

Biosynthesis of Ponasterone A, an Insect-Moulting Substance from *Podocarpus macrophyllus*

Ponasterone A is the first arthropod-moulting steroid discovered in the plant kingdom,¹⁾ and has hitherto been isolated from a number of vegetable sources. It seems to be most probable that this phytoecdysone is biosynthesized in the plants from cholesterol which is now accepted to exist in the plant kingdom. In order to confirm this assumption, we have undertaken an investigation on the biosynthesis of ponasterone A, using the conifer *Podocarpus macrophyllus* D. DON (Podocarpaceae), which is known to contain ponasterone A as the major phytoecdysone.²⁾

Cholesterol-4-¹⁴C was administered³⁾ twice a week for one month to the leaves of three *P. macrophyllus* seedlings grown in soil (total dose 1.54×10^7 counts/min). Four weeks after the last treatment, the leaves were harvested (610 g) and extracted with methanol. The ether-insoluble portion of the methanol extract was extracted with *n*-butanol to give the butanol extract (11.8 g, 4.75×10^5 counts/min) which was chromatographed over neutral alumina (60 g). Fractions eluted with ethyl acetate were combined (626 mg, 1.87×10^5 counts/min) and rechromatographed on silica gel (15 g). Elution with chloroform-methanol (10:1) afforded a crystal paste (111 mg, 9.30×10^4 counts/min) which showed on thin-layer chromatography only a single radioactive spot possessing the same mobility as ponasterone A. The paste was diluted with carrier, ponasterone A, and crystallized five times to furnish the radioactive ponasterone A having a constant specific activity ($9.7\text{--}10 \times 10^2$ counts/min/ μmole).

Thus the biosynthesis of ponasterone A from cholesterol has been proven.

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