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Note

Structural requirements for the electron capturing properties of ecdysones

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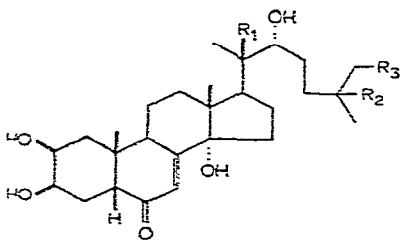
The ecdysones are a group of polyhydroxy sterols with a 5β-cholest-7-en-6-one framework represented by Formula I. In insects and crustaceans they play an important role as hormones controlling moulting. Over fifty ecdysones have been identified in arthropods and plants, differing chiefly in the number and position of the hydroxyl groups[1]. Their presence in very small quantities in arthropods demands a very sensitive technique for their determination as hormones. It has been found by chance that they can be detected at the picogram level with an electron capture detector after conversion of hydroxyl groups to trimethylsilyl (TMS) ethers for gas chromatography[2,3]. This high sensitivity to detection could not have been predicted, and to find the scope of the technique, the electrophore (the portion of the molecule responsible for electron adsorption) has been identified.



Formula I (a) $R_1, R_3 = H$ $R_2 = OH$
(b) $R_1, R_2 = OH$ $R_3 = H$
(c) $R_1, R_3 = OH$ $R_2 = H$

Steroids generally are not sensitive to an electron capture detector, and are converted to suitable derivatives, such as halogen-containing silyl ethers or acyl esters to make them sufficiently sensitive for detection at low levels[4,5]. The first five compounds of Table I indicate the sensitivity of typical sterols to electron capture detection, expressed as least detectable amount (LDA) producing a signal-to-noise ratio of 2, using a Pye Model 84 gas chromatograph, with ^{63}Ni electron capture detector, with pulse width 0.75 μsec, pulse period 50 μsec, pulse height 47–60 V and detector oven at 300°. The column oven was adjusted for each compound to produce the

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TABLE I

DETECTION LIMITS OF REPRESENTATIVE STEROLS BY ELECTRON CAPTURE METHOD

Compound	*LDA* ($g \times 10^9$)
TMS-cholesterol	200.0
17β-TMS-17α-methyltestosterone	5.0
Cholest-4-en-3,6-dione	5.0
5α-Cholestan-6-one	40.0
5α-Cholest-7-en-6-one	40.0
5α-Acetoxy-cholest-7-en-6-one	0.25
14α-Hydroxy-5α-cholest-7-en-6-one	0.07
14α-TMS-5α-cholest-7-en-6-one	0.03
2β,3β-Di-TMS-5α-cholest-7-en-6-one	1.0
2β,3β-Di-TMS-5β-cholest-7-en-6-one	1.0
2β,3β-Di-TMS-14α-hydroxy-5α-cholest-7-en-6-one	0.05
2β,3β-Di-TMS-14α-hydroxy-5β-cholest-7-en-6-one	0.05
2β,3β,14α-Tri-TMS-5α-cholest-7-en-6-one	0.005
2β,3β,14α-Tri-TMS-5β-cholest-7-en-6-one	0.005
Ecdysone (Ia) penta-TMS ether	0.005
20-Hydroxyecdysone (Ib) hexa-TMS ether	0.005
Inokosterone (Ic) hexa-TMS ether	0.005
Cyasterone penta-TMS ether	0.005
2β,3β,14α-Tri-TMS-5β-pregnen-7-en-6,20-dione	0.005

same peak width at half height on a 3-ft. column of 2% OV-101 on Gas-Chrom Q with a flow-rate of nitrogen of 85 ml/min.

No specific sensitivity is shown by 5α-cholestan-6-one or 5α-cholestan-7-en-6-one, but introduction of the 14α-hydroxyl group or its silyl ether gives a sharp increase in sensitivity (about 1,300 fold in the case of the 14α-TMS ether). The 2β,3β-di-TMS-5α (or β)-cholest-7-en-6-ones are 40 times more sensitive to detection than cholest-7-en-6-one, although the two trimethylsilyl ether groups are remote from the unsaturated ketone. Similarly, 2β,3β,14α-tri-TMS-5α (or β)-cholest-7-en-6-one is some six times more sensitive than 14α-TMS-5α-cholest-7-en-6-one. Thus, in the ecdysones, the electrophore is not simple, involving the 7-en-6-one group, the 14α-hydroxyl and smaller contributions from groups further removed from the ketone. There is no evidence that the weakly electron-capturing groups in the side chain exert an influence on the electrophore.

It has been previously noted that two or more groups which are not electron absorbing by themselves, when conjugated can confer electron-absorbing properties on the molecule[6–8]. Small further increases in electron-capture sensitivity with substituents remote from the conjugated group were attributed to electronic interaction across the saturated steroid framework[7,8]. To the best of our knowledge, the ecdysone possess the most sensitive conjugated steroid electrophore yet described.

A molecule can capture thermal electrons by two basic mechanisms[9], both temperature dependent:

$$AB + e^- \rightarrow AB^- \qquad \text{non-dissociative}$$
$$AB + e^- \rightarrow A\cdot + B^- \qquad \text{dissociative}$$

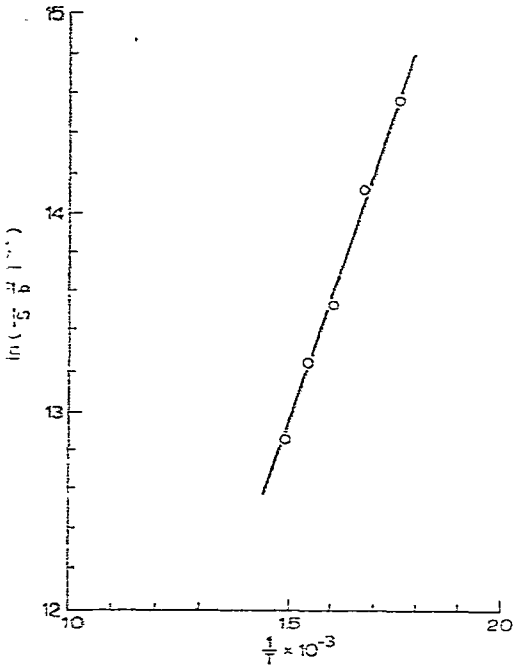

Fig. 1. Plot of $\ln\left(\frac{AF}{S}\frac{b_0}{b}T3/2\right)$ against $\frac{1}{T}$ for ecdysone penta-TMS ether (*cf.* ref. 9).

A plot of $\ln([AF/S][b_0/b]T^{3/2})$ against $1/T$ (Fig. 1) for ecdysones has a positive slope (where A = peak area in cm^2, F = gas flow-rate in ml/min, S = chart speed in cm/min, b_0 = standing current in A in the detector in the presence of pure carrier gas, b = standing current with column at operating temperature, and T is detector oven temperature in °K). This indicates a non-dissociative capture mechanism[9–11], in agreement with the finding of Durbin *et al.*[12] that conjugated electrophores capture electrons in a non-dissociative manner. The peak area measured by the detector decreases with increasing detector oven temperature, and for maximum sensitivity, the lowest practical detector temperature should be used. For the analysis of ecdysones as their TMS ethers, a detector temperature of 300° and column temperature of 270–280° is the best compromise. Under these conditions we have observed no loss in detector sensitivity in over one year of constant use.

The available evidence indicates that arthropods convert cholesterol and phytosterols to ecdysones by sequences which involve the early introduction of the unsaturated ketone and C-14 hydroxyl groups[13,14]. The electron capture method therefore provides an excellent means of detecting all the compounds in the later stages of ecdysone synthesis and is insensitive to other unrelated sterols.

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