The Microbiological Oxidation of Insect Moulting Hormones

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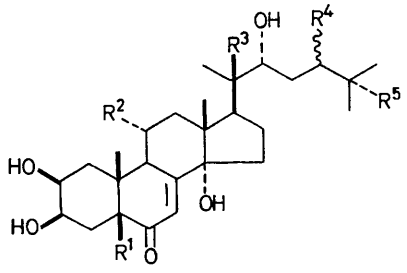
Summary Crustecdysone (I) is degraded by a lysed mycelium of the micro-organisms *Rhizopus arrhizus, Rhizopus nigricans* and *Curvularia lunata* to give poststerone (VI) which is then transformed into rubrosterone (VIII); makisterone A (II) behaves similarly, muristerone A (III) gives a new product, identified as 5β,11α-dihydroxypoststerone (VII), and ecdysone (IV) is not affected by these micro-organisms.

IT has been shown that crustecdysone (I)[1] and ponasterone A (V)[2] are catabolised in insects by C(20)–C(22) side-chain fission to 4-hydroxy-4-methylpentanoic acid lactone (IX) and poststerone (VI) respectively. The isolation of rubrosterone (VIII)[3] and poststerone (VI)[4] in some plants suggests that a similar fission occurs in vegetal tissues and that rubrosterone† arises from poststerone through C(17)–C(20) fission. In other plants, different oxidative processes, such as ring hydroxylation, can take place, giving rise to products, *e.g.* polipodine B[5] and muristerone A,[6] which are very active as moulting hormones.

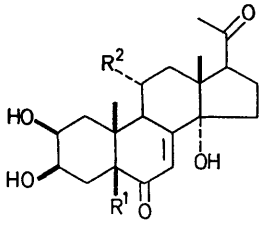
On the basis of these considerations, it seemed of interest to study the behaviour of some phytoecdysones, crustecdysone (I), makisterone A (II), muristerone A (III) and ecdysone (IV), in fermentation with typical hydroxylating micro-organisms *Rhizopus arrhizus* ATCC 11145, *Rhizopus nigricans* ATCC 6261 and *Curvularia lunata* ATCC 12017. The ecdysones are not transformed by the whole cells; in fact, if the mycelium obtained in a normal culture medium[7] is collected, washed and suspended in water, crustecdysone (I), makisterone A (II) and muristerone A (III) are transformed only when an extended lysis begins.

In a typical experiment crustecdysone (20 mg ml^{-1}) was incubated by shaking at 27° for 110 h with a *Rhizopus arrhizus* mycelium in water. After Bu^nOH extraction, silica gel chromatography and crystallization, poststerone (VI, 70%)‡ and rubrosterone (VIII, 3%)‡ were isolated.

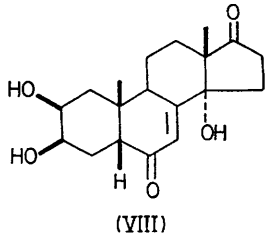
In a further experiment, the incubation liquid was acidified and continuously extracted with ether,[1] g.l.c. of the ether extract showed a peak identical with the one of an authentic sample of 4-hydroxy-4-methylpentanoic acid lactone.[8]



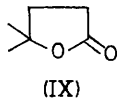
(I) $R^1=R^2=R^4=H$; $R^3=R^5=OH$
(II) $R^1=R^2=H$; $R^3=R^5=OH$; $R^4=Me$
(III) $R^1=R^2=R^3=OH$; $R^4=R^5=H$
(IV) $R^1=R^2=R^3=R^4=H$; $R^5=OH$
(V) $R^1=R^2=R^4=R^5=H$; $R^3=OH$



(VI) $R^1=R^2=H$
(VII) $R^1=R^2=OH$



(VIII)



(IX)

† Rubrosterone has not yet been isolated in insects and arthropoda.

‡ The identity of the products was confirmed by comparison of their physicochemical properties with the ones reported in the literature [3,4]

It is likely that rubrosterone is a transformation product of poststerone, in fact, the incubation of poststerone with *Rhizopus arrhizus* under the same conditions gives a complete transformation into rubrosterone.

Under the same conditions in which crustecdysone is degraded, makisterone A (II) is converted into poststerone, while muristerone A (III) gives 5β, 11α-dihydroxypoststerone (VII), amorphous, M^+ at *m/e* 394, n.m.r. [$^2H_6$]-DMSO δ 5·69 (1H, d, *J* 2·5 Hz), 2·10 (3H, s), 0·88 (3H, s), 0·48 (3H, s), identical with the product we prepared by CrO_3–pyridine oxidation at room temperature of muristerone A. The same results were obtained by oxidation of the ecdysones with *Rhizopus nigricans* and *Curvularia lunata*.

With crustecdysone, microbiological attack has led to the degradation of the side-chain and not to hydroxylation of the nucleus. The presence of a methyl group at C(24), as in makisterone A, or of two OH at C(5β) and C(11α), as in muristerone A, does not seem to affect the course of the microbic oxidation, while for the latter the presence of a preformed 20*R*,22*R*-diol system is required; in fact, under the reported conditions, ecdysone (IV) is not transformed by the three micro-organisms.

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