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Note

Quantitative determination of phytoecdysones as illustrated by application to species of *Helleborus*

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Phytoecdysones are insect-moulting hormones of plant origin. About forty of these steroidal compounds have been isolated from about eighty different plant families[1]. The yields range from 45×10^{-6}–7×10^{-2}% (ref. 2). The isolation procedures for these compounds from different plants vary and involve several extraction and purification stages. The crystallisation property of ecdysones is extremely poor and gravimetric results cannot reflect the actual levels at which these compounds occur.

Two phytoecdysones, namely ecdysterone (2β,3β,14α,20,22*R*,25-hexahydroxy-5β-cholest-7-en-6-one) and 5β-hydroxyecdysterone (polypodine B) were detected in species of *Helleborus*, where they occur with bufadienolides and saponins in one morphological group only[3]. Ecdysterone and 5β-hydroxyecdysterone were isolated from the aerial parts of *H. orientalis* hybrids in yields of 0.065 and 0.001% m.f.b. (moisture-free basis), respectively[3].

The method adopted for the quantitative assay of these steroids is based on the UV absorption at 254 nm of the α,β-unsaturated keto-chromophore which is common to all phytoecdysones. Five grams of powdered plant material were used and these were extracted with methanol. The concentrate was purified over a 20 × 1.5 cm column filled with 8 g Polyamide (Woelm). Elution was with 100 ml of water. Removal of the water under reduced pressure gave a mixture of steroids free of chlorophyll and other lipophylic compounds prior to preparative thin-layer chromatography. Ecdysterone and 5β-hydroxyecdysterone have R_F values of 0.25 and 0.27 in chloroform–methanol (4:1)[4], respectively. However, they were completely separated from one another, and from bufadienolides and saponins, by continuous development for 8 h on silica gel $Pf_{254+366}$ 1-mm-thick plates, using the solvent system dichloromethane–ethanol (5:1). The bands were visualised by UV at 254 nm. Other compounds showing absorption at this wavelength were at the solvent front. These were shown not to be ecdysones[5]. The ecdysterone-(lower R_F) and the 5β-hydroxyecdysterone-(higher R_F) containing adsorbent were each removed and extracted with methanol for 12 h in a Soxhlet apparatus. The concentrate was usually diluted 1:1000 with redistilled methanol and assayed in a SP 600 Pye Unicam spectrophotometer. The absorption was recorded at 254 nm and the concentration determined from a calibration curve which was in conformity with Lambert-Beer's law (Fig. 1). The

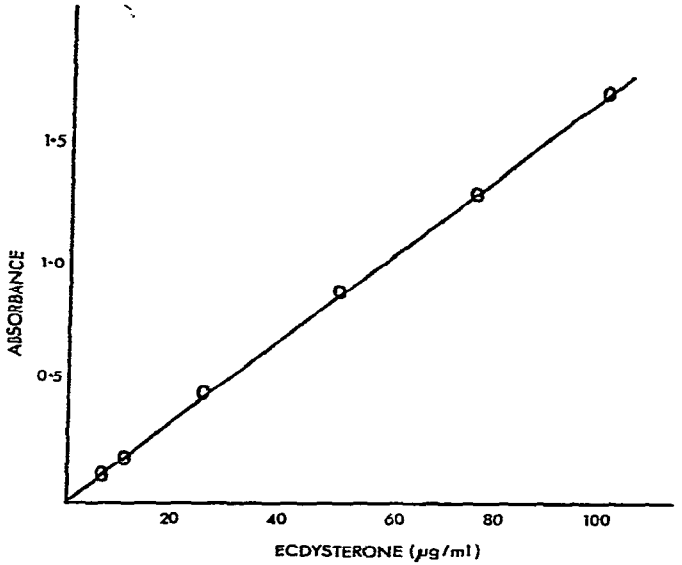

Fig. 1. UV spectrophotometric calibration curve of ecdysterone.

results are expressed as % m.f.b. in Table I. Results for other species have been published[3]. They were lower than for *H. viridis* L. ssp. *viridis*. The leaves of *H. orientalis* hybrids at flowering time are a good source of the two ecdysones.

The recovery of the phytoecdysones was tested using powdered *H. orientalis* hybrids (aerial parts) and ecdysterone isolated and purified from the same plant[3]. Five samples of 5 g each gave a mean ecdysterone concentration of 0.37% w/w (m.f.b.) ±0.006% S.D., representing a mean result of 18.5 mg. The recovery obtained from another five samples to which 10 mg of ecdysterone had been added (5 × 10 mg) gave a mean ecdysterone concentration of 0.57% w/w (96.4–103.5%) ±0.017% S.D., representing a mean result of 28.5 mg.

TABLE I

AMOUNTS OF PHYTOECDYSONES IN *HELLEBORUS* SP.

Helleborus sp.	*Ecdysterone (% m.f.b.)*	*5β-Hydroxy-ecdysterone (% m.f.b.)*
H. orientalis hybrids		
whole plant with flowers	0.45	0.12
leaves only	0.44	0.12
stem only	0.26	0.08
underground parts only	0.29	0.05
H. niger Ulbrich., whole plant without flowers	0.20	trace
H. niger, commercial root*	0.21	0.07
H. viridis L. ssp. *viridis*, whole plant without flowers	0.50	0.13

* Purchased from Brome and Schimmer, London, Great Britain.

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REFERENCES

1 E. Heftmann, *Lloydia*, 38 (1975) 195.
2 H. Hikino and Y. Hikino, *Fortschr. Chem. Org. Naturst.*, 28 (1970) 256.
3 R. Hardman and T. V. Benjamin, *Phytochemistry*, 15 (1976) 1515.
4 T. Matsuoka, S. Imai, M. Sakai and M. Kamanda, *Takeda Kenkyusho Nempo (Ann. Rep. Takeda Res. Lab.)*, 28 (1969) 221.
5 T. V. Benjamin, *Ph.D. Thesis*, University of Bath, Bath, 1976.