Psilocybe sarcocephala*, which also produces the corresponding alcohol (II).

Of the new compounds the diacid (VII; R = H) is of special interest as it contains the longest carbon chain (C_{14}) yet found in fungal polyacetylenes and because it possesses the rare structural feature of unsaturation separated from the main chromophore; several examples are now known amongst plant polyacetylenes.[5] It was the major acid present in the culture fluids and was eventually isolated by countercurrent distribution between light petroleum–ether (3 : 1) and phosphate buffer, after it had been found that its diester (VII; R = Me) decomposed on alumina. The acid was only obtained as an amorphous powder which rapidly decomposed at 20°. Homogeneity was established by the symmetrical distribution curve of its diester and, although no melting point of the

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* Part XIII, preceding paper.

[1] Gardner, Jones, Leeming, and Stephenson, *J.*, 1960, **691.**

[2] Cambie, Chapman, Hirschberg, Jones, and Lowe, unpublished work.

[3] Jones and Stephenson, *J.*, 1959, 2197.

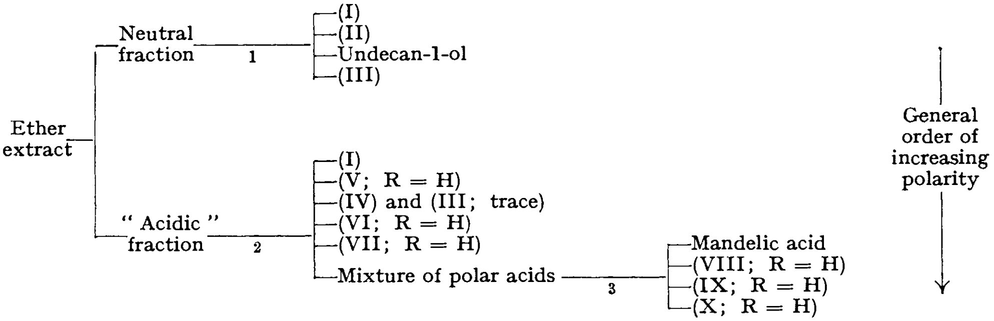
[4] Jones, *Proc. Chem. Soc.*, 1960, 199; Jones and Remers, unpublished work.

[5] Bohlmann, Arndt, Bornowski, Jastrow, and Kleine, *Chem. Ber.*, 1962, **95**, 1320.

ester could be determined as it decomposed even more rapidly than the acid itself, by reproducible ultraviolet absorption intensities after successive crystallisations.

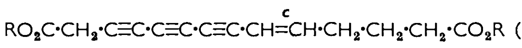
The ultraviolet spectra of the diacid (VII; R = H) and its diester (VII; R = Me) showed a triyne-carbonyl chromophore and each compound possessed a sharp band in the infrared spectra at *ca.* 740 cm.$^{-1}$ which could be assigned to a *cis*-double bond [6] but probably arose from the grouping $C{\equiv}C{\cdot}CO_2R$.[7] Carbonyl bands in the infrared spectrum of the diester (VII; R = Me) at 1707 and 1731 cm.$^{-1}$ showed the presence of both conjugated and non-conjugated ester groupings. Hydrogenation of the diacid and of the diester yielded tetradecanedioic acid and its dimethyl ester, respectively, thus establishing the chain length. Because of the great instability of the acid and its diester analytical data were of little value. However, titration gave a satisfactory equivalent weight while the presence of an isolated double bond and its location were indicated by the paper-chromatographic identification of glutaric acid in the products of acidic or alkaline permanganate oxidation. In the alkaline oxidation succinic and traces of malonic acid were also formed and although glutaric acid was unaffected under the reaction conditions the formation of lower homologues during degradation of the poly-yne is not surprising (cf. ref. 8).

Fractionation of *Poria sinuosa* extracts.



1, Chromatography on Al_2O_3. 2, Countercurrent distribution between light petroleum–ether and M/15-KH_2PO_4. 3, Countercurrent distribution between ether and M/15-KH_2PO_4.

The structure of the diacid (VII; R = H) was unequivocally established by the observation that its chromophore was changed to that of a conjugated enetriyne on treatment with alkali. This product was isolated by alkali-treatment of the crude acid fraction from *P. sinuosa* followed by countercurrent distribution and, although analytical data were not obtained, its diester, isolated by similar isomerisation of the ester (VII; R = Me), was fully characterised. Its infrared spectrum clearly showed the presence of a *cis*-double bond (732 cm.$^{-1}$) and carbonyl absorption only at 1735—1740 cm.$^{-1}$ consistent with


$$RO_2C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{c}{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2R$$ (XI)

the structure (XI; R = Me) for the isomerised product. It followed that the naturally occurring acid was *cis*-tetradec-9-ene-2,4,6-triynedioic acid (VII; R = H). The isomerisation of an αβ- to a βγ-acetylenic acid is a well-authenticated rearrangement [9] and di- and tri-acetylenic acids behave similarly; [10] with the acid (VII; R = H) rearrangement is facilitated by the double bond.

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[6] Bellamy, " The Infra-red Spectra of Complex Molecules," Methuen and Co. Ltd., London, 2nd edn., 1958, p. 34.
[7] Allan, Meakins, and Whiting, *J.*, 1955, 1874.
[8] Drummond and Waters, *J.*, 1953, 435.
[9] Jones, Whitham, and Whiting, *J.*, 1954, 3201.
[10] J. M. Thompson, Ph.D. Thesis, Manchester, 1954.

The ester (VI; R = Me) was isolated by countercurrent distribution and chromatography of esterified fractions possessing a triyn-enone chromophore. The ester was an unstable oil and owing to the small yield quantitative spectroscopic and analytical data were not obtained. However, the infrared spectrum showed bands of a free ethynyl group and a *trans*-double bond at 3278 and 947 cm.$^{-1}$, respectively. A band at 1730 cm.$^{-1}$ was assigned to the ester grouping, while further bands at 1680 and 3390 cm.$^{-1}$ suggested an amide linkage. This was confirmed when L-valine was identified by paper chromatography and optical rotation after acid hydrolysis. The ester could then be formulated as *N*-(*trans*-non-2-ene-4,6,8-triynoyl)-L-valine methyl ester (VI; R = Me), and this was confirmed when the perhydro-derivative of the ester (VI; R = Me) was found, on gas-liquid chromatography, to have the same retention time as synthetic nonanoyl-L-valine ester. Although methyl esters of polyacetylenic acids have been isolated from fungal metabolites, notably *Polyporus anthracophilus*,[11] this is the first example of a polyacetylenic acid linked with an amino-acid.

The acid (V; R = H), possessing an enetriyne chromophore, did not crystallise and was converted into its crystalline but extremely unstable methyl ester, whose infrared spectrum revealed the presence of a free acetylenic hydrogen and a *trans*-double bond, and that the ester grouping was αβ-unsaturated. Hydrogenation gave methyl nonanoate, and hydrogenation followed by reduction with lithium aluminium hydride gave nonan-1-ol, both identified by gas-liquid chromatography. The parent was therefore *trans*-non-2-ene-4,6,8-triynoic acid (V; R = H).

In addition to the above metabolites traces of a further three polar polyacetylenic compounds, not separated in the above countercurrent distribution, were observed in acid fractions. D-(—)-Mandelic acid also occurred in these fractions. These polyacetylenes were only isolated in workable quantities by repeated growth and extraction of *P. sinuosa* cultures. They were partially separated by redistribution between ether and phosphate buffer but were more readily purified by chromatography of the methyl esters on alumina.

The diacid (VIII; R = H) and its diester possessed a chromophore (see Experimental section) which differed from any previously known but would be expected for the grouping CO·CH=CH·C≡C·C≡C·C≡C·CO. The ester formed unstable needles but insufficient was obtained at any one time for analysis, and its purification was hampered by the co-occurrence of methyl mandelate in chromatogram fractions. The infrared spectrum showed the absence of hydroxyl groups and the presence of a *trans*-double bond and of conjugated ester groups; hydrogenation gave dimethyl decanedioate. The structure, *trans*-dec-2-ene-4,6,8-triynedioic acid (VIII; R = H), for the acid was confirmed by reduction of the diester with lithium aluminium hydride to the diol (XII), identical with the compound previously synthesised[12] and isolated[13] from natural sources. The dimethyl ester (VIII; R = Me) was synthesised by coupling *trans*-5-bromopent-2-en-4-ynoic acid with the previously unknown penta-2,4-diynoic acid.

$HO{\cdot}CH_2{\cdot}CH{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}CH{\cdot}CH_2{\cdot}OH$ (XII)

The methyl α-hydroxy-acid ester (IX; R = Me) was optically active, crystalline, and comparatively stable. Its analyses indicated a formula $C_{11}H_{10}O_4$ (although $C_{14}H_{10}O_5$ could not be excluded) and it had a triyne chromophore. From its analysis, polarity on alumina, and infrared spectrum a diol, rather than the alternative triol, structure was favoured. In the infrared region it showed a strong hydroxyl band at 3470 cm.$^{-1}$ and a non-conjugated ester band at 1740 cm.$^{-1}$. The ester was unaffected by periodic acid, and acetylation followed by alkali treatment had no effect on the chromophore. It therefore appeared probable that it was not a β-hydroxy-ester[14] and that the acid was 2,10-dihydroxydeca-4,6,8-triynoic acid (IX; R = H). Catalytic hydrogenation (6 mol.) of the ester

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[11] Bu'Lock, Jones, and Turner, *J.*, 1957, 1607.
[12] Heilbron, Jones, and Sondheimer, *J.*, 1947, 1586.
[13] Bew, Jones, and Lowe, unpublished work.
[14] Linstead, Owen, and Webb, *J.*, 1953, 1211.

gave a crystalline perhydro-derivative which after reduction with lithium aluminium hydride yielded a triol consuming 1 mol. of periodate. The structure was finally proved when methyl (±)-2,10-dihydroxydecanoate, prepared from 9-hydroxynonanal through the cyanohydrin, was found to possess an infrared spectrum identical with that of the perhydro-ester.

The α-hydroxy-acid (X; R = H), isolated as its methyl ester (X; R = Me), was obtained only in traces, and quantitative measurements were precluded. It had a disubstituted enediyne chromophore and absorbed 5 mol. on catalytic hydrogenation. From the polarity of the ester on alumina, the presence of a non-conjugated ester (1748 cm.$^{-1}$), a *trans*-double bond (953 cm.$^{-1}$), and hydroxyl band in the infrared spectrum, it appeared likely that the acid was the enediyne analogue of the triacetylenic acid (IX; R = H). This was rendered more probable by the identity of the perhydro-ester with the optically active methyl 2,10-dihydroxydecanoate derived from ester (IX; R = Me).

The principal constituent of neutral fractions, *trans*-dec-2-ene-4,6,8-triyn-1-ol (I), was readily isolated by chromatography on alumina. *trans*-Non-2-ene-4,6,8-triyn-1-ol (II) was present in small amounts and this, coupled with its extreme instability (cf. ref. 3), precluded crystallisation. Its presence in enriched fractions from chromatography was inferred from the typical enetriyne ultraviolet absorption, its stability to alkali, oxidation by manganese dioxide to an aldehyde with spectral characteristics identical with those recorded [3] for *trans*-dec-2-ene-4,6,8-triynal and by hydrogenation to nonan-1-ol. Gas–liquid chromatographic determination of the nonanol was hampered by the recognition of undecan-1-ol in fractions before and after hydrogenation. It was therefore necessary to ensure that it did not arise from a further polyacetylenic constituent. This was accomplished by addition of a known weight of dodecan-1-ol to the combined residues, containing the alcohols (I) and (II), followed by quantitative gas–liquid chromatography. The weight ratio of dodecan-1-ol to undecan-1-ol before and after hydrogenation of aliquot parts was virtually the same. Further, the ratio was unaltered when the alcohol (II) was removed from a further portion by treatment with ammoniacal silver nitrate before hydrogenation.

The more polar and more stable crystalline diol (III) was also isolated by chromatography. It possessed typical enediyne ultraviolet absorption and its comparative stability and infrared spectrum proved the absence of a free ethynyl group. The spectrum showed the presence of *trans*-ethylenic hydrogen, while broad bands at 3450—3050 cm.$^{-1}$ and the polarity of the compound suggested that it was a diol. This was confirmed, and the chain length established, by hydrogenation and gas–liquid chromatographic identification of nonane-1,9-diol as the major product. The ready conversion into a hydroxy-aldehyde, formulated as *trans*-9-hydroxynon-2-ene-4,6-diynal, on oxidation with activated manganese dioxide,[15] showed that one alcoholic group was allylic. The diol could then only be *trans*-non-2-ene-4,6-diyne-1,9-diol (III) and it was synthesised from but-3-yn-1-ol and *trans*-5-bromopent-2-en-4-yn-1-ol. It represents the second exception to the generalisation [3] that polyacetylenic fungal metabolites containing an odd number of carbon atoms also have an ethynyl grouping. The first exception was reported [16] in Part XI of this series, and several others are now known.[13]

The known polar diol (IV) was isolated along with traces of diol (III) during distribution of the "acidic" fractions but was not apparent in the neutral fractions. It was shown to be neutral by complete recovery from alkaline solution by ether-extraction and, although not obtained crystalline, possessed the spectral characteristics of authentic *trans*-dec-2-ene-4,6,8-triyne-1,10-diol.[3] It is noteworthy that on hydrogenation it gave a mixture of decane-1,10-diol and decan-1-ol, the latter arising from hydrogenolysis even under the mild conditions used. In the case of the diol (III) which also formed nonan-1-ol in addition to nonane-1,9-diol on hydrogenation, the hydrogenolysis was confirmed when synthetic material was also found to lead to two perhydro-derivatives.

[15] Attenburrow, Cameron, Chapman, Evans, Hems, Jansen, and Walker, *J.*, 1952, 1104.

[16] Jones, Leeming, and Remers, *J*, 1960, 2257.

EXPERIMENTAL

For general experimental methods and conditions of culture growth see Part IX[3] and earlier Parts of this series. Ultraviolet absorption spectra of pure compounds were measured for EtOH solutions on a Cary double-beam recording spectrophotometer. Infrared spectra, unless otherwise stated, were recorded for CS_2 solutions on a Perkin-Elmer 21 instrument, and m. p.s (corrected) were determined on a Kofler block. Deactivated alumina refers to P. Spence's grade " H " material, deactivated by treatment with 5% of 10% acetic acid, and acidic alumina to that supplied by Woelm. Light petroleum refers to the fraction with b. p. 60—80°.

Unless otherwise stated, identification of perhydro-compounds was by gas–liquid chromatography. Identity was considered to be established if the retention time in two or more runs did not vary from that of a standard under the same conditions by more than 1%. Alcohols and esters were run at 125—145°, diesters at 180—210°, diols at 210—230°, and diol dimethyl ethers at 160° on a 4 ft. column of 10 or 25% Apiezon " L " on Embacel. Paper chromatography of aliphatic dicarboxylic acids was on Whatman No. 1 paper in ethanol–ammonia (*d* 0·88)–water (16 : 1 : 3) with Bromocresol Green as spray reagent.

Acidity of constituents and stability to alkali, where applicable, were established by extraction of aliquot parts (10 c.c.) of their ethereal solutions with sodium hydrogen carbonate solution and 2N-sodium hydroxide, and back-extraction with ether, before and after acidification. Quantitative measurements in solution were determined spectrographically.

Isolation of Polyacetylenes.—The culture of *P. sinuosa* Fr. was No. 303 from the type culture collection of the Forest Products Research Laboratory and was grown as surface cultures on a 3% or 5% solution of malt extract. Reflood medium was a 4% glucose solution, 0·2M in sodium acetate. A particular account of the growth procedure is not possible as growth varied from batch to batch. Several batches were grown and the general isolation procedure only is described. The culture fluids (total volume ~200 l.) were continuously extracted with ether (3—4 × 24 hr.), and the concentrated extracts were separated into acidic and neutral fractions with sodium hydrogen carbonate solution.

The neutral fractions were chromatographed from benzene on acidic alumina (column A) and eluted with ether, or on deactivated alumina (column B) and eluted with benzene and benzene–ether.

Acidic fractions were evaporated to 30 c.c. and tars precipitated by addition of light petroleum (20 c.c.). The clarified solutions were distributed (this is referred to subsequently as distribution A) between light petroleum–ether (3 : 1) and M/15-potassium dihydrogen phosphate on a 120-tube Craig type countercurrent apparatus for 212 transfers, 92 fractions (each 40 c.c.) being collected. More polar acidic polyacetylenes were separated by redistribution (distribution B) of material from tubes 1—20 between ether and M/15-potassium dihydrogen phosphate for 120 transfers or by distribution of original solutions in the latter system and stripping the tubes of all other polyacetylenic acidic material. The polar acidic polyacetylenes were then esterified with 3% sulphuric acid in methanol and chromatographed from benzene on deactivated alumina. In the text below, acidic are treated before neutral constituents.

trans-*Non-2-ene-*4,6,8-*triynoic Acid* (V; R = H) (with Dr. W. A. REMERS).—This compound was isolated as its methyl ester (V; R = Me). Material containing enetriyne (9 mg.), recovered from fractions 36—92 and tubes 115—120 of distribution A, was esterified with 3% sulphuric acid in methanol and chromatographed in light petroleum on acidic alumina (5 g.). Elution with light petroleum–benzene (10 : 1) gave *methyl* trans-*non-*2-*ene-*4,6,8-*triynoate*, as pale yellow needles (from hexane), decomp. 20°, $\lambda_{max.}$ 3370 (ε 18,000), 3150 (ε 25,000), 2950 (ε 17,000), 2780 (ε 7900), 2630 (ε 4300), 2520 (ε 66,000), and 2410 Å (ε 47,000), $\nu_{max.}$ 3310 (HC≡C), 1733 (αβ-unsaturated CO_2Me), and 950 cm.$^{-1}$ (*trans*-CH=CH).

Hydrogenation over 5% palladium–charcoal gave methyl nonanoate, and lithium aluminium hydride reduction of the perhydro-ester in the usual manner gave nonan-1-ol.

Methyl Ester of N-(trans-*Non-2-ene-*4,6,8-*triynoyl*)-L-*valine* (VI; R = Me).—The concentrate containing triynenone (6 mg.) from tubes 65—52 of distribution A was esterified with 3% sulphuric acid in methanol and chromatographed from light petroleum on acidic alumina (5 g.). Elution with light petroleum–ether (1 : 1) gave *N*-(*trans*-non-2-ene-4,6,8-triynoyl)-L-valine methyl ester as an unstable oil, $\lambda_{max.}$ 3365, 3145, 2950, 2780, 2625, 2510, and 2400 Å, $\nu_{max.}$ 3390 (NH), 3278 (HC≡C), 1730 (CO_2Me), 1680 (CO·NH), and 947 cm.$^{-1}$ (*trans*-CH=CH). Insufficient material and its instability precluded analysis or quantitative measurements

The ester was hydrolysed with formic acid–5N-hydrochloric acid (1 : 1) under reflux for 16 hr. The diluted solution was extracted with ether, and the aqueous phase concentrated to 3 c.c. Comparative paper chromatography of the solution showed the presence of L-valine, R_F 0·46 in butan-1-ol–water–acetic acid (31 : 13 : 6), 0·71 in phenol–water (4 : 1), and 0·41 in butan-1-ol–water–ethanol (13 : 8 : 4) (ninhydrin spray reagent). The solution showed definite positive rotation but owing to the small amount a quantitative determination was not possible.

Hydrogenation of the ester in the presence of 10% palladium–charcoal gave an oil with retention time (15·7 min.) identical with that of synthetic nonanoyl-L-valine methyl ester (see below) on gas–liquid chromatography (25% Apiezon " L " column at 180°).

Synthetic N-*Nonanoyl*-L-*valine Methyl Ester*.—Nonanoyl chloride (700 mg.) was added to L-valine methyl ester [17] (500 mg.) in dry pyridine (3 c.c.) and kept at 20° for 12 hr. Isolation in the usual manner and distillation at 164—166° (bath-temp.)/0·03 mm. gave N-*nonanoyl*-L-*valine methyl ester* as needles (550 mg.), m. p. 35—37° (from light petroleum at $-40°$) (Found: C, 66·7; H, 10·9; N, 5·3. $C_{15}H_{29}NO_3$ requires C, 66·4; H, 10·8; N, 5·2%).

cis-*Tetradec*-9-*ene*-2,4,6-*triynedioic Acid* (VII; R = H).—Tubes 21—45 from distribution A contained material (40 mg.) exhibiting triynoic acid absorption. Repeated fractional precipitation from dichloromethane with hexane and cooling to $-10°$ gave cis-*tetradec*-9-*ene*-2,4,6-*triynedioic acid* as an amorphous solid (24 mg.) which decomposed with traces of melting at *ca.* 120°. The acid darkened rapidly at 20° and slowly decomposed even at $-35°$ [Found: equiv. (by titration), 121. $C_{12}H_{10}(CO_2H)_2$ requires equiv. 122]; it had $\lambda_{max.}$ 3280 (ε 2550), 3065 (ε 3950), 2880 (ε 3200), 2720 (ε 1900), 2560 (ε 1100), 2235 (ε 95,000), and 2100 Å (ε 85,000sh), $\nu_{max.}$ (in Nujol) 2200, 2120 (C≡C), 1695 (CO_2H, broad), and 747 cm.$^{-1}$ (C≡C·CO_2H and *cis*-CH=CH).

Hydrogenation of a further batch of the diacid (25 mg.) in ethanol with platinic oxide (28 mg.) and repeated crystallisation from ethyl acetate yielded tetradecanedioic acid (8 mg.), m. p. and mixed m. p. 123—126° (Found: C, 65·2; H, 9·9. Calc. for $C_{14}H_{26}O_4$: C, 65·1; H, 10·1%).

A solution of the diacid (6·5 mg.) in water (20 c.c.) and concentrated sulphuric acid (3 c.c.) was heated to 60° and 1% potassium permanganate solution (4 c.c.) added dropwise. Continuous extraction with ether for 15 hr. and evaporation of the solvent gave a residue which contained glutaric acid, identified by comparative paper chromatography. Oxidation of the diacid (10 mg.) in aqueous potassium carbonate solution with permanganate for 2 hr. at 20° and paper chromatography gave a residue which contained glutaric, succinic, and a trace of malonic acid. Alkaline oxidation of glutaric acid under the same conditions gave unchanged material.

Dimethyl cis-*Tetradec*-9-*ene*-2,4,6-*triynedioate* (VII; R = Me).—The acidic fraction from an ethereal extract of the culture medium was esterified with 3% sulphuric acid in methanol. Unchanged acids were removed with sodium hydrogen carbonate solution, the neutral fraction was washed with light petroleum (b. p. 30—40°), and the solution [containing 500 mg. of (VII; R = Me) from spectroscopic estimation] was distributed between light petroleum and methanol–water (4 : 1) in a 120-tube Craig countercurrent apparatus. After 220 transfers, spectroscopically pure dimethyl *cis*-tetradec-9-ene-2,4,6-triynedioate (250 mg.) was contained in fractions 85—100 and tubes 120—117. The pure material was crystallised three times from light petroleum (b. p. 32—36°) at $-70°$. The ester melted below $-10°$ and the colourless oil formed became dark brown in 10 min. at 20°; it had $\lambda_{max.}$ 3290 (ε 3200), 3080 (ε 4700) 2895 (ε 3800), 2725 (ε 2300), 2575 (ε 1700), 2270 (ε 74,000), 2180 (ε 66,000), and 2095 Å (ε 46,000sh), $\nu_{max.}$ 2198 (C≡C), 1731 (non-conjugated CO_2Me), 1707 (conjugated CO_2Me), and 739 cm.$^{-1}$ (C≡C·CO_2Me and *cis*-CH=CH).

Hydrogenation of the ester (50 mg.) in the usual manner gave dimethyl tetradecanedioate (42 mg.), m. p. and mixed m. p. 47—48°, as plates from hexane.

Isomerisation of cis-*Tetradec*-9-*ene*-2,4,6-*triynedioic Acid*.—An ethereal solution of the diacid, containing 30 mg. of partially purified material, was extracted with N-sodium hydroxide, the aqueous solution kept for 5 min., acidified, and re-extracted with ether. The isomerised acid was esterified with 3% sulphuric acid in methanol, and the product chromatographed from light petroleum (b. p. 30—40°; 20 c.c.) on deactivated alumina. Elution with light petroleum–benzene (7 : 3; 350 c.c.) yielded unisomerised material followed by dimethyl *cis*-tetradec-9-ene-3,5,7-triynedioate (XI; R = Me), pale yellow prisms (17 mg.), m. p. 15—16° [from light petroleum (b. p. 32—36°) at $-70°$]. The ester decomposed rapidly at 20° and had $\lambda_{max.}$ 3305

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[17] Brenner and Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.

(ε 12,000), 3080 (ε 17,000), 2895 (ε 13,000), 2730 (ε 7000), 2580 (ε 3850), 2435 (ε 110,000), 2315 (ε 70,000), 2240 (ε 35,000sh), and 2120 Å (ε 32,000), $\nu_{max.}$ 1735 (CO_2Me), 1740 (sh, CO_2Me), and 731 cm.$^{-1}$ (*cis*-CH=CH, broad).

cis-*Tetradec*-9-*ene*-3,5,7-*triynedioic Acid* (XI; R = H).—Spectroscopically pure material from isomerisation of the diacid (VII; R = H), similar to that described above, was fractionally precipitated from dichloromethane solutions with light petroleum (b. p. 32—36°) to yield cis-*tetradec*-9-*ene*-3,5,7-*triynedioic acid* (4·5 mg.) as an off-white amorphous powder, m. p. 117—120° (decomp.) (Found: C, 69·05; H, 5·0. $C_{14}H_{12}O_4$ requires C, 68·8; H, 4·95%), $\lambda_{max.}$ 3305 (ε 12,000), 3090 (ε 17,500), 2895 (ε 13,000), 2730 (ε 7000), 2580 (ε 4000), 2440 (ε 100,000), 2330 (ε 66,000), 2240 (ε 33,500sh), and 2120 Å (ε 29,500), $\nu_{max.}$ (in Nujol) 2200 (C≡C), and 1695 cm.$^{-1}$ (CO_2H, broad).

D-(−)-*Mandelic Acid*.—Solvent was removed from fractions and tubes immediately preceding the more polar acidic polyacetylenes from countercurrent distribution B. The residue was extracted with hot water, the extracts were treated with charcoal, and the solvent was removed. Further purification gave D-(−)-mandelic acid (26 mg.) as plates, m. p. 132—133°, $[\alpha]_D^{22}$ −146° (*c* 1·9 in H_2O), from water (lit.,[18] m. p. 133°, $[\alpha]_D$ −158° in H_2O) (Found: C, 63·1; H, 5·5. Calc. for $C_8H_8O_3$: C, 63·15; H, 5·3%). The ultraviolet and infrared spectra were identical with those of authentic inactive material. The methyl ester, needles from hexane, had m. p. 54—55°, $[\alpha]_D^{20}$ −209° (*c* 1·0 in CS_2) (lit.,[18] m. p. 55°, $[\alpha]_D$ −214° in CS_2). The m. p. was undepressed on admixture with the material isolated in the purification of *trans*-dec-2-ene-4,6,8-triynedioic acid (see below).

trans-*Dec*-2-*ene*-4,6,8-*triynedioic Acid* (VIII; R = H).—This compound possessed an unsymmetrical distribution curve and was only obtained pure as its dimethyl ester. A mixture of the ester and methyl mandelate was contained in initial fractions (eluted with benzene) from chromatography of the more polar acidic polyacetylenes. (For later fractions † see below.) Fractional precipitation from dichloromethane solutions with hexane gave dimethyl *trans*-dec-2-ene-4,6,8-triynedioate (VIII; R = Me) as an off-white amorphous powder (6 mg.). The ultraviolet and infrared spectra were identical with those of the synthetic ester (see below).

Hydrogenation of the ester in the usual manner gave dimethyl decanedioate.

Reduction of Dimethyl trans-*Dec*-2-*ene*-4,6,8-*triynedioate by Lithium Aluminium Hydride*.—The crude ester (*ca.* 5 mg.) in dry ether was treated with lithium aluminium hydride (10 mg.) at 20° for 5 min. The excess of reagent was decomposed and the mixture extracted with ether, to yield *trans-trans*-deca-2,8-diene-4,6-diyne-1,10-diol (4 mg.) as fine needles, m. p. 153—155° (decomp.) (lit.,[12] m. p. 155—156°) (from ether–hexane). Ultraviolet and infrared spectra were identical with those of authentic material.

Hydrogenation of the diol (4 mg.) over 10% palladium–charcoal gave decane-1,10-diol (2·5 mg.) as rods, m. p. and mixed m. p. 70—71° (obtained by slow evaporation of an ethanol solution). The infrared spectrum was identical with that of an authentic sample. Methylation of mother-liquors with methyl iodide in potassium t-butoxide gave 1,10-dimethoxydecane identified by gas–liquid chromatography.

Synthesis of Dimethyl trans-*Dec*-2-*ene*-4,6,8-*triynedioate* (VIII; R = Me).—A cooled and stirred solution of penta-2,4-diyn-1-ol (7·0 g.) in dry acetone (20 c.c.) was oxidised with 8N-chromic acid in the usual manner. Extraction of the acidic fraction with carbon disulphide gave penta-2,4-diynoic acid (860 mg.) which after repeated purification was obtained as plates, m. p. <0°. Further material was obtained by repeated re-oxidation of neutral fractions. The acid rapidly decomposed on exposure to light and air and correct analyses were not obtained; it had $\lambda_{max.}$ 2690 (ε 2700), 2540 (ε 4000), 2410 (ε 3900), 2300 (ε 2700), and 2200 Å (ε 2200), $\nu_{max.}$ 3226 (HC≡C), 2227 (C≡C), 2119 (C≡C), and 1701 cm.$^{-1}$ (CO_2H).

Penta-2,4-diynoic acid (100 mg.) in methanol (2 c.c.) was added to a mixture of cuprous chloride (12 mg.) and 30% aqueous ethylamine (10 c.c.), the blue colour of which had been destroyed by addition of hydroxylamine hydrochloride. *trans*-5-Bromopent-2-en-4-ynoic acid (200 mg.) in methanol (1 c.c.) was then added rapidly to the stirred and cooled mixture under a rapid stream of nitrogen. Stirring was continued for 15 min., then the mixture was diluted with water, acidified, and extracted with ether. Spectral analysis showed that the dried extract contained *trans*-dec-2-ene-4,6,8-triynedioic acid (*ca.* 100 mg., 50%) and *trans-trans*-deca-2,8-diene-4,6-diynedioic acid (*ca.* 50 mg.).

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[18] Heilbron and Bunbury, "Dictionary of Organic Compounds," Vol. III, 1953, p. 212.

The mixed acids were esterified with 5% sulphuric acid in methanol, and the ester fraction was chromatographed from light petroleum–benzene (1 : 1) on alumina. Fractions eluted with light petroleum–benzene (5 : 1) contained *dimethyl* trans-*dec-2-ene-4,6,8-triynedioate* which after repeated rechromatography was obtained as plates, m. p. $<0°$, from dichloromethane–hexane at $-70°$ (Found: C, 66·7; H, 3·6; $C_{12}H_8O_4$ requires C, 66·7; H, 3·7%), $\lambda_{max.}$ 3520 (ε 4,500), 3280 (ε 7400), 3080 (ε 7020), 2905 (ε 5200), 2560 (ε 38,600), 2440 (ε 50,400), and 2360 Å (ε 42,400), $\nu_{max.}$ 2208 (C≡C), 1717 (conjugated CO_2Me, broad), 954 (*trans*-CH=CH), and 740 cm.$^{-1}$ (C≡C·CO_2Me).

Methyl 2,10-*Dihydroxydeca*-4,6,8-*triynoate* (IX; R = Me).—Fractions † eluted from the column above with benzene–ether (1 : 1) crystallised from dichloromethane–hexane or carbon disulphide, to yield *methyl* 2,10-*dihydroxydeca*-4,6,8-*triynoate* as needles (42 mg.), m. p. 102—103° (decomp.), $[\alpha]_D^{17}$ +44° (*c* 0·45 in EtOH) (Found: C, 64·3; H, 4·9; O, 30·7. $C_{11}H_{10}O_4$ requires C, 64·1; H, 4·9; O, 31·0%), $\lambda_{max.}$ 2830 (ε 160), 2670 (ε 185), 2530 (ε 135), and 2110 Å (ε 180,000), $\nu_{max.}$ 3470 (OH), 1740 (non-conjugated CO_2Me), 1113, 1075, 1032, and 985 cm.$^{-1}$ (C–O). The compound decomposed slowly at 20°.

Hydrogenation of Methyl 2,10-*Dihydroxydeca*-4,6,8-*triynoate*.—The ester (5·0 mg.) in methanol (5 c.c.) was hydrogenated over pre-reduced 10% palladium–charcoal (50 mg.) at 20°. The uptake was 5·92 mol. Crystallisation from hexane yielded methyl 2,10-dihydroxydecanoate (4 mg.) as needles, m. p. 94—96°, $\nu_{max.}$ (CCl_4) 3425 (OH), 1724 (ester), 1263, 1089, and 1064 cm.$^{-1}$ (C–O) (see below).

The saturated ester (5 mg.) was heated under reflux with lithium aluminium hydride (15 mg.) in ether for 1 hr. Isolation of the product in the usual manner and crystallisation from water gave decane-1,2,10-triol (3·5 mg.), m. p. 100—101°, $[\alpha]_D$ $+16° \pm 4°$ (*c* 0·1 in EtOH), $\nu_{max.}$ (in Nujol) 3311, 3247 (OH), 1348, 1098, and 1050 cm.$^{-1}$ (C–O). Quantitative oxidation with sodium periodate resulted in the uptake of 1·02 mol.

Synthesis of (±)-*Methyl* 2,10-*Dihydroxydecanoate*.—The bisulphite derivative of 9-hydroxynonanal (7 g.; prepared from oleyl alcohol by Scanlen and Swern's method [19]) was treated with an aqueous solution of sodium cyanide (10 g.) at 20° and the mixture kept for 15 min. The oily nitrile, $\nu_{max.}$ (liquid film) 3497 (OH) and 2273 cm.$^{-1}$ (C≡N), which separated was heated under reflux with concentrated hydrochloric acid (50 c.c.) for 8 hr. with occasional shaking. The resulting crude hydroxy-acid, obtained as an oil, was esterified with 4% sulphuric acid in methanol for 24 hr. at 20° and the mixture was poured into water. Repeated crystallisation of the precipitate from hexane gave (±)-*methyl* 2,10-*dihydroxydecanoate* (1·2 g.) as needles, m. p. 89—91° (Found: C, 60·8; H, 10·3. $C_{11}H_{22}O_4$ requires C, 60·5; H, 10·2%). The infrared spectrum was identical with that of the perhydro-ester from ester (IX; R = Me). Reduction of the triol with lithium aluminium hydride gave the (±)-triol, m. p. 96—97°, with an infrared spectrum identical with that of the (+)-triol.

Methyl trans-2,10-*Dihydroxydec*-4-*ene*-6,8-*diynoate* (X; R = Me).—Fractions eluted with benzene–ether (1 : 1) from the column above, following those containing the ester (VIII; R = Me), yielded *methyl* trans-2,10-*dihydroxydec*-4-*ene*-6,8-*diynoate* (X; R = Me) which was an oil even at $-70°$. It had $\lambda_{max.}$ 2800, 2640, 2510, 2380, 2300, and 2080 Å, $\nu_{max.}$ (liquid film) 3448 (OH), 1748 (CO_2Me), and 953 cm.$^{-1}$ (*trans*-CH=CH).

The ester (5·4 mg.), estimated spectroscopically to contain $<0·2$ mg. of (IX; R = Me), was hydrogenated over pre-reduced 10% palladium–charcoal (50 mg.) at 20°. The uptake was 5·1 mol. The product crystallised from hexane to yield methyl 2,10-dihydroxydecanoate (2·3 mg.) as needles, m. p. and mixed m. p. 90—96°, after seeding with the compound derived from ester (IX; R = Me). The infrared spectrum was identical with that of the latter product.

trans-*Dec*-2-*ene*-4,6,8-*triyn*-1-*ol* (I).—Initial fractions (total vol. 1050 c.c.) eluted from column A with ether, and after separation from polymeric impurities, crystallised from hexane, to yield *trans*-dec-2-ene-4,6,8-triyn-1-ol (250 mg.) as pale yellow needles, m. p. and mixed m. p. 128—129° (decomp.). The infrared and ultraviolet spectra were identical with those of an authentic sample. Further alcohol (6 mg.) was obtained by working up fractions 2—14 from distribution A of the acidic fractions in a similar manner.

Hydrogenation over 5% palladium–charcoal gave decan-1-ol.

Undecan-1-*ol*.—Mother-liquors from the isolation of *trans*-dec-2-ene-4,6,8-triyn-1-ol gave a

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[19] Scanlen and Swern, *J. Amer. Chem. Soc.*, 1940, **62**, 2305.

yellow oil (280 mg.) which darkened on storage. Gas–liquid chromatography showed that this consisted mainly of undecan-1-ol (see below for quantitative estimation).

trans-*Non-2-ene-*4,6,8-*triyn-*1-*ol* (II).—The presence of this compound was inferred from the following experiments. Later fractions (total vol. 1400 c.c.) eluted from column A with ether gave an unstable oil exhibiting enetriyne absorption. The oil (22 mg.) was hydrogenated over 5% palladium–charcoal, to yield a mixture of nonan-1-ol, decan-1-ol, and undecan-1-ol. The oil (5 mg.) from fractions leading to hydrogenated material rich in nonan-1-ol was oxidised in chloroform solution with activated manganese dioxide (25 mg.) for 3 hr. The resulting solution showed ultraviolet absorption identical with that of *trans*-non-2-ene-4,6,8-triynal [3] and unaffected when the product was treated with alkali.

Dodecan-1-ol (100 mg.) was added to an ethereal solution (1500 c.c.) of the combined residues from column A after isolation of the bulk of the above neutral constituents. The weight ratio of dodecan-1-ol to undecan-1-ol determined by quantitative gas–liquid chromatography proved to be 1 : 2·4. Decan-1-ol was absent. Hydrogenation of an aliquot part (150 c.c.) gave a ratio of dodecan-1-ol : undecan-1-ol : decan-1-ol : nonan-1-ol of 1 : 2·44 : 1·50 : 0·30. The ratio after extraction of a further portion with ammoniacal silver nitrate solution (2 × 100 c.c.) and hydrogenation was 1 : 2·42 : 1·45 : 0·07.

trans-*Non-2-ene-*4,6-*diyne-*1,9-*diol* (III).—Fractions eluted with benzene–ether (2 : 1; 500 c.c.) from chromatography of the neutral fraction on column B were rechromatographed on deactivated alumina. Crystallisation of residues eluted with benzene–ether (2 : 1) gave *trans*-non-2-ene-4,6-diyne-1,9-diol (10 mg.) as plates, m. p. 57—60° (decomp.), from dichloromethane–hexane. Light absorption data were identical with those of the synthetic diol given below. Hydrogenation of the diol in ethanol over 5% palladium–charcoal gave nonane-1,9-diol and nonan-1-ol.

The diol was oxidised with activated manganese dioxide, and the product chromatographed from benzene–light petroleum (1 : 1) on deactivated alumina. Elution with benzene–ether (9 : 1) gave *trans*-9-hydroxynon-2-ene-4,6-diynal which decomposed rapidly at −10° in red light and had $\lambda_{\max.}$ (in ether) 3100, 2920, 2750, 2650sh, 2460, and 2240 Å, $\nu_{\max.}$ 3550 (OH), 2700 (aldehydic CH), 1690 (conjugated CH=O), 1110 (CH=CH·CH=O), 1050, 1040 (C–O), and 955 cm.$^{-1}$ (*trans*-CH=CH).

Synthesis of trans-*Non-2-ene-*4,6-*diyne-*1,9-*diol* (III).—But-3-yn-1-ol (7·0 g.) was added to a stirred solution of cuprous chloride (200 mg.) in 33% aqueous ethylamine (28 c.c.). To the mixture, under nitrogen at 35—45°, was added *trans*-5-bromopent-2-en-4-yn-1-ol (from 8 g. of *trans*-pent-2-en-4-yn-1-ol) in methanol (10 c.c.) during 10 min. Crystals of hydroxylamine hydrochloride were added as required to reduce cupric ion formed. The mixture was stirred for a further 10 min., poured into dilute aqueous hydrochloric acid (200 c.c.) and ice, and extracted with ether. The ether extracts were washed with ammoniacal silver nitrate solution and with water, and the product, in benzene, was adsorbed on acidic alumina (200 g.). Fractions eluted with benzene–ether (1 : 2) and ether crystallised from chloroform, to yield trans-*non-2-ene-*4,6-*diyne-*1,9-*diol* (10·5 g.), m. p. 56—58·5° (decomp.), undepressed on admixture with the natural product (Found: C, 71·9; H, 6·9. $C_9H_{10}O_2$ requires C, 72·0; H, 6·7%), $\lambda_{\max.}$ 2820 (ε 14,500), 2660 (ε 18,500), 2520 (ε 12,500), 2390 (ε 6000), 2275 (ε 3000), and 2125 Å (ε 49,500), $\nu_{\max.}$ (in Nujol) 3450—3050 (OH), 1050, 1025, 990 (C–O), and 962 cm.$^{-1}$ (*trans*-CH=CH). Hydrogenation gave nonane-1,9-diol and nonan-1-ol in the ratio 2·2 : 1.

trans-*Dec-2-ene-*4,6,8-*triyne-*1,10-*diol* (IV).—Material (7 mg.) from tubes 66—110 of distribution A was neutral and possessed the ultraviolet and infrared spectral properties of *trans*-dec-2-ene-4,6,8-triyne-1,10-diol.[3] The ultraviolet spectrum indicated a trace of material (III) as impurity. Hydrogenation over 5% palladium–charcoal gave decane-1,10-diol and decan-1-ol (5 : 1) and traces of nonane-1,9-diol and nonan-1-ol (cf. III).

Oxidation with activated manganese dioxide gave a solution which exhibited typical enetriynal ultraviolet absorption.[3]

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