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THE *IN VIVO* INCORPORATION OF TRITIUM FROM L[methyl-^{3}H]-METHIONINE INTO POLYPRENOIDS IN PEAS

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SUMMARY

Tritium from L[methyl-^{3}H]-methionine was incorporated *in vivo* into β-amyrin and into the side chain and likely into the tetracyclic moiety of β-sitosterol by *pisum sativum*.

We have reported on the *in vivo* incorporation of ^{14}C and ^{3}H from L[methyl-^{14}C, ^{3}H]-methionine into cholesterol and 5α-cholest-7-en-3β-ol in the rat [2, 3]. To evaluate the generality of the pathway from methionine to polyprenoids the biosynthesis of triterpenes and sterols from L[methyl-^{3}H]-methionine in the pea was investigated.

Thirty peas (Blue Bantam Variety) were germinated in a medium containing L[methyl-^{3}H]-methionine (250 μCi), methionine (5 mg) and water (3 ml). When the solution was absorbed, water was added in 3 ml portions as needed. The germination was continued at ambient temperature until the sprouts grew to 3–5 cm. in length (80–120 h). At the end of the incubation the peas were washed, ground and extracted continuously (soxhlet) with acetone (10 h). The acetone solution was concentrated and the residue was refluxed with 15% methanolic KOH (200 ml) under nitrogen for 3 h. Most of the methanol was removed, water (100 ml) was added and the mixture was extracted with ether (3 × 150 ml). Following a conventional workup a non-saponifiable residue was obtained (15 mg; $2{\cdot}9 \times 10^6$ d.p.m. of ^{3}H; 0·54% incorporation of ^{3}H).

To the residue β-amyrin (6 mg) and β-sitosterol (10 mg) were added and the mixture was fractionated by t.l.c. [silica gel; hexane–ethyl acetate (4: 1)]. The zone of β-amyrin ($3{\cdot}3 \times 10^5$ d.p.m.) and the zone of β-sitosterol ($2{\cdot}3 \times 10^5$ d.p.m.) were recovered.

The β-amyrin fraction was rechromatographed in the same system, and the obtained residue ($2{\cdot}3 \times 10^4$ d.p.m.) on crystallization (methanol–chloroform) gave a solid (6×10^3 d.p.m.). The solid was diluted with β-amyrin (15 mg) and crystallized four times (see Table 1).

A second incubation was carried out and the reaction mixture processed in a similar manner. The β-amyrin fraction (9×10^3 d.p.m.) was diluted with non-radioactive material and crystallized (Table 1). The combined mother liquor was acetylated, the acetates purified [t.l.c., silica gel, hexane–ethyl acetate (19:1)] and crystallized (see Table 1).

The β-sitosterol fraction (Experiment A; $2{\cdot}3 \times 10^5$ d.p.m.) on rechromatography in the same system gave a residue ($1{\cdot}3 \times 10^5$ d.p.m.). An aliquot of this material was diluted with non-radioactive β-sitosterol (20 mg) and crystallized (see Table 1).

To define whether tritium was present in the tetracyclic portion of the [^{3}H]-β-sitosterol, the radioactive material from several experiments was diluted with non-radioactive β-sitosterol, and the dilute sample (100 mg; 1200 d.p.m./mg) was incubated with Mycobacterium Sp (KNGSF 70) as previously described [3]. The recovered androsta-1,4-diene-3,17-dione ($3{\cdot}4 \times 10^3$ d.p.m.) was purified [3] and crystallized (see Table 1). A second incubation of β-sitosterol (30 mg; $4{\cdot}0 \times 10^4$ d.p.m. total) was carried out and the resulting androsta-1,4-diene-3,17-dione was purified [3] and crystallized (see Table 1).

Clearly the results indicate that the β-amyrin contains tritium and it can thus be concluded that the

Table 1. Tritium content of β-amyrin and β-sitosterol (and products derived from them) biosynthesized from L[methyl-^{3}H]-methionine in peas

Compound and experiment	[^{3}H]-S. A. (d.p.m. per mg) Crystallization 1	2	3	4
β-Amyrin	135	103	108	108
β-Amyrin	48	45	49	
β-Amyrin acetate	41	55	43	
β-Sitosterol	606	606	600	
androsta-1,4-diene-3,17-dione	25	16	12	15
Androsta-1,4-diene 3,17-dione	19	25	25	

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hydrogen atoms and presumably the carbon atom [2, 3] of the methyl group of L-methionine were incorporated into the triterpene.

It was anticipated that relatively large amounts of tritium of the L(methyl-^{3}H)-methionine would be incorporated into the C-24 ethyl moiety of β-sitosterol [4, 5] (see Table 1). However, it was not known whether tritium would be present in other parts of the molecule (e.g. tetracyclic moiety). Consequently cleavage of the phytosterol side chain with Mycobacterium Sp. was carried out. Removal of the β-sitosterol side chain by the microorganism proceeded poorly and androsta-1,3-diene-3,17 dione was obtained in low yield. The results in Table 1 indicate that the specimens of androsta-1,4-diene-3,17-dione derived from the two samples of [^{3}H]-β-sitosterol contained low but reproducible amounts of tritium. The low [^{3}H]-S. A. of the derived androsta-1,4-diene-3,17-dione is consistent with the view that the bulk of tritium was indeed present in the C-24 ethyl moiety. Although the results indicate that some tritium was incorporated into the tetracyclic portion of β-sitosterol, the evidence is less certain than that obtained for the pentacyclic triterpene β-amyrin.

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