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# Short Communication

## Studies on Insect Moulting Hormones: Biosynthesis of Ponasterone A and Ecdysterone from [2-$^{14}$C]Mevalonate in *Taxus baccata*

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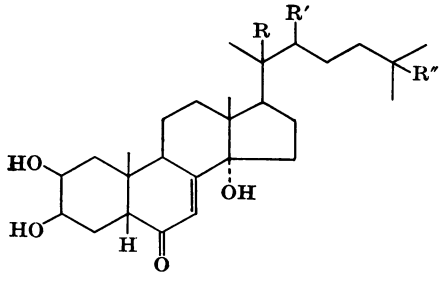
Ponasterone A (Ia) and ecdysterone (Ib) are two of several steroids related to ecdysone (Ic) with moulting-hormone activity in insects and crustaceans that have recently been found in the plant kingdom. They occur in members of the families Podocarpaceae (Nakanishi, Koreeda, Sasaki, Chang & Hsu, 1966; Galbraith & Horn, 1966; Imai, Fujioka, Nakanishi, Koreeda & Kurokawa, 1967), Amaranthaceae (Takemoto, Ogawa & Nishimoto, 1967*b*; Takemoto, Ogawa, Nishimoto & Taniguchi, 1967*d*), Polypodiaceae (Jizba, Herout & Šorm, 1967), Taxaceae (Imai *et al.* 1967; Takemoto, Ogawa, Nishimoto & Hoffmeister, 1967*c*; Takemoto, Hikino, Jin & Hikino, 1968; Hoffmeister, Heinrich, Staal & van der Burg, 1967), Pteridiaceae (Kaplanis, Thompson, Robbins & Bryce, 1967; Takemoto, Arihara, Hikino & Hikino, 1968), Aspidiaceae (Takemoto *et al.* 1967*a*), Verbenaceae (Rimpler & Schulz, 1967) and Osmundaceae (Takemoto, Hikino, Jin, Arai & Hikino, 1968). Ecdysterone is the more widely distributed of the two compounds and is the only reported moulting-hormone constituent of *Taxus baccata* (Takemoto *et al.* 1967*c*; Hoffmeister *et al.* 1967). The phytoecdysones presumably arise via the mevalonate → steroid pathway. The conversion of [4-$^{14}$C]cholesterol into ecdysterone in *Podocarpus elata* (Heftmann, Sauer & Bennett, 1968; Sauer, Bennett & Heftmann, 1968) and into ponasterone A in *Podocarpus macrophyllus* (Hikino, Kohama & Takemoto, 1969) has been reported.




(Ia) R = R′ = OH, R″ = H
(Ib) R = R′ = R″ = OH
(Ic) R = H, R′ = R″ = OH

We report here the biosynthesis of ponasterone A and ecdysterone from mevalonate in *Taxus baccata*.

*Experimental and results. Taxus baccata* L. seedlings were obtained from Boulton Brothers, Moddershall, Staffs. A solution of 1 $\mu$c of DL-[2-$^{14}$C]mevalonic acid lactone (6·4 mc/m-mole) in 0·2 ml. of aq. 0·01% Nonidet P42 (Shell TP7143) (Mercer & Pughe, 1969) was applied as droplets to the top cluster of leaves of five seedlings. Similar applications were made every alternate day until 10 $\mu$c of [2-$^{14}$C]mevalonic acid lactone was deposited on the leaves. Twenty days after the first application was made, the seedlings were worked up by methods described in the literature (Sauer *et al.* 1968; Hikino *et al.* 1969) and the phytoecdysones isolated by column chromatography on alumina (grade III). The radioactive phytoecdysones were separated on t.l.c. plates of silica gel developed in ethyl acetate–ethanol (4:1, v/v) and the plates were scanned for radioactivity. The radioactivity was found to be distributed between two peaks, the more polar coinciding with an authentic ecdysterone marker ($R_F$ 0·42) and the less polar with an authentic ponasterone A marker ($R_F$ 0·65). The bands were eluted with methanol. The radioactivity associated with the more polar band was $1{\cdot}32 \times 10^4$ c.p.m. and that with the less polar band was $9{\cdot}8 \times 10^3$ c.p.m. Carrier ecdysterone and ponasterone A were added to one-fifth portions of the less mobile and more mobile bands respectively. Acetates were prepared of each of the portions and separated on t.l.c. plates of silica gel developed in ethyl acetate–hexane (4:1, v/v). The plates were scanned for radioactivity. Radioactive peaks were found to coincide exactly with ecdysterone 2,3,22-triacetate ($R_F$ 0·25) and ecdysterone 2,3,22,25-tetra-acetate ($R_F$ 0·48) for the more polar phytoecdysone and with ponasterone 2,3,22-triacetate ($R_F$ 0·59) for the less polar phytoecdysone. No radioactive peaks were observed to coincide with authentic ecdysone 2,3,22-triacetate ($R_F$ 0·35) and ecdysone 2,3,22,25-

tetra-acetate ($R_F$ 0·64). The lower limit of our detection of radioactivity with ecdysone triacetate was 20% of that associated with the ecdysterone triacetate peak. Periodate oxidation of another portion of each of the bands and t.l.c. of the products also showed radioactive peaks with the same mobility as the major products of a similar reaction done on authentic samples of ecdysterone and ponasterone A. Carrier ecdysterone was then added to the remaining radioactivity associated with the more polar phytoecdysone and was crystallized to constant specific radioactivity (418 c.p.m./$\mu$mole). The triacetate was also prepared and crystallized to constant specific radioactivity (425 c.p.m./$\mu$mole). Lack of sufficient ponasterone A prevented a similar crystallization being done with the less polar radioactive phytoecdysone.

*Discussion.* The findings show the reality of the incorporation of [2-$^{14}$C]mevalonate into ponasterone A and ecdysterone. In further support of these results is the isolation by us (unpublished work) of both ecdysterone and ponasterone A (approx. 20% of the ecdysterone content) from the dried leaves of *Taxus baccata* (cf. Takemoto *et al.* 1967*c*; Hoffmeister *et al.* 1967). To our knowledge this is the first demonstration of the conversion of mevalonate into ecdysone-like compounds. The extents of incorporation of radioactivity into ponasterone A and ecdysterone, namely approx. 0·1% and 0·13% respectively of the available mevalonic acid, are sufficiently high to permit investigation of the pathways by which the ecdysones are formed. The use of specifically labelled mevalonic acids should make possible the elucidation of the specificity of the incorporation.

The incorporation of radioactivity into both the pentahydroxy compound (Ia) and the hexahydroxy compound (Ib), coupled with our observations of the relative proportions of the two compounds in the plant, indicate a possible precursor–product relationship between ponasterone A and ecdysterone. Though we were unable to detect association of radioactivity with ecdysone, also a possible precursor of ecdysterone, at extents more than 20% of the incorporation of radioactivity into ecdysterone, its involvement as a more readily metabolized precursor of ecdysterone cannot be ruled out.

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