300. *Chemistry of the Higher Fungi. Part XII.* The Enzymic Decarboxylation of an αβ-Acetylenic Acid.*

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A cell-free extract from the Basidiomycete *Coprinus quadrifidus* has been shown to convert the hydroxy-acetylenic acid (I; R = CO_2H) into the alcohol (I; R = H) containing the free ethynyl group. The significance of this conversion is discussed in connexion with the occurrence of polyacetylenes containing an odd number of carbon atoms.

IT has been suggested that the free ethynyl group, which has been observed only in those fungal polyacetylenes with odd-numbered carbon chains,† is formed either by the decarboxylation of an αβ-acetylenic acid of even chain-length, or, perhaps less probably, by elimination of formaldehyde from the acetylenic alcohol.[1,2] In an attempt to gain experimental support for this hypothesis, *Coprinus quadrifidus* has been examined for an enzyme system capable of bringing about such a conversion. This organism was chosen because it has been shown to produce, in addition to C_{10} compounds, a series of closely related C_9 compounds containing the free ethynyl group. The substrate selected was the hydroxy-acid (I; R = CO_2H) since it can be readily prepared and detected spectroscopically and since the enediyne chromophore of the expected product (I; R = H) would also be easily detected.

Cell-free extracts obtained by crushing the mycelium of *C. quadrifidus* in a Hughes press [3] showed no interfering absorption in the ultraviolet region and had pH between 4 and 5. The extracts were adjusted to pH 7 before and after the addition of the substrate (I; R = CO_2H). The amount of polyacetylene present was estimated spectroscopically. It was found that the hydroxy-acid (I; R = CO_2H) was converted into a product with the

(I) $R\cdot C{\equiv}C\cdot C{\equiv}C\cdot \overset{t}{C}H{=}CH\cdot CH_2OH$ $\qquad$ $R\cdot C{\equiv}C\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH_2\cdot CH_2\cdot CO_2H$ (II)

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

enediyne chromophore at approximately the same rate by equivalent concentrations of (*a*) blended mycelium, (*b*) crushed mycelial slurries, and (*c*) the supernatant solution from (*b*). The " cell solids " from (*b*) showed no activity. The cell-free extract (*c*) was used in the preparative experiments, and the enediyne produced after two days was isolated. It gave heptan-1-ol on hydrogenation and was oxidised by manganese dioxide to a diynenal. The infrared spectrum of the original product showed typical free ethynyl and *trans*-olefinic peaks, confirming its nature as the alcohol (I; R = H). In comparable small-scale experiments spectroscopic evidence was obtained which indicated that the related triynedicarboxylic acid (II; R = CO_2H) was similarly converted into the ethynyl-acid (II; R = H).

The isolation of the alcohol (I; R = H) in this experiment clearly supports the suggestion made from structural evidence,[1,2] that fungal metabolities containing the free ethynyl group are formed by cleavage at the terminal carbon atom of αβ-acetylenic metabolites. The absence of any intermediates such as the corresponding αβ-acetylenic aldehyde or alcohol and the common occurrence of decarboxylating enzymes in Nature renders the decarboxylation mechanism highly probable.

The enzyme system appears to be quite tolerant towards the gross structure of the

* Part XI, *J.*, 1960, 2257.

† One exception to this generalisation has recently been found. The biogenesis of this metabolite is being investigated.

[1] Jones, Pedler Lecture, 1959, *Proc. Chem. Soc.*, 1960, 199; Bu'Lock and Gregory, *Biochem. J.*, 1959, **72**, 322.

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

[3] Hughes, *Brit. J. Exp. Path.*, 1951, **32**, 97.

αβ-acetylenic acid as the products (I; R = H) and (II; R = H) are not natural metabolites of this organism. This enzyme system may therefore be of value in the synthesis of elaborate naturally occurring polyacetylenes such as mycomycin (III) in which the free ethynyl group represents a centre of considerable instability.

Experimental

For details of general experimental methods and conditions of culture growth see Part IX.[2]

8-Hydroxyoct-trans-6-*ene*-2,4-*diynoic acid* (I; R = CO_2H).—To a stirred solution of cuprous chloride (25 mg.) in 33% aqueous ethylamine (5·6 c.c.) was added an aqueous solution of 90% propiolic acid (0·89 g.). To this mixture in nitrogen at 15° *trans*-5-bromopent-2-en-4-yn-1-ol, prepared from *trans*-pent-2-en-4-yn-1-ol [4] (1·04 g.), was added during 10 min. Crystals of hydroxylamine hydrochloride were added as required to reduce any cupric ion formed. After being stirred for a further 10 min. the mixture was treated with potassium cyanide (0·25 g.), diluted with water (to 35 c.c.), and extracted with ether. The aqueous phase was acidified, and further ether-extraction gave the crude *hydroxy-acid* (I; R = CO_2H) (0·65 g., 67%). Crystallisation from ether–light petroleum afforded off-white prisms, slowly decomposing above 147° (Found: C, 63·6; H, 4·4. $C_8H_6O_3$ requires C, 64·0; H, 4·0%), $\lambda_{max.}$ (in EtOH) 3030 (ε 8050), 2850 (ε 11,350) 2700 (ε 9870), 2590 (ε 7900), 2495 (ε 7000), 2220 (ε 41,400), and 2165 Å (ε 44,000), $\nu_{max.}$ (in CS_2) 3340 (OH), 1650 (conjugated acid C=O stretching), and 940 cm.$^{-1}$ (*trans*-ethylenic hydrogen).

Cell-free Extract of C. quadrifidus.—After 15 days' growth, mycelium from six penicillin flasks of *C. quadrifidus* was washed with water, blended with 0·1M-phosphate buffer (pH 7·2) and crushed in a Hughes press.[3] On attaining room temperature the slurry obtained was centrifuged at 1800 ***g*** for 25 min. and the clear solution (*ca.* 100 c.c.) decanted from the " cell solids." The cell-free extract was used immediately.

trans-*Hept*-2-*en*-4,6-*diyn*-1-*ol* (I; R = H).—An aqueous solution (8 c.c.) of the acetylenic acid (I; R = CO_2H) (80 mg.) was added to the cell-free extract (90 c.c.), adjusted to pH 7·0 with saturated sodium hydrogen carbonate, and transferred to a sterile conical flask (500 c.c.) fitted with a sterile cotton-wool plug. After 48 hr. at 25° in the dark, when the spectrum of the ether extract of an acidified sample (1 c.c.) showed an optimum yield of the enediyne, the solution was diluted with water (100 c.c.) and extracted with ether. The dried ethereal solution was estimated spectroscopically to contain 25 mg. of enediyne. *The product* (I; R = H) was purified on a column of Woelm acidic alumina (1·5 × 6·0 cm.) and crystallised from hexane at −40° as fine colourless needles, m. p. 48—49°, rapidly becoming brown in light (Found: C, 77·0, 78·6; H, 6·1, 6·9. C_7H_6O requires C, 79·2; H, 5·7%), $\lambda_{max.}$ (in EtOH) 2785 (ε 10,600), 2640 (ε 13,800), 2500 (ε 9200), 2370 (ε 4900), 2260 (ε 2800), 2075 Å (ε 115,500), $\nu_{max.}$ (in CS_2) 3510 (OH), 3226 (≡CH), 951sh, and 942 cm.$^{-1}$ (*trans*-ethylenic hydrogen). Hydrogenation over 5% palladised charcoal gave heptan-1-ol, identified by vapour-phase chromatography. Oxidation with manganese dioxide in chloroform gave a product with typical diynenal spectrum, $\lambda_{max.}$ (in $CHCl_3$) 3040, 2860, 2700, and 2560 Å.

A control, prepared by heating a portion of the cell-free extract (2 c.c.) at 100° for 10 min., did not effect conversion of the hydroxy-acid (I; R = CO_2H) under the same conditions.

Enzymic Reaction of Acid (II; R = CO_2H).—The acetylenic dicarboxylic acid (II; R = CO_2H) (24 mg.; from *Merulius lacrymans* [5]) in a cell-free extract (50 c.c.; pH 5·9) from 27-day old mycelium of *C. quadrifidus* was incubated for 6 days at 22°. Extraction with ether did not remove residual substrate but gave an acidic fraction showing $\lambda_{max.}$ 2065 Å (in EtOH) (triyne) corresponding to *ca.* 2 mg. of the acetylenic acid (II; R = H). The methylated acid fraction showed a sharp band with $\nu_{max.}$ (in CS_2) 3300 cm.$^{-1}$ (C≡CH).

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[4] Chodkiewicz, *Ann. Chim.* (*France*), 1957, **2**, 852.
[5] Gardner, Joncs, Leeming, and Stephenson, *J.*, 1960, 691.