898. *The Biosynthesis of Polyacetylenes. Part V.* The Role of Malonate Derivatives, and the Common Origin of Fatty Acids, Polyacetylenes, and "Acetate-derived" Phenols.*

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Radioactivity from diethyl [α-^{14}C]malonate is incorporated specifically into $C_{(1)}$—$C_{(8)}$ of a C_{10} polyacetylene (III) and not into the terminal C_2 group. This is analogous to findings with fatty acids and with acetate-derived phenols, and a hypothesis linking the three types of biosynthetic product is suggested.

In earlier papers,[1,2] such polyacetylenes as nemotinic acid (I) and matricaria ester (II) were shown to be acetate-derived, inasmuch as the C_2 units of the characteristic unbranched chain are derived *in vivo* from an acetic acid derivative, presumably acetyl-CoA. It was observed [2] that such an origin superficially resembled that of natural fatty acids as then understood. More recently it has become clear [3] that in the biosynthesis of, for example, palmitic acid, only one of the eight C_2 units arises directly from acetyl-

$HC{:}C{\cdot}C{:}C{\cdot}CH{:}C{:}CH{\cdot}CH(OH){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$ (I)

$H_3C{\cdot}CH{:}CH{\cdot}[C{:}C]_2{\cdot}CH{:}CH{\cdot}CO_2Me$ (II)

$H_3C{\cdot}[C{:}C]_3{\cdot}CH{:}CH{\cdot}CH_2{\cdot}OH$ (III)

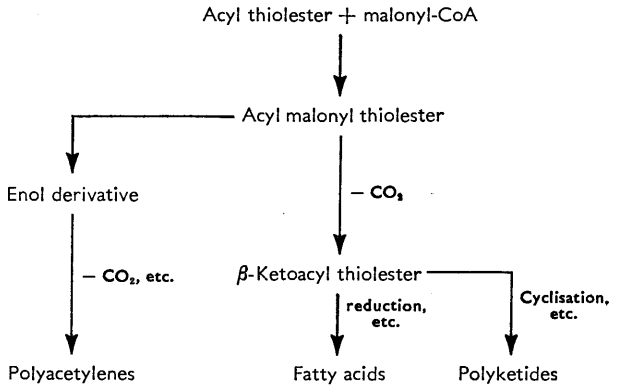
CoA; this furnishes a starter group [$C_{(16)}$ + $C_{(15)}$ in palmitate] to which the remaining C_2 units are attached as malonyl-CoA, formed from acetyl-CoA by carboxylations. Having established a similar mode of assembly in acetate-derived phenols,[4] evidence of the same kind was sought for the polyacetylenes. We had earlier observed marked stimulation of the biosynthesis of compound (I) by malonate,[5] but for the present work we employed dehydromatricarianol (III), a typical C_{10} polyacetylene isolated from *Tricholoma grammopodium* cultures by Jones and his co-workers [6] and suggested to us for this purpose.

* Part IV, Bu'Lock, Gregory, and Hay, *J.*, 1961, 3544.

[1] Bu'Lock and Gregory, *Biochem. J.*, 1959, **72**, 322.
[2] Allport, Bu'Lock, and Turner, *J.*, 1961, 1654.
[3] Cf. Stumpf, *Ann. Rev. Biochem.*, 1960, **29**, 261.
[4] Bu'Lock and Smalley, *Proc. Chem. Soc.*, 1961, 209 (cf. also Bentley and Keil, *ibid.*, p. 111); Bu'Lock, Smalley, and Smith, *J. Biol. Chem.*, 1962, **237**, 1778.
[5] Bu'Lock and Leadbeater, unpublished observations.
[6] Unpublished; cf. Gardner, Jones, Leeming, and Stephenson, *J.*, 1960, 691.

Both growth and polyacetylene synthesis were rather slow; neverthless a comparatively short incubation with diethyl [α-^{14}C]malonate was used to minimise confusing side-reactions. In the event, uptake of the ester was very low, presumably for reasons of permeability since very little radioactivity was incorporated into the mycelium at all, so that the low incorporation into polyacetylenes (about 0·1%) represents a relatively high proportion of the total uptake and does not invalidate our conclusions. The labelled alcohol (III) was separated chromatographically from traces of other polyacetylenes, hydrogenated, and oxidised to decanoic acid, successive Schmidt and Kuhn–Roth degradations then affording $C_{(1)}$ as carbon dioxide (inactive) and $[C_{(10)} + C_{(9)}]$ as acetic acid. The latter, representing the presumed " starter group," contained only 3% of the total radioactivity of compound (III), *i.e.*, 97% of the labelling from [α-^{14}C]malonate is incorporated into $C_{(1)}$—$C_{(8)}$ of the C_{10} chain, presumably in even-numbered carbon atoms since $C_{(1)}$ is inactive. This contrasts sharply with the uniform distribution of labelling from acetate found in polyacetylenes [1,2] and supports the analogy with fatty-acid synthesis.

It has been suggested,[7,8] and the suggestion has been supported by striking *in vitro* analogies,[8] that malonic acid derivatives can participate not only in the assembly of the carbon chain of polyacetylenes but also in the actual formation of the triple bonds, by a mechanism of concerted decarboxylation and elimination in enolic derivatives of acylmalonic acids. A further part of the framework for this hypothesis has now been substantiated, but the precise stage of assembly at which triple-bond formation occurs remains a matter of speculation. Three main categories of natural substances are now known to be assembled on the " acetate + *n* malonate " pattern, *viz.* fatty acids, the acetate-derived aromatics [4] (polyketides [9]), and polyacetylenes. Of the three, only the fatty acids are normal and general cell constituents, and their synthesis can be taken as the basic mechanism. Two essential and distinct stages in this synthesis are [10] (a) combination of acetyl thiolester and malonyl-CoA with simultaneous or subsequent



decarboxylation,* and (b) reduction of β-ketoacetyl thiolester by hydride transfer. Accepting the *in vitro* analogies, we may then visualise polyacetylene synthesis as a variation arising in step (a), by failure to decarboxylate until after the formation of an enolic derivative which allows the decarboxylation–elimination reaction. Correspondingly the polyketides would arise by a variation in stage (b), *viz.* omission of reduction steps,

* The observation [10] that one hydrogen atom of the malonyl-CoA is incorporated into fatty acids need not imply that decarboxylation is synchronous with the condensation step, since the later reactions of reduction and oxygen elimination are likely to be fully stereospecific.

[7] Jones, *Chem. and Eng. News*, 1961, **39** (No. 12), 46.
[8] Fleming and Harley-Mason, *Proc. Chem. Soc.*, 1961, 245; *Chem. and Ind.*, 1962, 560.
[9] Cf. Birch, *Proc. Chem. Soc.*, 1962, 3.
[10] Bressler and Wakil, *J. Biol. Chem.*, 1961, **236**, 1643.

leading to systems of β-diketone type apt for various cyclisation processes. Such variations may be summarised as shown.

Experimental

Growth and Incorporation of Carbon-14.—*T. grammopodium* from malt-agar plates was inoculated in culture flasks containing 500 ml. of Czapek–Dox medium with 3% malt extract, and allowed to grow at 25° for 6 weeks, after which the medium below the surface growth was replaced by 4% aqueous glucose. Such replacement cultures yield the maximum amount of compound (III) after 10—12 days. A total of 100 μc of diethyl [α-^{14}C]malonate in sterile water was added to 12 cultures 7 days after replacement, and after 2 days the culture medium was filtered off.

Isolation of Labelled Dehydromatricarianol (III).—The medium (6·5 l.) was extracted with hexane (3 × 650 ml.), and the combined extracts were dried, and evaporated to small volume under reduced pressure; benzene was added and the remaining hexane evaporated. The benzene solution (3 ml.) was put on to a column of silica gel which was eluted with light petroleum (b. p. 40—60°) containing increasing proportions of ether. With 65% of ether the alcohol (III) was eluted. By spectroscopic estimation the yield of (III) was 12 mg., giving a corrected thick-film count of 78·5 counts/sec. For comparison, the dried fungus mycelium gave a thick-film count of under 0·1 count/sec.

Degradation of the Alcohol (III).—The purified alcohol was taken up in hexane and hydrogenated over palladium–charcoal; the theoretical volume of hydrogen was absorbed. The solution was filtered and evaporated, and purified decan-1-ol (*ca.* 120 mg.) was added as diluent. The decanol (45 mg.) was oxidised, first with t-butyl chromate in t-butyl alcohol and then with 0·1N-permanganate in alkali; decanoic acid was isolated by steam distillation and purified as the sodium salt by ion-exchange chromatography.

The sodium decanoate was subjected to the Schmidt degradation by the method of Phares,[11] giving barium carbonate (inactive) and nonylamine, isolated by steam distillation, and converted *via* the hydrochloride into 2,4-dinitro-*N*-nonylaniline (64 mg.; R.M.A. 6·68 × 10^4). Normal Kuhn–Roth oxidation of this amine afforded acetic acid, which was purified by ion-exchange chromatography and converted into *p*-bromophenacyl acetate, which was counted after purification on a silica gel column (12 mg.; R.M.A. 1·95 × 10^3).

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[11] Phares, *Arch. Biochem. Biophys.*, 1951, **33**, 173.