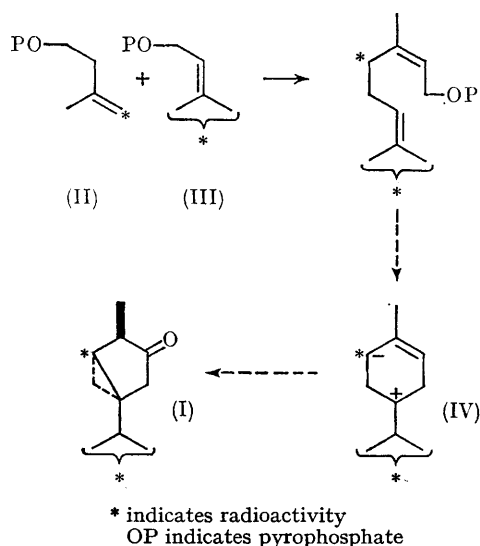


## The Biosynthesis of Thujane Derivatives in Higher Plants

By D. V. BANTHORPE and K. W. TURNBULL

(William Ramsay and Ralph Forster Laboratories, University College, Gower Street, London, W.C.1)

A RECENT study of the incorporation of [2-<sup>14</sup>C]-mevalonic acid (MVA) into (-)-thujone (I) in foliage of *Thuja occidentalis* L., has led to the conclusion that the skeleton of the ketone results from condensation of the labelled C<sub>5</sub> units (II) and (III), with the intermediacy of the remarkable zwitterion (IV), or its structural equivalent.<sup>1</sup> The only evidence for this scheme was that the carbonyl group of (I) was found not to be labelled with <sup>14</sup>C:



We have repeated this work and have located the position of tracer in thujane derivatives biosynthesized by three other species (of three different genera). A solution of [2-<sup>14</sup>C]MVA (0.075 mc, 0.015 mm) and ATP (0.1 mm) was stem-fed into small twigs (*ca.* 150 g.) of the species listed in the Table, which were then maintained in nutrient solution for eight days before harvesting. The extracted oils were diluted with carrier and pure samples of (I), the epimeric (+)-isothujone, (+)-sabinene (VI, X = H), and (+)-sabinyl acetate (VI, X = OAc) were obtained by preparative gas-liquid chromatography. The last two were converted into a mixture of thujones, and the carbonyl group in these and in the directly isolated thujones was cleaved under ultraviolet irradiation<sup>3</sup> to give (VII) together with its *cis*-isomer in equilibrium proportions. The specific activities (corrected for quenching) of the highly purified starting materials, and of the derived dienes are recorded. Thus, essentially all the tracer is located at the carbonyl group in all our examples. As time-incorporation studies in *Tanacetum vulgare* L. have shown that concerted bicyclization of C<sub>5</sub> units does not occur,<sup>4</sup> this is consistent with a biosynthetic pathway through (V), rather than through (IV). Also the C<sub>5</sub> unit, (II), derived directly from MVA, is apparently not appreciably converted into (III), but largely condenses with unlabelled (III) from the metabolic pool. The

TABLE

Specific activities of thujane derivatives and derived dienes

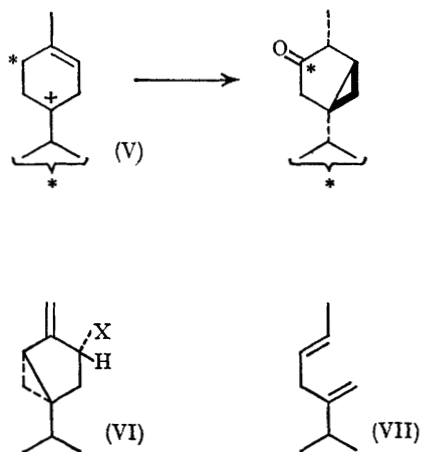
Compound	Source	Initial activity*	Activity of diene†
(-)-Thujone .. .. .	<i>Thuja occidentalis</i> L.	11737	10
(+)-Isothujone .. .. .	" " " "	1337	6
(-)-Thujone .. .. .	<i>Thuja plicata</i> L.	2518	12
(+)-Isothujone .. .. .	" " " "	4075	13
(+)-Isothujone .. .. .	<i>Tanacetum vulgare</i> L.	4927	218
(+)-Isothujone .. .. .	<i>Juniperus sabina</i> L.	306	26
(+)-Sabinene .. .. .	" " " "	216	0.3
(+)-Sabinyl acetate .. .. .	" " " "	3271	6
(+)-Sabinol .. .. .	" " " "	249	6

\* Activities are in counts/mm/min. The compounds were isotopically diluted to varying extents, therefore comparison of observed activities for different compounds leads to no conclusions concerning the relative degrees of labelling.

† Equilibrium mixture of *cis*- and *trans*-isomers.

The actual location of tracer was undetermined. An attractive route to (I) *via* cyclization of the ion (V) derived from terpinen-4-ol<sup>2</sup> was considered.

small residual activities in the dienes may well result from incorporation (in random positions) of labelled acetate resulting from a small percentage



of breakdown of the added MVA. In support of this, it was shown that the acetate group of (+)-sabinyl acetate contained tracer.

It is possible that the disagreement with the previous results is due to a seasonal difference, rather than to the operation of a different mechanism. An abundance of active enzyme catalysing the conversion of (II) into (III), or lack of the subsequent condensing enzyme, could enable these units to approach an equilibrium much in favour of (III), and condensation of labelled (III) with unlabelled (II) from the metabolic pool and reaction through (V) would lead to no tracer being situated at the carbonyl position.

(Received, February 2nd, 1966; Com. 063.)

<sup>1</sup> W. Sandermann, *Tetrahedron Letters*, 1962, 257.

<sup>2</sup> L. Ruzicka, *Experientia*, 1953, 9, 357.

<sup>3</sup> R. H. Eastman, J. E. Starr, R. S. Martin, and M. K. Sakata, *J. Org. Chem.*, 1963, 28, 2162.

<sup>4</sup> Ann M. Justice, unpublished results.