280. *Chemistry of the Higher Fungi. Part XVII.* Polyacetylenic Metabolites from* Clitocybe rhizophora *Velen.*

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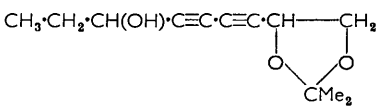
The isolation and structure determination of two C_9 polyacetylenic metabolites (I and II) are described. They are the first diacetylene derivative and the first polyacetylenic ketone to be isolated from micro-organisms. The stability and relatively large quantity of material available enabled the nuclear magnetic resonance spectra of their isopropylidene derivatives to be determined. The structure of the diacetylenic ketone (II) was established entirely by spectroscopic methods. Both metabolites disobey the "rule" that polyacetylenes with an odd-numbered carbon chain contain a free ethynyl group. This and their possible biosynthetic relationship with the widely distributed dehydromatricarianol (V) are discussed.

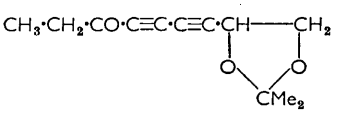
The Basidiomycete fungus *Clitocybe rhizophora* Velen was investigated as part of the screening programme described in Part X[1] of this series. The organism was grown as surface cultures on a malt medium, and was found to produce a diacetylenic triol (I) and the related keto-diol (II), the triol being the major component. These compounds were also produced on three successive glucose replacement media, when the ketone (II) became the major component. The triol (I) was first noticed (it has only a weak ultraviolet absorption spectrum) when it crystallised from a benzene solution of the neutral fraction of the ether extract from the original growths. The ketone (II), which has an intense and characteristic ultraviolet spectrum, was isolated by way of its isopropylidene derivative (IV). Subsequently, from ether extracts of the replacement media, the two metabolites were separated by alumina chromatography of their isopropylidene derivatives (III and IV).

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

$CH_3{\cdot}CH_2{\cdot}CO{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH(OH){\cdot}CH_2{\cdot}OH$ (II)



(III)



(IV)

The triol (I) formed needles which became only slightly coloured after several months in air at 20°. It is thus one of the most stable acetylenic derivatives to be isolated from natural sources. Ultraviolet (u.v.) and infrared (i.r.) absorption data indicated the metabolite to be a disubstituted polyhydroxy-diacetylene. It consumed one molecular equivalent of sodium periodate, to yield formaldehyde and a compound having u.v. absorption characteristic of a diynealdehyde,[2] thus establishing the presence of a terminal 1,2-glycol grouping adjacent to the diacetylene unit. The triol absorbed 3·8 moles of hydrogen, and periodate cleavage and chromic acid oxidation of the hydrogenated material gave 6-oxo-octanoic acid,[3] thereby establishing structure (I).

The triol (I) provided an opportunity of studying the nuclear magnetic resonance (n.m.r.) spectrum of a fungal polyacetylene since, contrary to our usual experience, the

* Part XVI, *J.*, 1963, 4120.

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

[2] Bohlmann, *Chem. Ber.*, 1953, **86**, 657; Bohlmann, Herbst, Arndt, Schönowsky, and Gleinig, *Chem. Ber.*, 1961, **94**, 3193.

[3] Blaise and Koehler, *Compt. rend.*, 1909, **148**, 489; *Bull. Soc. chim. France*, 1910, **7**, 222.

metabolite is very stable and was available in unusually large amounts. It was decided to investigate the isopropylidene derivative (III), since this eliminated two of the hydroxyl protons and permitted examination in carbon tetrachloride. The spectrum gave a clear indication of the presence of an ethylcarbinol group, confirmed by the spectrum of pent-1-yn-3-ol (see Table). The resonance absorption from the ketal ring clearly showed the non-equivalence of the *gem*-dimethyl group and ketal ring protons, owing to the asymmetric substitution of the rigid cyclic structure. The *gem*-dimethyl protons gave two lines, and the three ring-protons formed a 10-line ABX system. This analysis was confirmed through examination of the spectrum of 1,2-*O*-isopropylidenebut-3-yne-1,2-diol (see Table), which exhibited similar characteristics, having an ABX system of 12 lines due to the different

Nuclear magnetic resonance data of the metabolites and related compounds.

τ (p.p.m.)	*J* (c./sec.)	Assignment
1,2-*O*-Isopropylidenenona-3,5-diyne-1,2,7-triol (III)		
8·99 triplet	7·2	$-CH_2\cdot CH_3$
8·66 singlet, 8·55 singlet	—	$>CMe_2$
8·30 quintet	7·2	$-CH(OH)\cdot CH_2\cdot CH_3$
6·50 singlet	—	−OH
6·10 *, 5·90 * septet	$J_{AB} = 8·1$	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}-$
5·68 triplet	6·2	$\equiv C\cdot CH(OH)\cdot CH_2-$
5·27 * triplet	$J_{AX} = J_{BX} = 5·9$	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}-$
1,2-Dihydroxy-1,2-*O*-isopropylidenenona-3,5-diyn-7-one (IV)		
8·88 triplet	6·7	$CH_3\cdot CH_2\cdot CO-$
8·68 singlet, 8·56 singlet	—	$>CMe_2$
7·47 quartet	7·4	$CH_3\cdot CH_2\cdot CO-$
6·14 quartet *, 5·96 quartet *	$J_{AB} = 8·0$	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}-$
5·32 triplet *	$J_{AX} = J_{BX} = 5·7$	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}\cdot C\equiv C-$

τ (p.p.m.)	*J* (c./sec.)	Assignment
Pent-1-yn-3-ol		
8·99 triplet	7·3	$-CH_2\cdot CH_3$
8·28 quintet	6·9	$-CH(OH)\cdot CH_2\cdot CH_3$
7·60 doublet	2·2	$H\cdot C\equiv C\cdot CH(OH)-$
6·02 singlet	—	−OH
5·73 double triplet	6·2, 2·0	$HC\equiv C\cdot CH(OH)\cdot CH_2-$
1,2-*O*-Isopropylidenebut-3-yne-1,2-diol		
8·69 singlet, 8·59 singlet	—	$>CMe_2$
7·64 doublet	2·2	$HC\equiv C\cdot CHO-$
6·22 quartet *, 5·98 quartet *	$J_{AB} = 7·6$, $J_{AX} = J_{BX} = 6·2$	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}-$
5·48 double triplet *	6·2, 2·0	$\overline{O\cdot CMe_2\cdot O\cdot CH_2\cdot CH}\cdot C\equiv CH$
Hydrogenated (IV)		
8·96 triplet	7·4	$CH_3\cdot CH_2\cdot CO-$
8·69 singlet, 8·65 singlet	—	$>CMe_2$
7·60 triplet	6·2	$-CO\cdot CH_2\cdot CH_2-$
7·60 quartet	7·3	$CH_3\cdot CH_2\cdot CO-$

* ABX system.

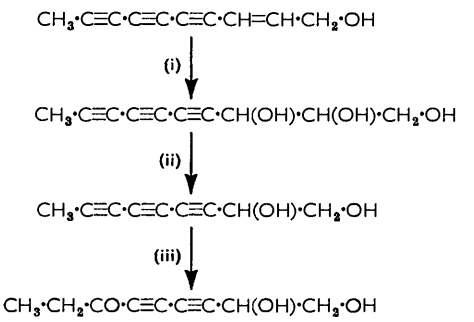
substitution of the ketal ring. Thus, the structure of the isopropylidene derivative (III), and therefore that of the triol (I), was satisfactorily confirmed.

The ketone (II) also formed an isopropylidene derivative (IV), whose structure was determined entirely from spectral data. The presence of a conjugated diacetylenic ketone was established from its u.v. and i.r. spectra. The n.m.r. spectrum (see Table) established the presence of an ethyl group adjacent to either an acetylenic linkage or a carbonyl group, together with a resonance system from the ketal group very similar to that found in the spectrum of the isopropylidene derivative (III). Hydrogenation of compound (IV) gave an isopropylidene derivative whose n.m.r. spectrum (see Table) resulted from a very similar ethyl group, thus establishing ethyl–carbonyl propinquity and the structure of the natural ketone as (II).

The two metabolites (I) and (II) are seen to be closely related; indeed, oxidation of the derivative (III) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gave the ketone isopropylidene derivative (IV). Reduction of the ketone derivative (IV) with sodium borohydride resulted in the formation of a mixture of diastereoisomers of the isopropylidene derivative of the triol (I). This mixture had spectroscopic (u.v., i.r., and n.m.r.) properties identical with those of the natural triol derivative (III).

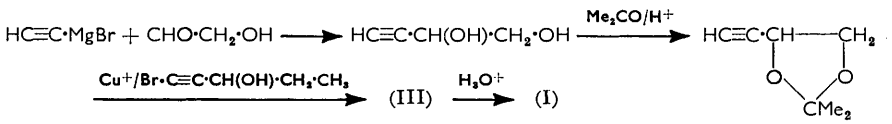
The biosynthetic relationship between metabolites (I) and (II) is obvious although it

is by no means certain which is the precursor of the other.* Their relationship to the main group of C_9 and C_{10} fungal polyacetylenes is less clear. However, both metabolites disobey the erstwhile " rule " that fungal polyacetylenes with an odd number of carbon atoms contain a free ethynyl group. From the increasing number of " exceptions," there is reason to suppose that a mechanism is available to several fungi for the removal of a carbon atom (in as yet an unknown form) from the carbon terminus finally attached to



the coenzyme A. The derivation from, *e.g.*, dehydromatrocarianol (V) is then seen to be formally through three steps (order unknown), *viz.*, (i) hydroxylation of the double bond [cf., nona-4,6,8-triyne-1,2,3-triol (VI) from *Coprinus quadrifidus*,[4] (ii) carbon–carbon cleavage (cf. dimethyl *trans*-undec-2-ene-4,6-diyne-1,11-dioate from *Drosophila subatrata*,[5] *trans*-non-2-ene-4,6-diyne-1,9-diol from *Poria sinuosa* [6] and *Aleurodiscus roseus*,[7] and several other unpublished examples), and (iii) hydration of a triple bond. The biosynthetic relationships between the metabolites themselves and with dehydromatricarianol are being investigated.

The synthesis of the racemic diastereoisomers of the diynetriol (I) was achieved thus:



The reaction between ethynylmagnesium bromide and glycollaldehyde gave the known but-3-yne-1,2-diol,[8] the isopropylidene derivative of which reacted [9] with 1-bromopent-1-yn-3-ol. Hydrolysis of the product gave a mixture of the racemic diastereoisomers of the triol (I) from which the natural racemic diastereoisomer was separated by crystallisation.

Experimental

Ultraviolet (Cary model 14M double-beam recording spectrophotometer) and infrared (Perkin-Elmer 21) spectra were recorded in ethanol and carbon tetrachloride, respectively, except where otherwise stated. Optical rotations were measured in ethanol using an ETL–NPL (Ericksson) automatic polarimeter type 143A. M. p.s (corrected) were determined on a Kofler block. Nuclear magnetic resonance data were obtained in carbon tetrachloride (tetramethylsilane internal reference) on an A.E.I. RS 2 spectrometer operating at 60 Mc./sec. The shift of spectral lines from that of tetramethylsilane was measured by the " superimposition method," assuming a linear sweep. Alumina for chromatography was Woelm " neutral "

* [*Added in Proof.*] Recent evidence now suggests that the ketone (II) is the biosynthetic precursor of the triol (I).

[4] Jones and Stephenson, *J.*, 1959, 2197.
[5] Jones, Leeming, and Reemers, *J.*, 1960, 2257.
[6] Cambie, Gardner, Jones, Lowe, and Read, *J.*, 1963, 2056.
[7] Cambie, Hirschberg, Jones, and Lowe, *J.*, 1963, 4120.
[8] Lespieau, *Compt. rend.*, 1925, **180**, 442; *Bull. Soc. chim. France*, 1928, **43**, 203.
[9] Chodkiewicz, *Ann. Chim.* (*France*), 1957, **2**, 852.

deactivated by 10% of water. Chemical drying was effected by anhydrous magnesium sulphate, and evaporations were carried out under reduced pressure, unless otherwise stated.

Growth of Clitocybe rhizophora.—The fungus [obtained from the Type Culture Collection, Baarn (Netherlands)] was grown as surface cultures on a medium of 3% malt extract. Maximum concentrations, after 35—44 days, of diynetriol and diyne ketone were *ca.* 45 and *ca.* 5 mg./l., respectively. Two subsequent replacements of the medium by 4% solutions of glucose each produced *ca.* 8 mg./l. of diynetriol and *ca.* 20 mg./l. of diyne ketone after *ca.* 21 days. A third replacement afforded only 4 and 8 mg./l. of triol and of ketone, respectively, after 26 days.

Isolation of Polyacetylenes.—(*a*) For original growths, the aqueous medium (20 l.) was decanted and continuously extracted with ether (2 l.) for 24 hr. The extract was evaporated to 200 c.c. and separated into acidic and neutral fractions with sodium hydrogen carbonate solution. The neutral components were transferred to benzene, from which the triol crystallised at 4°. The benzene solution was then extracted with water and the combined aqueous extracts were extracted with ether. After drying and evaporation, the residue from the ethereal extracts was heated under reflux in acetone (40 c.c.) with toluene-*p*-sulphonic acid (5 mg.) for 18 hr.; an excess of solid sodium carbonate was then added and the mixture kept at 15° for 1 hr. The residue, after filtration and evaporation of the acetone solution, was chromatographed in light petroleum (b. p. 30—40°) on alumina (10 g.), and the purified isopropylidene derivative (31 mg.) was eluted in the same solvent (150 c.c.).

(*b*) For the replacement media, ethereal extracts of the culture fluid were washed with sodium hydrogen carbonate and transferred to benzene (200 c.c.). This solution was then extracted with water, and the aqueous extracts were extracted with ether. The dried ethereal extracts were evaporated, and the residue was treated with acetone as described above. The crude products were chromatographed on alumina (18 g.), to give the diyne ketone isopropylidene derivative with light petroleum (b. p. 30—40°; 250 c.c.), and the diynetriol isopropylidene derivative with light petroleum (b. p. 40—60°)–benzene (1 : 1; 120 c.c.).

The free triol and keto-diol were obtained from their isopropylidene derivatives by treatment with 2N-hydrochloric acid (10 c.c.) for 30 min., and extraction of the mixture with ether (3 × 20 c.c.). The extracts were dried to give solutions of the triol (I) and keto-diol (II).

Nona-3,5-diyne-1,2,7-triol (I).—Recrystallisation from ether–hexane afforded *needles*, m. p. 71·5—72·5°, $[\alpha]_D^{26}$ −21° (*c* 0·53) (Found: C, 64·5; H, 7·45. $C_9H_{12}O_3$ requires C, 64·25; H, 7·2%), $\lambda_{max.}$ 2560 (ε 230), 2420 (ε 370), 2280 (ε 450), and 2200 Å (ε 410), $\nu_{max.}$ (in Nujol) 3130 (O–H), 1082 and 1028 cm.$^{-1}$ (C–O).

Oxidation of the Triol (I) *with Sodium Periodate.*—(*a*) The triol (57·0 mg., 0·34 mmole with an excess of sodium periodate solution (*ca.* 4 g./l.; 40 c.c.) was kept at 15° for 12 hr. The solution was then reduced to about one third of the original volume by distillation, and the distillate collected under saturated aqueous dimedone (*ca.* 0·8 mmole). The distillate mixture was kept at *ca.* 95° for 10 min. and then set aside at 0°. The precipitate (80 mg.), m. p. 150—165° and 190—192°, was recrystallised from aqueous ethanol to give needles (45 mg., 45%), m. p. and mixed m. p. 191—192°, of formaldehyde dimedone derivative.

(*b*) The triol (32 mg.) was treated with an excess of sodium periodate solution (20 c.c.; 4 g./l.) at 15° for 16 hr., the solution was extracted with ether (3 × 25 c.c.), and the ethereal extracts were washed with water (3 × 100 c.c.) and dried. Evaporation yielded an unstable yellow oil (27 mg.), $[\alpha]_D^{19}$ −21° (*c* 0·70) [an ethanolic solution of the oil (0·02% w/v) lost 95% of its u.v. absorption intensity at 15° during 8 hr.], $\lambda_{max.}$ 2865 (ε 4500), 2705 (ε 5600), 2565 (ε 4100), 2435 (ε 2600), and 2325 Å (ε 1700) (cf. ref. 2), $\nu_{max.}$ 3597 (O–H), 2732 (C–H of aldehyde), 2232, 2174 and 2110 (C≡C), and 1668 cm.$^{-1}$ (C=O).

Quantitative Hydrogenation.—The triol (6·40 mg.) was hydrogenated in ethanol over platinum (from 9 mg. of platinic oxide) in a Hösli microhydrogenator. The uptake of hydrogen at s.t.p. was 3·22 c.c., 95% of that calculated for a diacetylene with *M* 168. In a second run, the triol (6·57 mg.) absorbed 3·28 c.c. at s.t.p., 94% of the calculated value.

Large-scale Hydrogenation of the Triol (I).—The triol (393 mg.) was hydrogenated in ethanol (100 c.c.) over 5% palladised charcoal (42 mg.) for 3 hr. The solution was filtered and evaporated, to yield an oil (409 mg.) which could not be crystallised, $[\alpha]_D^{22}$ +15° (*c* 0·78), $\nu_{max.}$ (liquid film), 3330 (broad; intermolecular-bonded OH), 1119 and 1071 cm.$^{-1}$ (C–O–H).

Oxidation of the Hydrogenated Triol with Sodium Periodate.—(*a*) To the perhydro-triol (43 mg.) in aqueous ethanol (25 c.c.) was added sodium periodate solution (25 c.c.; 0·4%).

This solution was kept at 15° and titrations of samples indicated consumption of 81 mole % of periodate after 1 hr. and 95 mole % after 24 hr.

(*b*) The perhydro-triol (176 mg., 1·00 mmole) was dissolved in sodium periodate solution (0·4%; 188 c.c., 3·50 mmoles) containing sodium carbonate (415 mg., 3·00 mmoles) and potassium permanganate (21 mg., 0·13 mmole) and kept at 15° for 16 hr. The solution was acidified, extracted with ether, and the extracts were dried and evaporated. The residue (166 mg.) was dissolved in acetone (15 c.c.), and 8N-chromic acid–12N-sulphuric acid (0·5 c.c.) was added with stirring during 35 min. The mixture was stirred at 15° for a further 2 hr., and the acetone solution was decanted and evaporated to 5 c.c. The separated solids were washed with ether, and the ethereal and acetone solutions combined and extracted twice with saturated sodium hydrogen carbonate solution. These extracts were acidified, and isolation with ether yielded a semi-solid mass which was extracted with hot hexane (2 × 10 c.c.). On cooling, prisms (5 mg.) of 6-oxo-octanoic acid, m. p. 49—51°, were deposited, $\nu_{max.}$ 3500—2642 (CO_2H), and 1751, 1712sh, and 1703 cm.$^{-1}$ (carbonyl and carboxyl), (lit.,[3] m. p. 52°). The semicarbazone formed needles, m. p. 189—190° (from aqueous ethanol) (lit.,[3] 190°).

The Isopropylidene Derivative (III) *of the Triol* (I).—The crude triol (98 mg.) in acetone (50 c.c.) was heated under reflux with toluene-*p*-sulphonic acid (20 mg.) for 18 hr. An excess of solid sodium carbonate was then added, and the mixture was kept at 15° for 2 hr., filtered, and evaporated to dryness. The residue was adsorbed from light petroleum (b. p. 40—60°)–benzene (1 : 1) on to alumina (15 g.), and the *isopropylidene compound* (59 mg.) eluted in the same solvent (100 c.c.) as an oil, $[\alpha]_D^{24}$ −61° (*c* 0·23) (Found: C, 68·75; H, 8·05. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·75%), $\lambda_{max.}$ 2575 (ε 350), 2440 (ε 560), 2315 (ε 580), and 2200 Å (ε 470), $\nu_{max.}$ 3584 (O–H), 2150 (weak C≡C), 1381 and 1373 (CMe_2), and 1210, 1151, and 1065 cm.$^{-1}$ (C–O–C).

Hydrogenation of the Isopropylidene Compound (III).—The isopropylidene compound (27 mg.) in ethanol (15 c.c.) was hydrogenated over 5% palladised charcoal (20 mg.) for 4 hr. The product was chromatographed on alumina (9 g.) and eluted in benzene as an oil (15 mg.), $[\alpha]_D^{21}$ 0° ± 0·3° (*c* 0·38), $\nu_{max.}$ 3610 (O–H), 1380 and 1372 (CMe_2), and 1211, 1157, and 1061 cm.$^{-1}$ (C–O–C).

1,2-*Dihydroxynona*-3,5-*diyn*-7-*one* (II).—Crystallisation from ether–hexane afforded the *keto-diol* as needles, m. p. 34·5—35°, $[\alpha]_D^{24}$ −30° (*c* 2·52) (Found: C, 65·25; H, 6·15. $C_9H_{10}O_3$ requires C, 65·05; H, 6·05%), $\lambda_{max.}$ 2825 (ε 4300), 2670 (ε 5700), 2535 (ε 4200), 2410 (ε 2300), and 2275 Å (ε 1400), $\nu_{max.}$ 3553 (broad; O–H), 2240 and 2150 (C≡C), 1683 (C=O), and 1103 and 1068 cm.$^{-1}$ (C–O).

The Isopropylidene Derivative (IV) *of the Ketone* (II).—(*a*) Prepared as described in the isolation and purification, the *isopropylidene derivative* was isolated, after chromatography, as an oil, $[\alpha]_D^{21}$ −65° (*c* 0·35) (Found: C, 69·75; H, 7·3. $C_{12}H_{14}O_3$ requires C, 69·9; H, 6·85%), $\lambda_{max.}$ 2825 (ε 4700), 2670 (ε 6500), 2525 (ε 4700), 2400 (ε 2500), and 2270 Å (ε 1600), $\nu_{max.}$ 2230 and 2141 (C≡C), 1675 (C=O), 1379 and 1371 (CMe_2), and 1206, 1147, and 1065 cm.$^{-1}$ (C–O–C).

(*b*) The isopropylidene compound (III) (*ca.* 200 mg.) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (387 mg.) in benzene (30 c.c.) were kept at 15° for 40 hr. The mixture was then adsorbed directly on to alumina (10 g.) and eluted with carbon tetrachloride (50 c.c.). The eluate contained the ketone (IV) (28 mg.), $[\alpha]_D^{23}$ −57°(*c* 1·1; visual polarimeter), $\lambda_{max.}$ 2825 (ε 4000), 2665 (ε 5600), 2525 (ε 4900), 2395 (ε 3700), and 2260 Å (ε 3000), $\nu_{max.}$ 2230, 2137 (C≡C), 1677 (C=O), 1381 and 1373 (CMe_2), and 1210, 1148, and 1066 cm.$^{-1}$ (C–O–C).

Reduction of the Ketone (II) *with Sodium Borohydride*.—The ketone (45 mg.) was treated with an excess of sodium borohydride in methanol (10 c.c.) at 0° for 30 min. The mixture was then evaporated to 2—3 c.c., and poured into water (40 c.c.), and isolation with ether yielded a colourless uncrystallisable oil (32 mg.), $[\alpha]_D^{23}$ −15° (*c* 1·58), $\lambda_{max.}$ 2555 (ε 350), 2420 (ε 540), 2310 (ε 530), and 2200 Å (ε 420), $\nu_{max.}$ 3400 (broad; O–H), and 1096, 1042, and 1016 cm.$^{-1}$ (C–O–H).

Reduction of the Isopropylidene Derivative (IV) *with Sodium Borohydride*.—The isopropylidene derivative (84 mg.) was treated with an excess of sodium borohydride as described above, and the product, isolated from ether, had $[\alpha]_D^{22}$ −50° (*c* 0·29), $\lambda_{max.}$ 2575 (ε 350), 2435 (ε 540), 2315 (ε 560), and 2190 Å (ε 490), $\nu_{max.}$ 3571 (O–H), 1378 and 1370 (CMe_2), and 1205, 1148, and 1063 cm.$^{-1}$ (C–O–C).

Hydrogenation of the Ketone Isopropylidene Derivative (IV).—The ketone isopropylidene compound (150 mg.) was hydrogenated over 5% palladised charcoal (43 mg.) in ethanol (15 c.c.) at 15° for 6 hr. The product was eluted from alumina (10 g.) by light petroleum (b. p.

40—60°)–benzene (6 : 1) as an oil (120 mg.), $[\alpha]_D$ −7° (*c* 0·24), $\nu_{max.}$ 1723 (C=O), 1379 and 1371 (CMe_2), and 1210, 1156, and 1061 cm.$^{-1}$ (C–O–C).

Synthesis of Racemic Nona-3,5-diyne-1,2,7-triol (I).—1,2-O-*Isopropylidenebut-3-yne-1,2-diol.* Ethyl bromide (2·28 g.) in tetrahydrofuran (11 c.c.) was added in portions, under nitrogen, to a stirred mixture of magnesium turnings (0·53 g.) in tetrahydrofuran (20 c.c.) at 15°, during 30 min. The mixture was heated under reflux for 1 hr., cooled, and filtered through glass wool into a tap funnel, from which it was added dropwise, under acetylene, to a stirred saturated solution of acetylene in dry tetrahydrofuran (60 c.c.) (prepared by passing in acetylene for 20 min. at 0°). The addition was complete in 30 min., after which the solution was stirred for 30 min. under acetylene (cf. ref. 10). The acetylene was then replaced by nitrogen, and glycollaldehyde hydrate (0·45 g.) in tetrahydrofuran (35 c.c.) was added dropwise during 1 hr. After stirring for 16 hr. the clear orange solution was treated with saturated ammonium chloride solution (50 c.c.), and stirred for a further 30 min., and the two layers were separated. The top layer was evaporated to small bulk, and the aqueous phase extracted continuously with ether for 12 hr. The ethereal and tetrahydrofuran solutions were combined, dried, and evaporated. The residue was dissolved in acetone (60 c.c.) containing toluene-*p*-sulphonic acid (0·02 g.), and heated under reflux for 15 hr. An excess of sodium carbonate was then added, and the mixture kept at 15° for 1 hr. The filtered solution was evaporated, and the residue chromatographed in light petroleum (b. p. 30—40°) on alumina (8 g.). The *isopropylidene derivative* (0·13 g., 14% from glycollaldehyde hydrate) was eluted, in the same solvent, as a mobile oil (Found: C, 66·75; H, 8·35. $C_7H_{10}O_2$ requires C, 66·65; H, 8·0%), $\nu_{max.}$ 3274 (≡C–H), 1378 and 1370 (CMe_2), and 1206, 1151, and 1063 cm.$^{-1}$ (C–O). Hydrolysis of an aliquot with 2N-hydrochloric acid gave but-3-yne-1,2-diol, plates (from carbon tetrachloride), m. p. 38—39° (lit.,[8] 39·5—40·5°).

1-*Bromopent-1-yn-3-ol.* Pent-1-yn-3-ol (2·00 g.) was dissolved in a few drops of 2N-sodium hydroxide solution, and sodium hypobromite solution [14 c.c. of a solution from ice (20 g.), 10N-sodium hydroxide (10 c.c.), bromine (2·2 c.c.), and water (4 c.c.)] was added during 5 min. with stirring. After stirring for 30 min., isolation in the usual way yielded the *bromo-alcohol* (2·6 g.), b. p. 56°/1·5 mm., n_D^{23} 1·4953 (Found: C, 36·85; H, 4·45; Br, 49·3. C_5H_7BrO requires C, 36·85; H, 4·35; Br, 49·05%), $\nu_{max.}$ 3610 (O–H) and 2208 cm.$^{-1}$ (C≡C).

(±)-*Nona-3,5-diyne-1,2,7-triol* (with R. C. Cambie). To a stirred solution of cuprous chloride (5 mg.) in aqueous ethylamine (33%; 10 c.c.) was added 1,2-*O*-isopropylidenebut-3-yne-1,2-diol (5·0 g.) in methanol (5 c.c.) under nitrogen at 17°. Hydroxylamine hydrochloride was added in sufficient quantity to maintain the copper in the reduced form. 1-Bromopent-1-yn-3-ol (6·0 g.) in methanol (5 c.c.) was added dropwise during 10 min., hydroxylamine hydrochloride being added periodically as required. After stirring for 20 min., an excess of potassium cyanide was added, the mixture poured into water, and the product isolated with ether. The product, in light petroleum (b. p. 40—60°)–benzene (20 : 1), was adsorbed on to alumina (5 g.). and the oily isopropylidene derivative (3·1 g.) was eluted with light petroleum (b. p. 40—60°)–benzene (1 : 1) and further purified by chromatography, $\nu_{max.}$ (liq. film) 3390 (O–H), 1370 and 1362 (CMe_2), and 1214, 1152, and 1063 cm.$^{-1}$ (C–O).

The synthetic isopropylidene derivative (3·0 g.) was treated with 2N-hydrochloric acid (20 c.c.) at 15° for 16 hr. The product was isolated with ether, and fractionally crystallised from ether–hexane and then ether–chloroform, to yield nona-3,5-diyne-1,2,7-triol (final yield 80 mg.) as needles, m. p. 68° (Found: C, 64·1; H, 7·1. Calc. for $C_9H_{12}O_3$: C, 64·25; H, 7·2%). The compound had an i.r. spectrum identical with that of the *C. rhizophora* natural product, m. p. 71·5—72·5° and mixed m. p. 69·5—71°, $\lambda_{max.}$ 2560 (ε 216), 2425 (ε 370), 2300 (ε 368), and 2180 Å (sh.; ε 275). The more soluble fraction from the crystallisation contained the other racemic diastereoisomer which was not obtained crystalline.

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[10] Jones, Skatteböl, and Whiting, *J.*, 1956, 4765.