Catabolism of Ponasterone A to Ecdysterone, Inokosterone, and Poststerone in *Bombyx mori*

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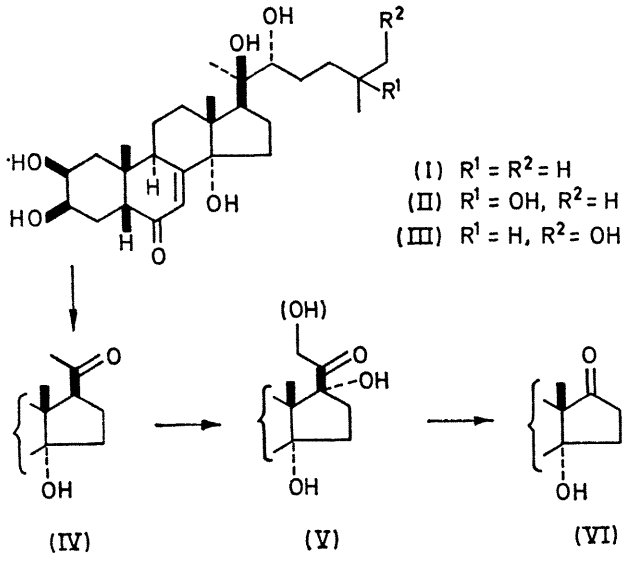
Summary Ponasterone A is catabolized in *Bombyx mori* larvae by hydroxylation at C-25 and C-26 to ecdysterone and inokosterone and by C-20:C-22 side-chain fission to poststerone.

THE levels of moulting hormones in insects undergo periodic rise and fall synchronized with life cycles.[1] The decrease in moulting hormone titre is considered to be due to enzymatic catabolism of the hormones. When Horn *et al.*[2] isolated crustecdysone [ecdysterone (II)] from a crayfish, *Jasus lalandei*, they suggested that it might be catabolized to the methyl ketone (IV), but later they concluded that this was unlikely since no methyl ketone (IV) could be detected in the crayfish extracts. Shortly thereafter, however, we[3] isolated rubrosterone (VI) from a plant, *Achyranthes rubrofusca* (Amaranthaceae), when we postulated that a metabolic pathway from the arthropod moulting steroids such as ecdysterone (II) to rubrosterone (VI) *via* the methyl ketone (IV) and the hydroxy-methyl ketone (V) is operating at least in the plant kingdom. In support of this supposition, we[4] recently isolated the postulated intermediate, poststerone (IV), from another plant, *Cyathula capitata* (Amaranthaceae). In the meantime, Galbraith *et al.*[5] found that in prepupae of *Calliphora stygia*, ecdysterone (II) was catabolized partly by the C-20:C-22 side-chain scission to 4-hydroxy-4-methylpentanoic acid. Although this finding implies the concurrent formation of poststerone (IV), no direct proof has been given. We now present evidence that an arthropod moulting steroid is catabolized in larvae of the silkworm (*Bombyx mori*) to poststerone (IV) in high efficiency.

[1α,2α-$^3H_2$]Ponasterone A was prepared in a similar manner as described previously[6] but using [1α,2α-$^3H_2$]-cholesterol instead of [4-^{14}C]cholesterol. The labelled ponasterone A (12·5 mg, 1·46 × 10^6 d.p.m.) was injected

into 500 fifth instar larvae and the animals were extracted with methanol 48 h later. The methanol-soluble portion was extracted with ethyl acetate. After washing with light petroleum, the ethyl acetate extract was submitted to successive liquid chromatography using alumina, silica gel, and Amberlite XAD-2.[7] The radioactive fractions having the same retention times as ecdysterone (II), poststerone (IV),



and rubrosterone (VI), respectively, were co-crystallized several times with the corresponding unlabelled carriers [60 mg for (II), 60 mg for (IV), and 30 mg for (VI)]. As a result, ecdysterone (II) and poststerone (IV) reached constant specific activity [$4{\cdot}97 \times 10^5$ d.p.m./mmole for (II) and $1{\cdot}34 \times 10^5$ d.p.m./mmole for (IV)], but rubrosterone (IV) lost the radioactivity. The identity of ecdysterone (II) and poststerone (IV) thus obtained was further confirmed by acetylation of each radioactive steroid, the radioactivity being retained in the corresponding acetate. The mother-liquor of ecdysterone (II) might possibly contain some inokosterone (III); it was combined with the radioactive fraction having the same retention time as inokosterone (III) and the mixture was acetylated. The resulting radioactive tetra-acetate was diluted with the carrier inokosterone tetra-acetate (35 mg) and subjected to repeated crystallization to constant specific activity ($1{\cdot}77 \times 10^5$ d.p.m./mmole).

Although ponasterone A (I) may not be a normal major intermediate in the sterol metabolism of *Bombyx mori*, these data demonstrate that ponasterone A (I) is catabolized by hydroxylation at C-25 and C-26 to ecdysterone (II) and inokosterone (III), and by C-20:C-22 side-chain scission to poststerone (IV). Furthermore, the radioactivity isolated in the form of the catabolite, poststerone (IV), represents 2% of the total activity administered. Since considerable amounts of ponasterone A (I) ($15{\cdot}0 \times 10^4$ d.p.m.), ecdysterone (II) (6.21×10^4 d.p.m.), and inokosterone (III) ($0{\cdot}95 \times 10^4$ d.p.m.), which had not yet lost their side-chains, were still present, it is concluded that direct C-20:C-22 bond cleavage constitutes a major metabolic pathway in *Bombyx mori* larvae and is one of the main mechanisms of inactivation of the moulting hormones.

In this experiment, however, catabolism of ponasterone A (I) to rubrosterone (VI) by C-17:C-20 side-chain fission could not be proven.

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