438. *Chemistry of the Higher Fungi. Part IX.* Polyacetylenic Metabolites from* Coprinus quadrifidus.

By E. R. H. Jones and J. S. Stephenson.

Seven polyacetylenic compounds have been isolated from the culture medium of *Coprinus quadrifidus*. The structures and stereochemistry of four of these products, C_9 compounds (I, II, III, and VIII), have been elucidated.

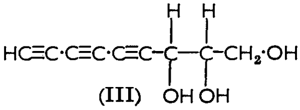
A brief examination of the antibiotics produced by the Basidiomycete *C. quadrifidus* in stirred, aerated culture has been reported by Doery, Gardner, Burton, and Abraham.[1] They obtained evidence for the presence of several moderately active but toxic substances and, since those that were purified were unstable in the crystalline state, the study was discontinued. Our interest in these products was stimulated by this instability and by the ultraviolet absorption spectra quoted for two fractions obtained from the mixture. Both the light sensitivity of the crystalline materials and the spacing of the sharp maxima of the spectra at *ca.* 2100 cm.$^{-1}$ are characteristic of polyacetylenic compounds.[2]

$HC\equiv C\cdot C\equiv C\cdot C\equiv C\cdot CH\overset{t}{=}CH\cdot CHO$

(I)

$HC\equiv C\cdot C\equiv C\cdot C\equiv C\cdot CH\overset{t}{=}CH\cdot CH_2\cdot OH$

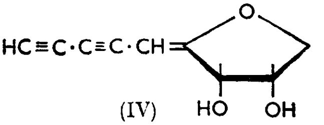
(II)



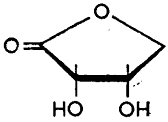
$HC\equiv C\cdot C\equiv C\cdot C\equiv C-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-CH_2\cdot OH$

(III)

The fungus was grown in surface culture and the mixture of compounds in the medium was separated into two main groups by continuous extraction, first with light petroleum (b. p. 30—40°) and subsequently with ether. The less polar fraction was resolved either by chromatography on alumina or by counter-current distribution between hexane and water. Four compounds were thus isolated which, in order of increasing polarity were (*a*) the aldehyde (I), exhibiting the ultraviolet absorption of a carbonyl-ene-triyne chromophore, (*b*) the alcohol (II) (ene-triyne chromophore), (*c*) a compound with maxima at 3095, 2910, 2735, and 2585 Å, and (*d*) another with absorption maxima at 3510, 3280, 3050, 2900, 2610, and 2480 Å. The last compound was at first thought to be the hydroxy-aldehyde (IX) corresponding to the diol (VIII; see below) but when this was obtained by oxidation of the diol with manganese dioxide, its ultraviolet absorption maxima were found to be slightly differently located from those of the natural material. The most likely formula for this compound is the isomeric hydroxy-aldehyde structure (X). No further investigation of materials (*c*) and (*d*) has been possible as only minute quantities were isolated.



(IV)



(V)

The major constituent of the less polar fraction, the alcohol (II), was present in a typical culture medium in a concentration of *ca.* 2 mg./l. It formed unstable colourless crystals from light petroleum at —70°; its lability and its reaction with alcoholic silver nitrate indicated a free ethynyl group. The infrared spectrum confirmed this and established the presence of a hydroxyl group and a *trans*-disubstituted double bond in the molecule. Since catalytic reduction gave nonan-1-ol, structure (II) was established unequivocally.

The substance containing the carbonyl-ene-triyne chromophore, was present mainly

---

* Part VIII, Bu'Lock, Jones, and Turner, *J.*, 1957, 1607.

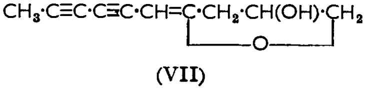
[1] Doery, Gardner, Burton, and Abraham, *Antibiotics and Chemotherapy*, 1951, **1**, 409.

[2] Jones, Whiting, Armitage Cook, and Entwistle, *Nature*, 1951, **168**, 900; Bohlmann, *Chem. Ber.*, 1951, **84**, 545, 785.

in the early stages of growth and was available only in very small amounts. Some 10 mg. of material were eventually accumulated during numerous isolations of the other constituents and, after the determination of the structure of the alcohol (II), its investigation was considerably simplified. The compound was purified by chromatography followed by crystallisation at —30°. The pale yellow crystals thus obtained were extremely unstable; at 35° in daylight they were converted almost instantaneously into a bright purple, insoluble polymer. The infrared spectrum showed the molecule to contain a free ethynyl group, an aldehyde group, and a *trans*-disubstituted double bond (a strong band at 1110 cm.$^{-1}$ is regarded as characteristic of an aldehyde grouping in such an environment [3]). The ultraviolet spectrum was compatible with structure (I) and final confirmation was obtained by preparation of the aldehyde by oxidation of the alcohol (II) with manganese dioxide.

$CH_3{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}CMe{\cdot}OMe$

(VI)



$CH_3{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2$ (C and CH_2 joined through O)

(VII)

$HO{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$

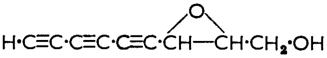
(VIII)

$HO{\cdot}CH_2C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CHO$

(IX)

$CHO{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH\overset{t}{=}CH{\cdot}CH_2{\cdot}OH$

(X)



(XI)

The more polar material in the ether extract was shown to consist of at least three polyacetylenic compounds, by counter-current distribution between ether and M/15-phosphate buffer [pH 5·9; used because of the tendency of the triyne-triol to isomerise under mildly basic conditions (see below)]. The major polyacetylene produced by *C. quadrifidus* is the triyne-triol (III). It is present in the culture medium to the extent of *ca.* 50 mg./l. and when pure could be stored for considerable periods at —30° in darkness without appreciable decomposition. Exposure to daylight at 20° caused rapid polymerisation in the crystal lattice (virtually complete in *ca.* 30 min.) whilst when heated quickly to *ca.* 80° the material exploded. Its instability suggested the presence of a free ethynyl group (confirmed with alcoholic silver nitrate), but its infrared spectrum in solution could not be determined owing to the insolubility of the triol in suitable solvents. Hydrogenation (6·0 mol. of hydrogen absorbed) gave a nonane-1 : 2 : 3-triol, characterised as its tribenzoate, and further identified by periodate oxidation (2·05 mol. consumed) to heptaldehyde. The only structure for a triyne-triol compatible with the above evidence is (III).

Advantage was taken of a transformation of the triol (III) which occurred under alkaline conditions to determine the absolute configuration of the asymmetric centres. The product of this reaction exhibited ultraviolet light absorption (see Table) characteristic of a diyne-enol ether (*e.g.*, VI).[4] On the basis of this correlation and by analogy with similar rearrangements of pent-4-yn-1-ol [5] and of deca-4 : 6 : 8-triyne-1 : 2-diol, the latter yielding (VII),[6] a cyclic enol ether structure (IV) was indicated for the product; this was supported by infrared absorption observations. The initial configuration of the two hydroxyl groups is retained in (IV) and this on ozonolysis gave L-erythronolactone (V). The full structure of the triol is thus given by the Fischer projection (III) [(2*D* : 3*D*)-nona-4 : 6 : 8-triyne-1 : 2 : 3-triol on Linstead's convention (see *J.*, 1950, 3333)].

The frequency (1755 cm.$^{-1}$) of the carbonyl band in the infrared spectrum of L-erythronolactone presents a good example of the bathochromic effect of hydrogen bonding. The band appears at 1800 cm.$^{-1}$ in the dibenzoate, a rather high value, possibly due to strain

---

[3] Personal communication from Dr. M. C. Whiting.

[4] Bohlmann and Viehe, *Chem. Ber.*, 1955, **88**, 1017.

[5] Paul and Tchelitcheff, *Compt. rend.*, 1950, **230**, 1872.

[6] Jones, Turner, and Whiting, unpublished results.

induced in the five-membered ring by two bulky *cis*-substituents or to the close proximity of three carbonyl groups as in glycerol triesters.[7]

The most polar compound present in the poly-yne mixture showed typical ene-diyne absorption maxima at 2805, 2650, 2510, 2385, 2275, and 2100 Å. Little work has been possible on this compound, but it seems likely that it is not simply the partially reduced derivative of the main component, the triyne-triol, since the product obtained on catalytic reduction appears to differ from the nonane-1 : 2 : 3-triol obtained from (III).

The least polar compound of this group is present in small quantities and its ultraviolet spectrum (ene-triyne chromophore) shows maxima similar to those of the ene-triynol (II) but displaced to somewhat longer wavelengths. This spectrum and the considerable stability of the compound suggested the absence of a free ethynyl grouping. This was confirmed by the infrared spectral data which also showed the double bond to have a *trans*-configuration. Catalytic hydrogenation of the compound and subsequent oxidation afforded sebacic acid together with some decanoic acid (formed by hydrogenolysis of a hydroxyl group). The compound was therefore formulated as the diol (VIII) and this was confirmed synthetically by the coupling [8] of penta-2 : 4-diyn-1-ol and *trans*-5-bromopent-2-en-4-yn-1-ol.

The variation in oxidation states of the 9-carbon atom chain present in compounds (I), (II), and (III) is of interest and may be significant in relation to the biogenetic processes occurring with *C. quadrifidus*. During preliminary growth studies it was observed that the ene-triyn-ol (II) was accompanied by significant quantities of the aldehyde (I) only in the very early stages of growth. On the other hand the concentration of the alcohol (II) reached a maximum after approximately 30 days, suggesting that it may be formed by reduction of the aldehyde (I). A plausible route to the triol (III) would be by *trans*-hydroxylation of the *trans*-double bond in (II), probably *via* an epoxide. Some evidence is available, which it is hoped to augment in due course, that the constituent (*c*) of the less-polar fraction (p. 2197) is the *trans*-epoxide (XI).

Ultraviolet spectra.

(I)	$\lambda_{max.}$	2105	2200 †	2280	2400	2570	2710	2870	3060	3270	
	$10^{-3}\varepsilon$	20·2		60·5	86·5	3·05	6·55	13·1	17·1	12·3	
(II)	$\lambda_{max.}$ *			2330	2430	2550		2830	3000	3200	3430
	$10^{-3}\varepsilon$			28·5	47·5	73·5		9·1	19·4	29·5	23·0
(III)	$\lambda_{max.}$	2080				2540	2695	2865	3050		
	$10^{-3}\varepsilon$	122				0·16	0·22	0·25	0·17		
(IV)	$\lambda_{max.}$		2160	2240			2650 †	2785	2930		
	$10^{-3}\varepsilon$		18·1	23·4			12·5	17·5	14·3		
(VII)	$\lambda_{max.}$		2170	2240			2650	2780	2935		
	$10^{-3}\varepsilon$		22·5	30·0			13·0	13·5	15·0		
(VI)	$\lambda_{max.}$			2220			2645	2770	2950		
	$10^{-3}\varepsilon$			27·4			10·3	15·0	12·0		
(VIII)	$\lambda_{max.}$	2050	2120	2310	2435	2590	2790	2905	3095	3305	
	$10^{-3}\varepsilon$	37·5	37·0	71·5	91·5	3·55	6·4	11·2	13·2	8·1	
(IX)	$\lambda_{max.}$				2470	2595	2875	3050	3250	3485	
	$10^{-3}\varepsilon$				46·5	69·5	8·2	16·8	24·8	19·5	

* In hexane. † Shoulder.

The isolation of these C_9 compounds (I, II, and III) makes possible a generalisation on a relation between chain-length and structure of the fungal polyacetylenes. Up to the present all the compounds containing an odd number of carbon atoms also have a free ethynyl grouping; none of those of even chain length contains such a system. So far, seven odd-numbered compounds have been described (3 in this paper, biformin,[9] nemotin and nemotinic acid,[10] and the C_{13} mycomycin [11]) but several more examples known to us will be reported in a subsequent publication.

---

[7] Shreve, Heether, Knight, and Swern, *Analyt. Chem.*, 1950, **22**, 1498.
[8] Chodkiewicz, *Ann. Chim.* (*France*), 1957, **2**, 819.
[9] Anchel and Cohen, *J. Biol. Chem.*, 1954, **208**, 319.
[10] Bu'Lock, Jones, and Leeming, *J.*, 1956, 3767.
[11] Celmer and Solomons, *J. Amer. Chem. Soc.*, 1952, **74**, 1870.

This generalisation and the occurrence together as metabolic products of *C. quadrifidus* of C_{10} derivatives (VIII) and (X) with oxygen functions at both ends, along with the C_9 compounds (I), (II), and (III) suggest that the terminal methyl group of polyacetylenic derivatives of even chain length is removed by ω-oxidation followed by decarboxylation. The alcohol (II) might arise thus:

$$CH_3{\cdot}[C{\equiv}C]_3{\cdot}CH{=}CH{\cdot}CO_2H \longrightarrow HC{\equiv}C{\cdot}[C{\equiv}C]_2{\cdot}CH{=}CH{\cdot}CH_2{\cdot}OH$$

by consecutive oxidation, decarboxylation and reduction steps. Some evidence has already been obtained of the existence of an enzyme system in cell-free extracts of *C. quadrifidus* capable of decarboxylating a triyne-acid.

Bu'Lock and Gregory [12] have provided a clear demonstration that the carbon chain of the C_{11} nemotinic acid is built up from acetate units. Working with [1-^{14}C]acetate they found that the radioactivity was confined to the odd-numbered carbon atoms and suggested that the twelfth carbon atom (which remains in the accompanying odyssic acid) is eliminated by oxidation followed by decarboxylation. In support of this they draw attention to the apparent interconvertibility of C_{10} compounds with CH_3, $CH_2{\cdot}OH$, and CO_2H terminal groups in cultures of *Polyporus anthracophilus*.[13] It appears that the enzyme system responsible for decarboxylation is not present in this fungus.

EXPERIMENTAL

Ultraviolet absorption spectra (cf. Table) were measured for ethanol solutions, unless otherwise stated (chromatographic and distribution fractions were examined directly), on a Cary Recording Spectrophotometer Model 14 M. Infrared absorption spectra were obtained on a Perkin-Elmer Model 21 spectrophotometer, and m. p.s (corrected) determined 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 a fraction with b. p. 60—80°. Evaporations were done under reduced pressure in nitrogen. Crystallisations of poly-ynes were carried out in a cold room (−9°) in red light.

The culture of *C. quadrifidus* was obtained from the Type Culture Collection, Baarn (Netherlands). The fungus was grown as surface cultures on a 5% solution of malt extract, and the culture medium was decanted and extracted after 30 days' growth at 24°. Subsequent replacement of the medium with a 4% solution of glucose [14] or a 4% glucose solution containing sodium acetate (0·2M) resulted in an enhanced rate of poly-yne production, comparable concentrations to those obtained during the initial growth period being produced after 7 to 14 days' growth.

The Less Polar Poly-yne Fractions.—In a typical experiment 36 l. of the culture medium (12 l. batches) were continuously extracted with light petroleum (b. p. 30—40°). The extract was evaporated to 160 c.c. and placed in the first 4 tubes of a 50 plate Craig countercurrent apparatus (volumes of upper and lower phases, 40 c.c.) and distributed between light petroleum and water. After 70 transfers the alcohol (II) appeared in tubes 28—50 and in fractions 9—20 removed from the apparatus. The less polar aldehyde (I) appeared in fractions 1—12, whilst the two polar fractions, one with ultraviolet absorption bands at 3510, 3280, 3050, 2900, 2610, and 2485 Å and the other showing maxima at 3095, 2900, 2735, and 2585 Å, were found in tubes 1—14 and tubes 14—22 respectively. The last material was adsorbed from benzene (10 c.c.) on alumina (50 g.). It was eluted with benzene–ether (19 : 1; 50 c.c.) and exhibited $\lambda_{max.}$ 2095, 2585; 2735, 2950, and 3095 Å and $\nu_{max.}$ 3580 (O–H), 3300 (≡C–H), 1087, 1065 (C–O), 904, 874, 844, and 755 cm.$^{-1}$. The partition coefficient between light petroleum–benzene (3 : 1) and water at 17° is 1·0. The ratio of the intensity of the absorption band at 2095 to that at 3095 Å is 120 : 1, much lower than the corresponding ratio, 717 : 1, observed in the case of the triyne-triol (III).

trans-*Non-2-ene-*4 : 6 : 8-*triyn-*1-*ol* (II).—The fractions (14—20) and contents of tubes (32—50) rich in material with ene-triyne absorption were combined; the aqueous phase was

---

[12] Bu'Lock and Gregory, *Biochem. J.*, 1959, in the press. We are indebted to Dr. J. D. Bu'Lock for keeping us informed about the progress of these closely related studies.

[13] Bu'Lock, Jones, and Turner, *J.*, 1957, 1607.

[14] Bu'Lock and Leadbeater, *Biochem. J.*, 1956, **62**, 476.

extracted with ether. The ether and light petroleum solutions were evaporated to 50 c.c., and, on cooling to −70°, trans-*non-2-ene-4 : 6 : 8-triyn-1-ol* (II; 70 mg.) separated as colourless needles. The material was then recrystallised from hexane at −70° and a sample dried at 10^{-2} mm. for 30 min. at −9°. The ultraviolet extinction coefficients of the ene-triyne alcohol (see Table) were then obtained after direct and rapid weighing on a torsion balance. In light at room temperature the poly-yne (II) was first converted into a purple polymer which rapidly became black (*ca.* 3 min.) and totally insoluble in the usual organic solvents. The alcohol had $\nu_{max.}$ (in CS_2) 3600 (O–H), 3290 (≡C–H), 1090 and 1027 (C–O) and 943 $cm.^{-1}$ (=C–H); (in CCl_4): 2190 (–C≡C–) and 2040 $cm.^{-1}$ (–C≡C–H). The distribution coefficient at 17° between hexane and water was 1·35.

Hydrogenation of trans-*Non-2-ene-4 : 6 : 8-triyn-1-ol* (II).—A solution of the alcohol (II) (48·0 mg.) in ethanol (30 c.c.) was hydrogenated over pre-reduced platinic oxide (56·7 mg.) [54 c.c. (N.T.P.) hydrogen absorbed ≡6·5 mol.], to give nonan-1-ol (41·5 mg.) shown to be homogeneous and identified by vapour-phase chromatography. (*a*) By use of a column at 160°, length 300 cm., packed with silicone rubber E301 (20 parts) on firebrick (80 parts; 30—40 mesh), the retention time of the only fraction was 28 min. (octan-1-ol, 17 min.; nonan-1-ol, 27 min.; decan-1-ol, 42 min.). (*b*) On use of a column at 180°, length 483 cm., packed with silicone oil MS 550 (20 parts) on firebrick (80 parts; 50—90 mesh) the retention time was 44·5 min. (nonan-1-ol, 44·5 min.).

Hydrogenation of the alcohol (II) (81·4 mg.), treatment of the product with *p*-phenylazobenzoyl chloride in pyridine, and crystallisation from ethanol gave *nonyl* p-*phenylazobenzoate* (167 mg.), laths, m. p. 54—55° (Found: C, 75·2; H, 8·0; N, 7·7. $C_{22}H_{28}O_2N_2$ requires C, 74·95; H, 8·0; N, 7·95%). The m. p. was undepressed on admixture with a specimen made from authentic alcohol. *Decyl* p-*phenylazobenzoate* (laths from ethanol) has m. p. 59·5—60° (Found: C, 75·1; H, 8·3; N, 7·5. $C_{23}H_{30}O_2N_2$ requires C, 75·35; H, 8·25; N, 7·65%). A mixed m. p. with nonyl derivative was 52—59°.

trans-*Non-2-ene-4 : 6 : 8-triyn-1-al* (I).—Material obtained during numerous isolations of the ene-triyne (II) was combined and adsorbed from light petroleum on alumina (100 g.). Elution with this solvent and careful evaporation at 10 mm. at −9° in red light gave trans-*non-2-ene-4 : 6 : 8-triyn-1-al* (I) which was further purified by crystallisation from light petroleum (b. p. 30—40°) at −30°. The crystalline compound (6 mg.; estimated spectroscopically) was dried at −9°/10^{-2} mm. for 10 min. A sample was then weighed rapidly (torsion balance) for the determination of ultraviolet absorption data (see Table). $\nu_{max.}$ (in CS_2) were at 3290 (≡C–H), 2740 (OC–H), 1693 (C=O), 1110 (C–CHO), and 950 $cm.^{-1}$ (=C–H).

Manganese Dioxide Oxidation of trans-*Non-2-ene-4 : 6 : 8-triyn-1-ol* (II).—A solution of the alcohol (II) (85 mg.) in methylene dichloride (120 c.c.) was shaken with active manganese dioxide (1·2 g.) for 15 hr. at 20°. The solvent was then removed at −9°/10^{-2} mm., and the residual material adsorbed from light petroleum on alumina (40 g.). Elution with light petroleum (50 c.c.) afforded a fraction (4 mg.), having ultraviolet and infrared absorption spectra identical with those of the natural aldehyde (I).

The More Polar Poly-yne Fractions.—The culture medium from 100 flasks (75 l.) was continuously extracted (in 10 l. batches) with ether for 72 hr., the ethereal solution was evaporated to *ca.* 2 l., M/15-phosphate buffer (150 c.c.) was then added, and evaporation continued until *ca.* 500 c.c. of ether remained. Benzene (1 l.) was then added to ensure that poly-acetylenic compounds with low water-solubility would remain in solution, and the residual ether was removed. The aqueous phase, containing the bulk of the polar poly-acetylenes, was separated and placed in the first four tubes of a 50-plate Craig counter-current apparatus (volumes of upper and lower phases, 40 c.c.) and distributed between ether and M/15-phosphate buffer (pH 5·9). After 50 transfers the most polar compound, that with ene-diyne absorption, remained in tubes 1—9 whilst the main component, the triol (III), appeared in tubes 19—35. A small amount of material with the ultraviolet absorption characteristics of an ene-triyne was in the tubes 43—48 whilst non-polar material moved with the solvent front.

(2D : 3D)-*Nona-4 : 6 : 8-triyne-1 : 2 : 3-triol* (III).—The ethereal solution (2·5 l.) from tubes 19—35 was stored at −9°. To ensure homogeneity of the product, portions of this solution (800 c.c.) were evaporated to 40 c.c. and redistributed in the same solvent system. After 130 transfers the triol (III) appeared in fractions 32—62, and ultraviolet spectral measurements showed that it was almost pure. These fractions were combined and evaporated to 30 c.c., hexane was added dropwise to the cooled (−9°) solution, the *triyne-triol* (III) (1·15 g.) separating as needles (decomp. *ca.* 40°). The original 75 l. of culture fluid must have contained a minimum of 3·5 g. of triol.

Reproducible ultraviolet absorption data (see Table) were obtained as before. Solutions were then standardised by means of their absorption intensities. $[\alpha]_D$ was +6° (*c* 0·82) in ethanol. The partition coefficient between ether and water at 21° was 1·2. With alcoholic silver nitrate the triol gave a colourless precipitate which darkened rapidly on exposure to light. The acid liberated was detected by the Methylene Blue–Methyl Red indicator [15] but reproducible titres were not obtained.

(2D : 3D)-*Nonane*-1 : 2 : 3-*triol.*—Hydrogenation of the triol (III) (138 mg.) in ethyl acetate (50 c.c.) over pre-reduced platinic oxide [110 c.c. (N.T.P.) hydrogen absorbed $\equiv$6 mol.], and crystallisation of the product from ether gave the *triol* (110 mg.), m. p. 103—104°, $[\alpha]_D$ +15° (*c* 1·0) in ethanol, $\nu_{max.}$ (in Nujol) 3300 (v. broad) (O–H) and 1070 cm.$^{-1}$ (C–O) (Found: C, 61·2; H, 11·5. $C_9H_{20}O_3$ requires C, 61·3; H, 11·4%).

Benzoylation of the triol (80 mg.) with pyridine–benzoyl chloride gave the *tribenzoate* (160 mg.), needles (from light petroleum), m. p. 94—95°, $[\alpha]_D$ −22° (*c* 0·8 in chloroform) (Found: C, 74·0; H, 6·4. $C_{30}H_{32}O_6$ requires C, 73·8; H, 6·6%), $\nu_{max.}$ (in CS_2) 2930 (C–H), 1735 (C=O), 1605, 1455, 1250, 1105, and 708 cm.$^{-1}$.

Periodate Oxidation of (2D : 3D)-*Nonane*-1 : 2 : 3-*triol.*—A solution of the triol (22·1 mg.) in water (25 c.c.) was used in a quantitative periodate estimation 2·05 mol. consumed). Heptaldehyde was isolated by ether-extraction and gave a 2 : 4-dinitrophenylhydrazone (25 mg.), m. p. 105—106°, undepressed by admixture with an authentic specimen but depressed to 94—102° and 90—98° by octaldehyde and hexaldehyde 2 : 4-dinitrophenylhydrazones respectively.

(3L : 4L)-*Tetrahydro*-2-(*penta*-2 : 4-*diynylidene*)*furan*-3 : 4-*diol* (IV).—A solution of the triol (III) (320 mg.) in water (225 c.c.) was treated with N-sodium hydroxide solution (25 c.c.) and kept in darkness at 20° under nitrogen for 5 hr. The resulting solution was extracted continuously with ether for 15 hr. and the extract dried, evaporated to 20 c.c., and cooled to −9°. Careful addition of hexane afforded crystals (300 mg.); two recrystallisations from ether–hexane at −9° gave the pure *diol* (IV), decomp. 94°, $[\alpha]_D$ −165° (*c* 0·5 in ethanol) (Found: C, 65·7 H, 4·8. $C_9H_8O_3$ requires C, 65·85; H, 4·9%, $\nu_{max.}$ (in CCl_4) 3580 (O–H), 3300 (≡C–H), 2200 (C≡C), 1655 (=C–O), 1125, 1035, 975, and 874 cm.$^{-1}$ (for ultraviolet absorption data (see Table).

L-*Erythronolactone* (V).—A solution of (V) (850 mg.) in ethyl acetate (250 c.c.) at −35° was treated with ozonised oxygen (11 l./min.; 2%) until a permanent blue colour appeared. The mixture was then evaporated to 20 c.c. at 20° and 0·05N-hydrochloric acid (100 c.c.) was added. The remaining ethyl acetate was then removed and the solution left at 20° for 12 hr. Evaporation at 10^{-2} afforded a gum which was distilled at 120°/0·1 mm. The distillate (305 mg.) was crystallised from ethyl acetate, to give L-erythronolactone (V) (160 mg.), m. p. 100—102°, $[\alpha]_D$ +73° (*c* 0·85 in water) (Jelinek and Upson [16] give m. p. 102°, $[\alpha]_D$ +72·5°), $\nu_{max.}$ (in Nujol) 3450 (O–H), 3280 (hydrogen bonded O–H), and 1755 cm.$^{-1}$ (γ-lactone C=O) + many in 1200—800 cm.$^{-1}$ region. Benzoylation gave the dibenzoate, m. p. 108·5—109·5°, $[\alpha]_D$ +182° (*c* 1·0 in chloroform) (Jelinek *et al.*[16] give m. p. 110—111°, $[\alpha]_D$ +176°) (Found: C, 65·9; H, 4·35. Calc. for $C_{18}H_{14}O_6$: C, 66·25; H, 4·3%), $\nu_{max.}$ (in Nujol) 1800 (γ-lactone C=O) and 1725 cm.$^{-1}$ (benzoate C=O), (in CS_2) 1815, 1739 cm.$^{-1}$.

trans-*Dec*-2-*ene*-4 : 6 : 8-*triyne*-1 : 10-*diol* (VIII).—To obtain significant amounts of this compound a different procedure to that used in the isolation of the triol (III) was adopted. After continuous extraction with light petroleum (b. p. 30—40°) to remove the less polar constituents, the culture medium (50 l.) was continuously extracted with ether. The polyacetylenes in the ethereal extract were transferred to a benzene–water system (80 c.c. of each phase): a considerable amount of the triol (III) was precipitated and thus removed. The solution was then placed in the first two tubes of a 50-plate counter-current apparatus. After 53 transfers the contents of the tubes 26—40 were combined, and the aqueous layer was separated and extracted with ether. The polyacetylene present in the benzene layers and the ethereal extract was adsorbed from benzene (100 c.c.) on alumina (130 g.). Benzene–ether (1 : 1; 200 c.c.) eluted the *diol* (VIII) (16 mg.) which crystallised as colourless needles (from chloroform), decomp. 138° (Found: C, 75·5; H, 5·0. $C_{10}H_8O_2$ requires C, 75·0; H, 5·0%), $\nu_{max.}$ (in Nujol) 3200 (O–H), 1080 and 1013 (C–O), and 944 cm.$^{-1}$ (=C–H) (for ultraviolet absorption data see Table), partition coefficient 1·12 between benzene and water at 21°.

---

[15] Eglinton and Whiting, *J.*, 1953, 3055.

[16] Jelinek and Upson, *J. Amer. Chem. Soc.*, 1938, **60**, 355.

Hydrogenation of trans-*Dec*-2-*ene*-4 : 6 : 8-*triyne*-1 : 10-*diol* (VIII).—A solution of the diol (VIII) (47·3 mg.) in ethanol (200 c.c.) was hydrogenated over 5% palladised charcoal (37·8 mg.) [44 c.c. of hydrogen (N.T.P.) absorbed ≡6·7 mol.]. The product was sublimed at 80°/3 × 10^{-2} mm. to give decanoic acid (10 mg., formed by hydrogenolysis of a hydroxyl group) characterised as its anilide (m. p. 67°). The residue after sublimation was sebacic acid (35 mg.), m. p. and mixed m. p. 130—134° (from ethyl acetate).

Synthesis of Diol (VIII).—A solution of *trans*-5-bromopent-2-en-4-yn-1-ol (735 mg.) in methanol (5 c.c.) was added under nitrogen during 15 min. to a stirred solution of penta-2 : 4-diyn-1-ol (350 mg.) and cuprous chloride (30 mg.) in 33% aqueous ethylamine (10 c.c.), at 25—30°. Hydroxylamine hydrochloride was added in small portions as the reaction proceeded to ensure that the copper remained in the reduced form. After the addition was complete stirring was continued for a further 5 min., then aqueous potassium cyanide solution was added, and the mixture extracted with ether. The yield of the diol (VIII) as estimated spectroscopically was 300 mg.

The material (150 mg.) was distributed between benzene and water (the amount treated being limited by the fairly low solubility of the compound). Thus purified, the diol (100 mg.) crystallised from chloroform (Found: C, 74·9; H, 5·4. Calc. for $C_{10}H_8O_2$: C, 75·0; H, 5·0%) and was identical with the natural material.

trans-*Dec*-2-*ene*-4 : 6 : 8-*triyn*-10-*ol*-1-*al* (IX).—A solution of the diol (VIII) (60 mg.) in methylene dichloride (250 c.c.) was shaken at 20° for 24 hr. with active manganese dioxide (3·0 g.). The oxidant was then removed and the solvent carefully evaporated under reduced pressure. The residue was adsorbed from benzene (20 c.c.) on alumina (70 g.). Benzene (100 c.c.) eluted a trace of material which must be a dialdehyde. Benzene–ether (19 : 1; 200 c.c.) eluted the *hydroxy-aldehyde* (IX) (24 mg.) and starting material (21 mg.) was recovered by elution with ether. The hydroxy-aldehyde (IX) crystallised from ether–hexane as off-white needles, m. p. *ca.* 90° (decomp.), which slowly became reddish-brown in light (Found: C, 75·55; H, 4·2. $C_{10}H_6O_2$ requires C, 75·95; H, 3·8%), $\nu_{max.}$ (in Nujol) 3350 (O–H), 1665 (C=O), 1095 and 1023 (C–O), and 958 cm.$^{-1}$ (=C–H) (for ultraviolet absorption data see Table).

This work was supported financially by the Rockefeller Foundation. The authors acknowledge their indebtedness to Miss B. Crompton for the associated mycological work. The work was carried out during the tenure (by J. S. S.) of a Pressed Steel Research Fellowship.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, February 2nd,* 1959.]