Juvenile Hormone Biosynthesis: Identification of 3-Hydroxy-3-ethylglutarate and 3-Hydroxy-3-methylglutarate in Cell-free Extracts from *Manduca sexta* Incubated with Propionyl- and Acetyl-CoA

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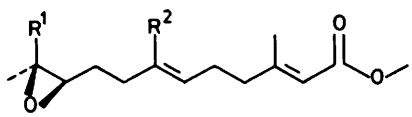
Summary The identification of 3-hydroxy-3-ethylglutarate and 3-hydroxy-3-methylglutarate *via* g.l.c.–m.s. and high-resolution liquid chromatography, and their mode of radiolabelling from [1-^{14}C]propionyl- plus acetyl-CoA (or [1-^{14}C]acetyl- plus propionyl-CoA) incubated with cell-free preparations of *Manduca sexta* corpora cardiaca–corpora allata complexes, is in agreement with their hypothetical role as intermediates of juvenile hormone biosynthesis.

THE pattern of incorporation of [2-^{14}C]acetate,[1] [1- or 2-^{14}C]propionate,[1-3] [5-^{3}H]homomevalonate,[4] and [2-^{14}C]-mevalonate[1] into the acid unit of the juvenile hormones (**1**), (**2**), and (**3**) (JH I, II, and III) is compatible with a modified sesquiterpenoid biosynthetic scheme in which propionyl-CoA (or related form) replaces the primary acetyl-CoA unit to produce homoisoprenoids *via* propionoacetate, 3-hydroxy-3-ethylglutarate (HEG), and homomevalonate.[1-6] However, none of these early key intermediates have been isolated or identified in systems responsible for JH biosynthesis. We now provide strong evidence for the validity of this biosynthetic pathway and report on the identification of both HEG and 3-hydroxy-3-methylglutarate (HMG) (or their metabolic equivalents) as products of acetyl-CoA and propionyl-CoA metabolism in *M. sexta*.



(1) $R^1 = R^2 = Et$ (JH I)
(2) $R^1 = Et, R^2 = Me$ (JH II)
(3) $R^1 = R^2 = Me$ (JH III)

3-Hydroxy-3-ethylglutaric acid was prepared according to published methods for HMG.[7] Corpora cardiaca–corpora allata complexes (cc–ca)[8] were homogenized in a TenBroeck apparatus in 0·1M phosphate buffer, pH 7·0, and centrifuged at 89,000 × *g* for 100 min. The clear supernatant liquor of cytosolic enzymes was incubated with either [1-^{14}C]acetyl-CoA (400 μM) plus unlabelled propionyl-CoA (435 μM) or similar concentrations of [1-^{14}C]propionyl-CoA with unlabelled acetyl-CoA. After 20 h at 27 °C, thioesters were hydrolysed by treatment with excess of 1M KOH (1 h). Free acids were isolated,[9] treated with distilled diazoethane in ether, and subjected to t.l.c. The zone corresponding to HEG and HMG diethyl esters was eluted for subsequent analysis.

According to anticipated metabolic pathways, both HMG and HEG should be radiolabelled from incubations containing [1-^{14}C]acetyl-CoA as one of the substrates. With propionyl-CoA as the radiolabelled substrate, ^{14}C in the products should be confined to HEG, although HMG should be formed.

G.l.c.–m.s. of products biosynthesized from [1-^{14}C] acetyl-CoA and unlabelled propionyl-CoA revealed total ion current peaks corresponding to the R_T of HMG and HEG diethyl esters (7·1 and 9·2 min, respectively) and mass spectra were obtained which agreed closely with those of the standard esters except for the presence of additional fragment ions due to ^{14}C enrichment: notably, the ions at *m/e* 159 (4%), 133 (16), and 87 (13) from enriched HMG diethyl ester and *m/e* 159 (20%), 147 (5), and 117 (23) from the enriched HEG diethyl ester. Analysis of another aliquot portion of material by high-resolution liquid chromatography (h.r.l.c.) on μBondapak C_{18} (MeOH–H_2O, 45 : 55, v/v) showed two main radioactive fractions which co-eluted with the HMG and HEG diethyl ester standards. About 214 ng HMG and 40 ng HEG were produced from 24 cc–ca gland pairs.

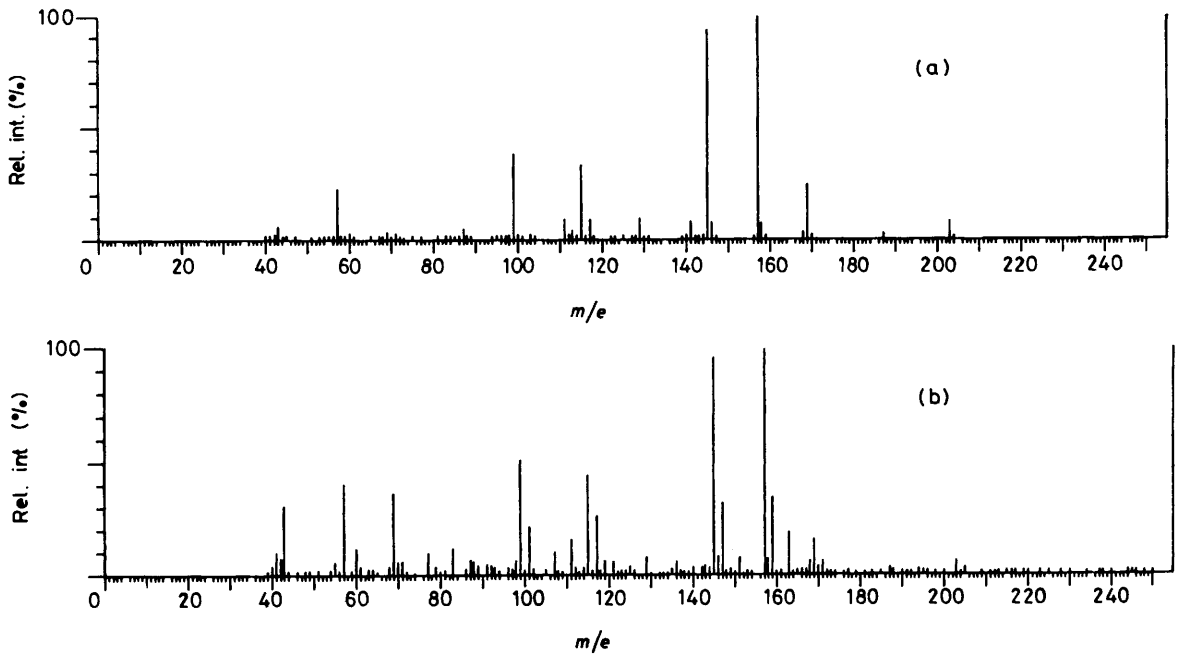


FIGURE. Mass spectrum of (a) unenriched HEG diethyl ester, and (b) biosynthesized ^{14}C-enriched HEG diethyl ester. Analyses were carried out on a 2 m 10% OV-101/0·1% Carbowax column, 120—200 °C at 4 °C per min, in a Hewlett Packard Model 5984A g.l.c.–m.s. data system; E.I. 70 eV.

G.l.c.–m.s. analysis of products from [1-^{14}C]propionyl-CoA and unlabelled acetyl-CoA provided very clear-cut data. Mass spectra of both HMG and HEG diethyl esters were clearly detectable; the HMG diethyl ester showed absolutely no ^{14}C enrichment, but the HEG diethyl ester gave fragment ions at *m/e* 203, 169, 157, 145, 115, and 99, accompanied by strongly enhanced *m/e* +2 ions (Figure, a and b). H.r.l.c. of the products showed that virtually all the recovered ^{14}C was co-eluted with HEG diethyl ester. We obtained 105 ng of HEG from 43 gland pairs.

Apart from verifying the biosynthesis of HMG and HEG by cc–ca from *M. sexta*, the data from a number of experiments indicated that molar ratios of HMG:HEG were generally in the range of 5—8:1, a value consistent with the known physiological levels of JH III and II at this stage of development of *M. sexta*.[8]

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