Juvenile Hormone Biosynthesis: Homomevalonate and Mevalonate Synthesis by Insect Corpus Allatum Enzymes

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Summary Centrifuged homogenates of corpora allata from *Manduca sexta* convert [1-^{14}C]acetyl-CoA and non-labelled propionyl-CoA into labelled (3*R*)-mevalonate and chiral homomevalonate; when [1-^{14}C]propionyl-CoA and non-labelled acetyl-CoA are used as substrates, labelled homomevalonate is synthesized, but not labelled mevalonate.

Two of the three known insect juvenile hormones[1] are biogenetically unique since their skeletons are assembled from homoisoprene, as well as isoprene, units. Based on incorporation and degradation studies, propionate has been well established as the source of the ethyl 'side-branch' in JH II[2,3] and JH I[3,4] from endocrine gland cultures of *Manduca sexta*[2] and *Heliothis virescens*,[3] and from *Hyalophora cecropia in vivo*.[4] Corpora allata of *M. sexta* incorporate [5-^{3}H]homomevalonate (HMev) into JH II without randomization[5] supporting the hypothesis[2] that HMev (**4**), yet unknown in nature, serves as a pivotal intermediate in JH I and II biosynthesis. We now report that crude enzyme systems[6,7] prepared from corpora allata are capable of producing HMev and mevalonate (Mev) in the presence of acetyl- and propionyl-CoA.

In a typical experiment, 20—30 corpora allata–corpora cardiaca complexes of *M. sexta* were obtained[8] and homogenized (TenBroeck apparatus, 5—7 strokes) in 0·1 M pH 7

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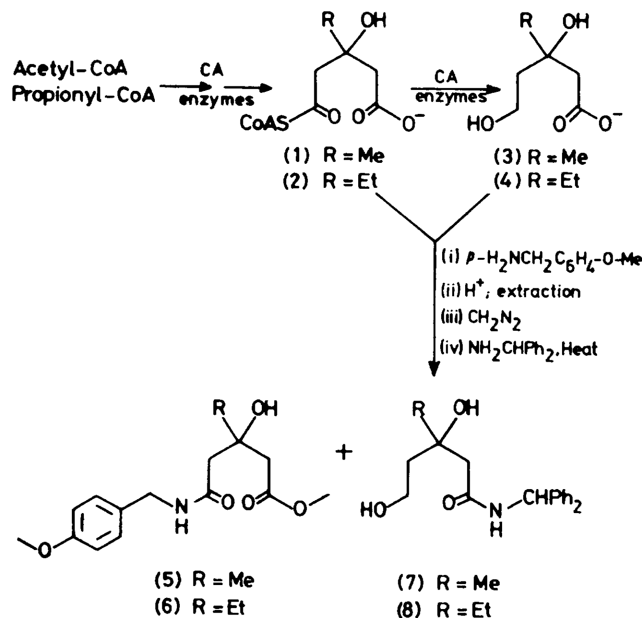
phosphate buffer containing $MgCl_2$, nicotinamide, and dithiothreitol (DTT), and the homogenate was centrifuged ($10{,}000 \times g$, 10 min). The supernatant liquor (nominally cytosolic and microsomal enzymes) was supplemented with an NADPH-generating system,[7] bovine serum albumin (1 mg/100 μl), [1-^{14}C]acetyl-CoA (0·05 mM), and propionyl-CoA (0—3·2 mM). After incubation at 27 °C for 20 h, the clear solution (pH 6·5) was diluted with known quantities of 3-hydroxy-3-methylglutaryl-CoA (**1**), Mev (**3**), and HMev (**4**), and successively treated as shown in the Scheme. After addition of standard (**6**), the mixture of the derivatives (**5**), (**6**), (**7**), and (**8**) (obtained in 40—50% chemical yield) was analysed by high-resolution liquid chromatography (h.r.l.c., Zorbax-SIL, ether–pentane 5 : 1, 90% water saturated) with radioassay of collected fractions. The isolated peaks corresponding to (**5**), (**6**), (**7**), and (**8**) were individually rechromatographed on reversed-phase h.r.l.c. (μBondapak C_{18}, MeOH–H_2O, 55 : 45) with radioassay of fractions, followed by a final micropreparative isolation by h.r.l.c. (Zorbax-SIL, ether–CH_2Cl_2 5 : 1, 90% water saturated). The specific radioactivity of each radiolabelled species did not change significantly after the first h.r.l.c. separation.



Mev is the major enzymatic product (20—30 pmol/gland pair) in the presence of [1-^{14}C]acetyl-CoA (with or without propionyl-CoA). Smaller (but consistent) amounts of HMev are synthesized from [1-^{14}C]acetyl-CoA with non-labelled propionyl-CoA as co-substrate. More HMev is formed with higher concentrations (1·6 mM) of propionyl-CoA (*ca.* 1 pmol/gland pair). Use of [1-^{14}C]propionyl-CoA and non-labelled acetyl-CoA results in the production of labelled HMev, but not labelled Mev. Small amounts of radioactivity are frequently detected in derivatives of 3-hydroxy-3-methylglutarate (**5**) and 3-hydroxy-3-ethylglutarate (**6**).‡

Carrier-diluted, enzymatically synthesized Mev was converted into the derivative (**9**) *via* amidation of the lactone at 120 °C, followed by routine acetylation. Analysis of the diastereomeric 1-(+)-α-(1′-naphthyl)ethylamide 5-acetates by h.r.l.c. (Zorbax-SIL, pentane–EtOAc 7 : 3, 90% water saturated) and radioassay, together with h.r.l.c. analysis of the corresponding derivatives of (3*R*)- and (3*S*)-mevalonolactone, revealed the biosynthetic Mev to possess the conventional (3*R*)-configuration (Figure 1).

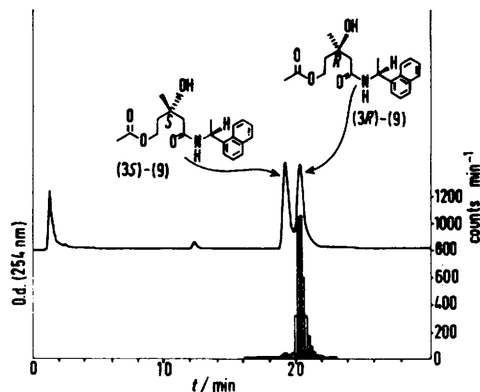


FIGURE 1. H.r.l.c. separation (u.v. absorbance detection) of diastereomeric derivatives from racemic carrier mevalonate (Mev). The single diastereomer from biosynthesized (3*R*-Mev was detected by radioassay of 0·2 min fractions.

Similar analysis of the corresponding HMev derivative (**10**) established that biosynthetic HMev is optically active (Figure 2). Determination of the HMev absolute configuration requires further study.

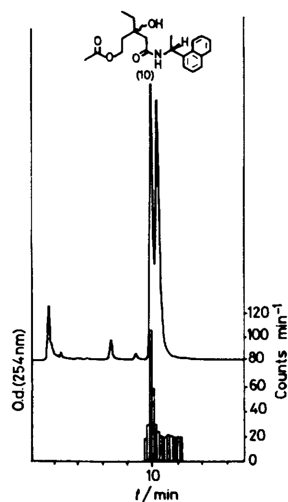


FIGURE 2. Same as Figure 1, except the analysis is of diastereomeric derivatives of homomevalonate (HMev).

‡ The assay conditions suggest that such intermediates are bound as thioesters, but not specifically as CoA thioesters. Rigorous proof of the identity of 3-hydroxy-3-methylglutarate and the previously unknown 3-hydroxy-3-ethylglutarate is reported in the following communication.

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[1] See articles in 'The Juvenile Hormones,' ed. L. I. Gilbert, Plenum Press, New York, 1976.

[2] D. A. Schooley, K. J. Judy, B. J. Bergot, M. S. Hall, and J. B. Siddall, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2921.

[3] R. C. Jennings, K. J. Judy, D. A. Schooley, M. S. Hall, and J. B. Siddall, *Life Sci.*, 1975, **16**, 1033.

[4] M. G. Peter and K. H. Dahm, *Helv. Chim. Acta*, 1975, **58**, 1037.

[5] R. C. Jennings, K. J. Judy, and D. A. Schooley, *J.C.S. Chem. Comm.*, 1975, 21.

[6] Homogenates of insect corpora allata have been used to study the final steps in JH biosynthesis: D. Reibstein and J. H. Law, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 266; B. D. Hammock, *Life Sci.*, 1976, **17**, 323.

[7] Mevalonate biosynthesis has been studied with insect tissue homogenates: R. D. Goodfellow, G. C. K. Liu, J. P. Stein, and K. Harker, *Insect Biochem.*, 1973, **3**, 113.

[8] K. J. Judy, D. A. Schooley, L. L. Dunham, M. S. Hall, B. J. Bergot, and J. B. Siddall, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 1509.