Biosynthesis of the Homosesquiterpenoid Juvenile Hormone JH II [Methyl (2*E*,6*E*,10*Z*)-10,11-epoxy-3,7,11-trimethyltridecadienoate] from [5-^{3}H]Homomevalonate in *Manduca sexta*

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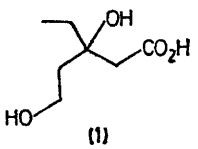
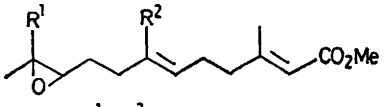
Summary Radioactivity from [5-^{3}H]homomevalonate is incorporated into the ethyl-branched or homosesquiterpenoid juvenile hormone JH II of *Manduca sexta*, but not into the co-occurring sesquiterpenoid juvenile hormone JH III; degradation experiments show that the incorporation of label apparently is specific and in accordance with that expected from a modified terpenoid pathway.

THE role of homomevalonic acid (**1**) in the biosynthesis of the homosesquiterpenoid juvenile hormones (**2**) and (**3**) (JH I and JH II) has been discussed.[1] Our recent results[2]



(1)

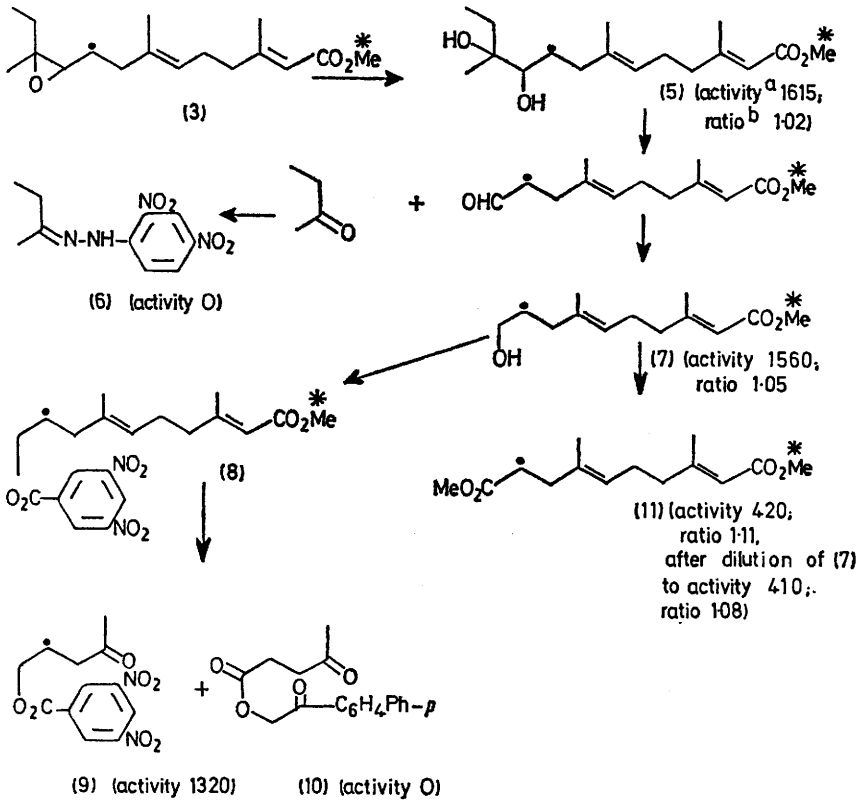


(2) $R^1 = R^2 = Et$ JH I
(3) $R^1 = Et, R^2 = Me$ JH II
(4) $R^1 = R^2 = Me$ JH III

on the incorporation of [1-^{14}C]propionate into JH II produced by *in vitro* cultures of corpora allata from the tobacco hornworm, *Manduca sexta*, provided the first evidence that homomevalonate could be involved. We report here that [5-^{3}H]homomevalonate is incorporated specifically into JH II in the same *in vitro* system in agreement with our hypothesis[2] for the biosynthesis of these compounds.

[5-^{3}H]Homomevalonolactone was synthesized by a modification of the routes used previously to synthesize [5-^{3}H]mevalonolactone.[3] Homomevaldic acid dimethyl acetal was synthesized in 70% yield by the reaction of 3-oxopentanal dimethyl acetal with the lithium enolate of ethyl acetate[4] in hexane–tetrahydrofuran at −78° and hydrolysis of the resulting hydroxy-ester with aqueous $Ba(OH)_2$. The barium salt of homomevaldic acid dimethyl acetal was isolated and treated with dilute acid and the resulting homomevaldic acid was neutralised and then reduced with [^{3}H]$NaBH_4$ (Amersham-Searle, 250 mCi, 15·6 Ci/mmol). Acidification and extraction of the reaction mixture afforded [5-^{3}H]homomevalonolactone in 75% radiochemical yield.

A total of 60 corpora allata-corpora cardiaca complexes were removed from adult female *M.sexta* (0—48 h old) and were cultured *in vitro* as described[5] previously. Potassium [5-^{3}H]homomevalonate (prepared from the lactone by hydrolysis) (3 μCi/ml) and L-[*Me*-^{14}C]methionine (2·5 μCi/ml) were added to the culture medium. After an



SCHEME. Label distribution from L-[*Me*-^{14}C]methionine (*) and [5-^{3}H]homomevalonate (●). [a] ^{3}H specific activity (d.p.m./μmol); [b] ^{3}H:^{14}C ratio.

appropriate incubation time the medium was extracted and the extract purified by t.l.c.[5] Elution of the zone with an R_f corresponding to that of the authentic hormones gave a doubly labelled material which was purified further by high-resolution liquid chromatography[2,5] and the fractions corresponding to the three authentic, synthetic juvenile hormones (**2**), (**3**), and (**4**) were collected and assayed for

TABLE

	^{3}H/d.p.m. $\times 10^4$	^{14}C/d.p.m. $\times 10^4$
Crude extract	24·8	36·5
JH I	1·25	0·41
JH II	14·5	10·4
JH III	0	16·9

radioactivity (Table). Not only does JH II contain both ^{3}H and ^{14}C but also JH III is completely devoid of tritium. The apparent production by *M. sexta* of traces of labelled JH I has been observed previously[2] but this material has not been characterised rigorously.

The pattern of incorporation of mevalonate into terpenoid compounds is well known and, by analogy, if our hypothesis[2] is correct [5-^{3}H]homomevalonate should give rise to JH II labelled at C-9 with tritium. The JH II was converted into the diol (**5**) which was then degraded after dilution with carrier. The methods used were largely as described[2] and the degradation scheme and results are shown in the Scheme.

All the tritium activity was found in the laevulinyl fragment (**9**) which originally contained C-9 of JH II, the other isolated fragments of the molecule being devoid of tritium. Attempts to exchange the tritium in the unstable aldehydic precursor of (**7**) under acidic conditions proved unsatisfactory, so to locate the label more satisfactorily, a portion of the alcohol (**7**) was diluted with cold carrier and converted into the diester (**11**) by oxidation followed by methylation. All the tritium activity was retained indicating that no tritium is located at C-10. Lack of material precluded further degradation of (**9**) but since the other carbon atoms in the fragment have been shown[2] to arise from acetate and mevalonate, and since the JH III produced in this system is devoid of tritium activity, C-9 is the only reasonable location for the tritium label.

Thus, in this system at least, [5-^{3}H]homomevalonate, unknown as a natural intermediate, is incorporated into JH II exactly as predicted[2] and hence it may be argued that the ethyl branches of the homoterpenoid juvenile hormones have their origins in homomevalonate.

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