Short Communications

Incorporation of Tritium from [(4R)-4-³H]Mevalonate into Absciscic Acid

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Noddle & Robinson (1969) have already demonstrated the incorporation of radioactivity from [2-¹⁴C]mevalonate into the plant hormone absciscic acid. Assuming the accepted scheme of isoprenoid biosynthesis, a molecule of absciscic acid should be formed from three molecules of mevalonate, via a C₁₅ pyrophosphate produced by the condensation of isopentenyl pyrophosphate with geranyl pyrophosphate. The double bond so generated would correspond in position with the *cis*-double bond between C-2 and C-3 in the side chain of absciscic acid, and the question is whether the C₁₅ precursor is 2-*cis*-farnesyl pyrophosphate, the geometry of the double bond being preserved in absciscic acid, or whether it is 2-*trans*-farnesyl pyrophosphate, the double bond undergoing isomerization at a later stage of the biosynthesis. It has been shown, by using mevalonates stereospecifically labelled with hydrogen isotopes, that chain elongation by the condensation of isopentenyl pyrophosphate with prenyl pyrophosphates involves the loss of a proton from C-2 of isopentenyl pyrophosphate (originally C-4 of mevalonate), and that the stereochemistry of this elimination is related to the geometry of the resulting double bond (Popják & Cornforth, 1966). Thus H_S is eliminated from C-4 of mevalonate if *trans*-double bonds are formed, as in the biosynthesis of squalene, phytoene and farnesol (Goodwin & Williams, 1965, 1966; Archer *et al.* 1966); if the double bonds have *cis*-geometry H_R is eliminated, as in rubber (Archer *et al.* 1966); and when compounds containing both *cis*- and *trans*-double bonds are produced the two types of elimination are observed concurrently, as in polyprenols (Gough & Hemming, 1967; Stone & Hemming, 1967). We have used [(4R)-4-³H]- and [(4S)-4-³H]-mevalonate to determine the origin, in the sense outlined above, of the *cis*-double bond in the side chain of absciscic acid.

Suitable amounts of [2-¹⁴C]mevalonic acid were mixed with [(4R)-4-³H]- and [(4S)-4-³H]-mevalonic acid (kindly provided by Dr Rita H. Cornforth) and each mixture was incubated with fruits of avocado (*Persea gratissima*) as described by Noddle & Robinson (1969). The isolated absciscic acid biosynthesized from the 4R-isomer had a ³H/¹⁴C ratio

close to 2:3 (relative to a 1:1 ratio in the substrate), and that biosynthesized from the 4S-isomer a ratio approaching zero (Table 1). These ratios were maintained on methylation. When the absciscic acid derived from [2-¹⁴C,(4R)-4-³H]mevalonate was treated with NaOH in H₂O or NaOD in D₂O, whereby six carbon-bound hydrogen atoms of the ring moiety, but none in the side chain, were exchanged with the medium (B. V. Milborrow, unpublished work), the ³H/¹⁴C ratio fell to 1:3 (Table 1).

Although we have no rigorous proof of the position of the labelled atoms, these results are consistent with the retention of tritium at C-2 and C-5' in the absciscic acid when [(4R)-4-³H]-mevalonate is the substrate, and corresponding expulsion of tritium from [(4S)-4-³H]mevalonate. (The third carbon atom derived from C-4 of mevalonate is C-1' and carries no hydrogen atoms.) The pattern of retention and elimination of the alkali-labile tritium, presumed to be at C-5', is as expected from the known stereochemistry of isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate (Popják & Cornforth, 1966). From the pattern of retention and elimination of the other tritium label, presumed to be at C-2, we conclude that absciscic acid is biosynthesized from a C₁₅ precursor having a *trans*-double bond corresponding in position to the *cis*-2,3 double bond of absciscic acid, and that at some stage of the biosynthesis a net isomerization takes place (or, conceivably, the addition of an extra carbon atom that becomes C-1 of absciscic acid, migration of the labelled hydrogen to another position in the side chain, and loss of what was C-1 of the C₁₅ precursor). *trans-trans*-Farnesyl pyrophosphate could fill the role of the C₁₅ precursor. Since it also gives rise to carotenoids (Jungalwala & Porter, 1967) the possibility that absciscic acid is a degraded carotenoid, suggested by the obvious similarity between the acid and the termini of several carotenoids, cannot be excluded.

We thank Dr B. V. Milborrow for making available details of the base-catalysed hydrogen exchanges in absciscic acid.

Table 1. $^3\text{H}/^{14}\text{C}$ ratios in abscisic acid biosynthesized by avocado fruit from $[2\text{-}^{14}\text{C},(4R)\text{-}4\text{-}^3\text{H}]$ and $[2\text{-}^{14}\text{C},(4S)\text{-}4\text{-}^3\text{H}]\text{-mevalonic acid}$

One avocado fruit was incubated for 4 days with $3\cdot77\text{ }\mu\text{moles}$ of $(RS)\text{-}[2\text{-}^{14}\text{C},(4R)\text{-}4\text{-}^3\text{H}]\text{mevalonic acid}$ ($2\cdot46 \times 10^8$ d.p.m. of ^3H , $3\cdot12 \times 10^7$ d.p.m. of ^{14}C) and a second fruit was similarly incubated with $5\cdot06\text{ }\mu\text{moles}$ of $(RS)\text{-}[2\text{-}^{14}\text{C},(4S)\text{-}4\text{-}^3\text{H}]\text{mevalonic acid}$ ($4\cdot95 \times 10^8$ d.p.m. of ^3H , $3\cdot37 \times 10^7$ d.p.m. of ^{14}C). The biosynthesized abscisic acids were isolated as described by Noddle & Robinson (1969). Isotope ratios were measured: after chromatography of the free acids in toluene-ethyl acetate-acetic acid (15:3:1, by vol.) (steps 1 and 2); after methylation with ethereal diazomethane and chromatography in *n*-hexane-ethyl acetate (1:1, v/v) (step 3); after treatment of the acids with *m*-NaOH for 6 hr. at room temperature followed by chromatography in toluene-ethyl acetate-acetic acid (step 4); and, with the acid derived from $[2\text{-}^{14}\text{C},(4R)\text{-}4\text{-}^3\text{H}]\text{mevalonate}$, after mixing with 1 mg. of unlabelled abscisic acid, treatment with *m*-NaOD in D_2O , methylation and chromatography in *n*-hexane-ethyl acetate (step 5). (The position of incorporated deuterium was established by n.m.r. spectroscopy.) All chromatography was on $250\text{ }\mu\text{m}$. precoated Kieselgel-F₂₅₄ plates previously washed in ethanol. Radioactive material was eluted from the gel with redistilled methanol, and samples of the eluates were evaporated to dryness for counting in 10 ml. of toluene-2-methoxyethanol (3:2, v/v) containing 0.8 g. of naphthalene and 0.06 g. of 2,5-bis-(5-tert.-butylbenzoxazol-2-yl)thiophen scintillator [Ciba (A.R.L.) Ltd., Duxford, Cambs.]. Counting efficiencies of 9.0 and 0.54% for ^3H and 11.2 and 49.0% for ^{14}C in the respective channels were obtained with the Packard 314E liquid-scintillation spectrometer.

Step	From $[2\text{-}^{14}\text{C},(4R)\text{-}4\text{-}^3\text{H}]\text{mevalonate}$		From $[2\text{-}^{14}\text{C},(4S)\text{-}4\text{-}^3\text{H}]\text{mevalonate}$	
	$^3\text{H}/^{14}\text{C}$ ratio (normalized to substrate)	Presumed structure*	$^3\text{H}/^{14}\text{C}$ ratio (normalized to substrate)	Presumed structure*
1. Initial isolation	1.86:3†		0.14:3‡
2. Rechromatography	1.84:3	As above	0.12:3	As above
3. Methylation and chromatography	1.93:3	As above, methyl ester	0.05:3	As above, methyl ester
4. Treatment with <i>m</i> -NaOH and chromatography	0.92:3		0.02:3	As above, free acid
5. Treatment with <i>m</i> -NaOD; methylation and chromatography	0.98:3		—	—

* Positions of ^{14}C shown by asterisks, and that of the ^3H label by T.

† Total radioactivity at step 1: $1\cdot16 \times 10^5$ d.p.m. of ^3H ; $2\cdot38 \times 10^4$ d.p.m. of ^{14}C .

‡ Total radioactivity at step 1: $1\cdot48 \times 10^4$ d.p.m. of ^3H ; $2\cdot18 \times 10^4$ d.p.m. of ^{14}C .

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