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ABSOLUTE CONFIGURATION OF INOKOSTERONE, AN INSECT-MOULTING SUBSTANCE FROM ACHYRANTHES FAURIEI

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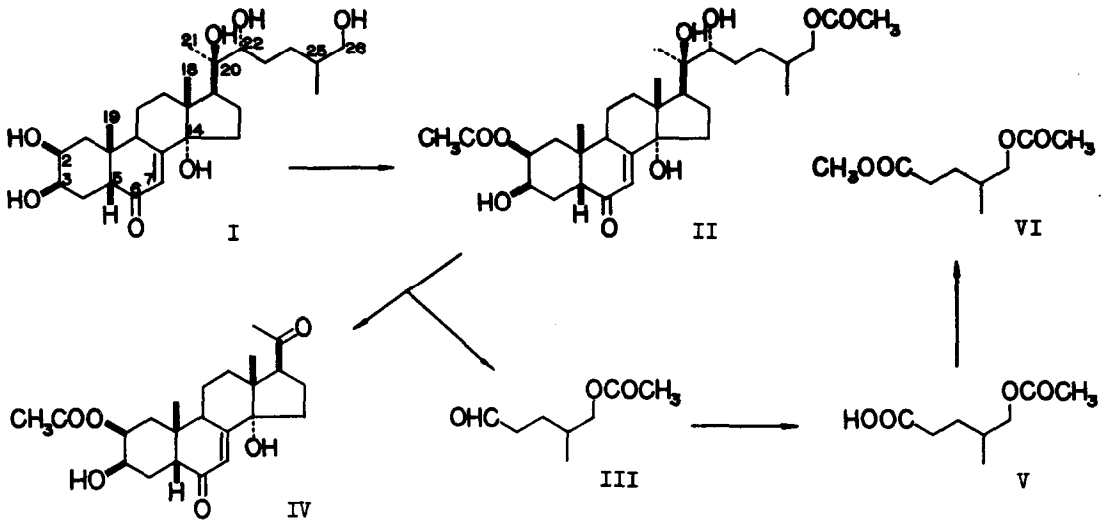
Inokosterone is a steroid isolated from a plant source, Achyranthes fauriei Léveillé et Vaniot (Amaranthaceae).[1,2] It has been revealed that inokosterone exhibits high moulting hormone activity in insects[1,3,4] and shrimps,[5] and high protein anabolic potency in mice.[6]

The structure of inokosterone has been assumed as 2,3,14,20,22,26-hexahydroxy-cholest-7-en-6-one.[1,2] Although some preliminary results[1,2] suggest that the configurations at C-5, 10, 13, and 14 of inokosterone are the same as those of the insect moulting hormone ecdysone, 2β,3β,14α, 22(R),25-pentahydroxy-coprost-7-en-6-one[7] and its analogues,[8-15] no conclusive proof for the complete stereochemistry has been given. The present communication deals with evidence for the absolute configuration of inokosterone.

Periodate oxidation of inokosterone gave a hydroxy-aldehyde which on further oxidation with potassium permanganate in water yielded (±)-α-methylglutaric acid.[2] It was possible, however, that the asymmetric center α to the carbonyl group had been racemized during oxidation in alkaline medium. Therefore, in order to eliminate this possibility, inokosterone was treated with acetic anhydride and pyridine in chloroform to give the 2,26-diacetate (II), m.p. 163-164°, IR (KBr): 3450 (hydroxyl), 1720, 1240 (acetoxyl), and 1650 cm^{-1} (cyclohexenone), NMR ($CDCl_3$):[*1] two 3H singlets at 2.04 and 2.08 p.p.m. (CH_3-CO-O-). On periodate oxidation the diacetate (II) furnished the acetoxy-aldehyde (III) and the methyl ketone (IV). The aldehyde (III) was subjected to oxidation with chromic acid followed by treatment with diazomethane to afford methyl γ-methyl-δ-acetoxy-valerate (VI), IR ($CHCl_3$): 1720 cm^{-1} (acetoxyl and methoxycarbonyl), NMR ($CDCl_3$): 3H doublet at 0.95 (CH_3-CH<), 3H singlet at 2.03 (CH_3-CO-O-), 3H singlet at 3.64 (CH_3-O-CO-),



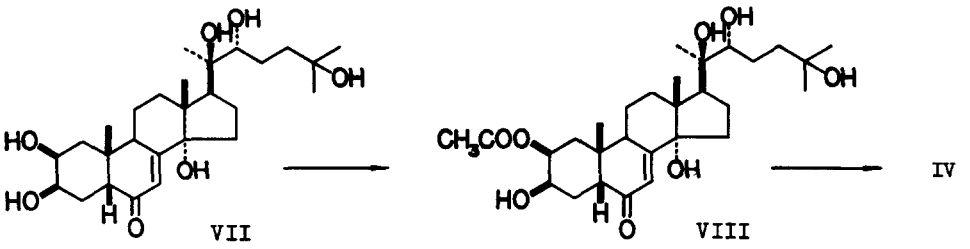
and 2H doublet at 3.89 p.p.m. (CH_3-CO-O-$C\underline{H}_2$-CH<). There had been no opportunity for the -CH_2-$C^*H(CH_3)CH_2OH$ moiety to racemize during the conversion of inokosterone to the valerate (VI). However, the ester (VI) thus prepared was again found to be optically inactive.*2 These facts indicate that inokosterone consists of a mixture of C-25 epimers.

The methyl ketone (IV), m.p. 240~242°, IR (KBr): 3400 (hydroxyl), 1736, 1241 (acetoxyl), 1695 (acetyl), and 1640 cm^{-1} (cyclohexenone), was identified with 2β-acetoxy-3β,14α-dihydroxy-5β-pregn-7-ene-6,20-dione, derived from ponasterone A,[9] pterosterone,[16] and cyasterone[17] in a similar manner, and the stereostructure (IV) has been established by hydrolysis to give 2β,3β,14α-trihydroxy-5β-pregn-7-ene-6,20-dione[9] produced as an intermediate en route for the synthesis of ecdysone.[18] Consequently, the absolute stereochemistry of the nucleus of inokosterone is established.

Inokosterone, as ecdysterone (VII), readily gives the 20,22-acetonide and rapidly consumes two moles of periodate. The chemical shifts of the C-18, 21, and 22 proton signals of inokosterone 2,3,22,26-tetraacetate (0.85, 1.24, and 4.85 p.p.m., respectively)*3 are very similar to those of ecdysterone 2,3,22,25-tetraacetate (0.86, 1.25, and 4.84 p.p.m., respectively). It is most likely that inokosterone is biosynthesized from ponasterone A, 2β,3β,14α,20(R),22(R)-pentahydroxy-coprost-7-en-6-one.[8~10] Therefore, the absolute configurations at C-20 and 22 are indicated to be both R.

The combined evidence leads to the conclusion that inokosterone is an epimeric mixture of 2β,3β,14α,20(R),22(R),26-hexahydroxy-25(R and S)-coprost-7-en-6-ones (I).

Ecdysterone is another moulting hormone isolated originally from animal sources and later from plant sources.[19] Its structure was elucidated by physico-chemical studies.[12,13] The stereochemistry of ecdysterone, however, has been tentatively assigned to be the same as ecdysone, mainly on the basis that ecdysterone is most probably biosynthesized from ecdysone. An unequivocal proof was required. Then ecdysterone was partially acetylated by acetic anhydride and pyridine in chloroform to yield the 2-acetate (VIII), IR ($CHCl_3$): 3620, 3420 (hydroxyl), 1720 (acetoxyl), and 1653 cm^{-1} (cyclohexenone), NMR ($CHCl_3$): 3H singlet at 2.07 p.p.m. ($\underline{CH_3}$-CO-O-), which on periodate oxidation furnished the same methyl ketone (IV), m.p. 240~242°. Thus the absolute stereochemistry of the tetracycle of ecdysterone is determined chemically as represented by stereoformula VII.



Quite recently, the stereochemistry of the nucleus of crustecdysone (ecdysterone) has been investigated in a similar manner by Siddall et al.[18] who arrived at the same conclusion as our own. The complete stereostructure (VII) of crustecdysone (ecdysterone) has been established by its synthesis.[14]

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FOOTNOTES AND REFERENCES

*1 NMR spectra were determined using a Hitachi H-60 or a Varian HA-100 spectrometer. Chemical shifts are given in p.p.m. downfield from internal TMS.

*2 The active methyl γ-methyl-δ-acetoxy-valerate produced from diosgenin has $[\alpha]_{300}$ +13.0°.

*3 The NMR spectra of inokosterone and its derivatives will be discussed in detail in a forthcoming paper.

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