Biosynthesis of Fern-9-ene in *Polypodium vulgare* Linn.

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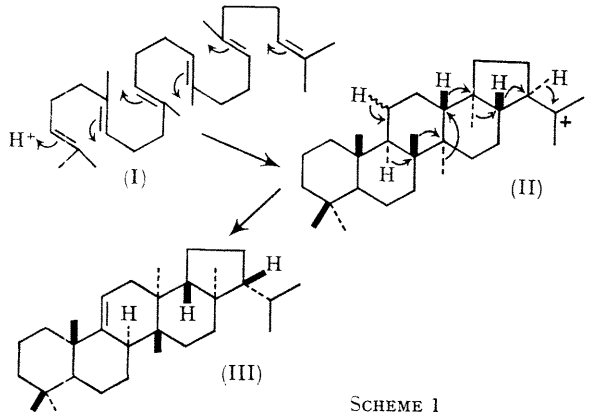
2,3-OXIDOSQUALENE is an intermediate in the biosynthesis of many steroids and of some triterpenoids.[1] Diterpenoids, on the other hand, are normally formed by proton-catalysed cyclisation of geranylgeranyl pyrophosphate.[2] For the relatively small number of triterpenoids[3] that are not oxygenated at C-3 a proton-induced cyclisation of squalene has been suggested.[3,4] Recent work by Caspi and his collaborators[5] has confirmed that the protozoan triterpenoid tetrahymanol is derived by cyclisation of squalene and not squalene oxide. We now report that the fern triterpene fern-9-ene (III) is similarly derived from squalene and not squalene oxide.

The fern *Polypodium vulgare* Linn. produces several triterpene hydrocarbons with the hopane and fernane skeletons, together with a number of sterols,[3b] including β-sitosterol† (IV). The most abundant hydrocarbon

† β-Sitosterol has not previously been isolated from *P. vulgare*. It was identified by comparison with an authentic sample.

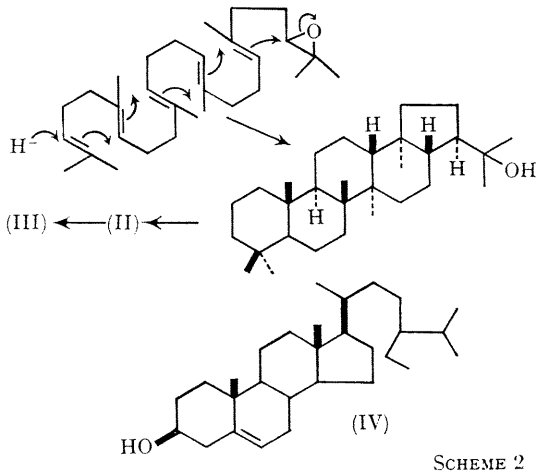
present, fern-9-ene (III) could arise by a concerted cyclisation of squalene (I) to the pentacyclic carbonium ion (II), which could undergo a series of hydride and methyl shifts to fern-9-ene (III) (Scheme 1).[6] Conceivably a "reverse



SCHEME 1

cyclisation" of 2,3-oxidosqualene could also be involved, giving rise to the same carbonium-ion intermediate (II) (Scheme 2).

applied this technique to whole *P. vulgare* plants. The undersides of the leaves were painted with acetone solutions of the labelled precursors in two parallel experiments. The



SCHEME 2

to be made between squalene and 2,3-oxidosqualene observed incorporations (Table 2) allow a clear distinction

TABLE 1

Feedings of [2-^{14}C]*mevalonic acid*

No.	Month	Feeding technique	Duration (Days)	Incorporations[a] % Squalene[b]	Fern-9-ene	β-Sitosterol
(1)	Jan.	Rhizome slices	2	0·066	0·008	0·000
(2)	Apr.	Rhizome slices	2	—	0·006	0·000
(3)	June	Rhizome slices	2	—	0·010	—
(4)	Oct.	Rhizome slices	1	2·68	0·20	0·12
(5)	Oct.	Rhizome slices	4	1·00	0·22	—
(6)	Oct.	Rhizome slices	11	0·30	0·06	—
(7)	Oct.	Sliced leaves	4	1·22	0·28	—
(8)	Nov.	Excised whole fronds	20 hr.	32·80	0·16	—
(9)	Oct.	Excised whole fronds	4	3·78	0·34	—

[a] Allowing for the utilisation of only one optical isomer.
[b] Counted as an isomeric mixture of hexahydrochlorides.

We therefore sought methods of incorporating precursors into fern-9-ene in *P. vulgare*, initially using sodium [2-^{14}C]-mevalonate in phosphate buffer at pH 7·4. The successful technique are summarised in Table 1. It was found that the optimum period of fern-9-ene formation was in October, with 4-day feedings (Table 1, Feeding Nos. 5, 7, and 9), to sliced rhizomes, sliced leaves, or excised whole fronds. Feedings for a longer period (Table 1, Feeding No. 6) resulted in a considerable decrease in the activity of the fern-9-ene.

[1,24,25,30-$^3H_4$]Squalene and 2,3-oxide-[1,24,25,30-$^3H_4$]-squalene prepared by a previously described route,[1f] were administered as Tween 80 emulsions by the techniques used for mevalonate feedings, but no incorporations were observed. Clearly, these precursors were not reaching the site of triterpenoid biosynthesis and penetration of the cell walls from micelles would appear to be prohibited.

It has been suggested that nonpolar precursors may be retained in the outer waxy layer during intact-leaf feedings. In order to circumvent this difficulty, Bennett and Heftmann[7] simultaneously defatted the leaf surface and effected feeding by painting on acetone solutions of the precursors. We

TABLE 2

Feedings of squalenoids

Precursor	Incorporations (%) Fern-9-ene	β-Sitosterol[a]
[1,24,25,30-$^3H_4$]Squalene	0·015	0·070
	0·008	0·036
2,3-Oxido[1,24,25,30-$^3H_4$]squalene	0·000	0·046
	0·000	0·048

[a] Allowing for the loss of two labelled methyl groups.

metabolism. Squalene is transformed into both fern-9-ene and β-sitosterol, but 2,3-oxidesqualene is converted only into the sterol, and not the hydrocarbon. In addition, a feeding of labelled 2,3-oxidosqualene in the presence of inactive squalene gave totally inactive recovered squalene, implying that there is no feedback of activity. Thus it would appear that β-sitosterol is produced by the usual sterol route,[1] but fernene must arise from squalene by a pathway not involving the oxide. The low incorporations observed do not allow degradation of the fernene for proof of the

specificity of the labelling, but the complete lack of incorporation from 2,3-oxidosqualene into fernene suggests that no scrambling has occurred. The use of other specifically labelled squalenes should allow a proof of the specificity of the incorporation to be determined and work is in hand on this aspect of the problem.

The fern *P. vulgare* is remarkable in its ability to cyclise squalene by both the oxidative and the proton-catalysed routes. The study of this phenomenon in other lower plants is in progress.

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