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SHORT COMMUNICATION

ISOLATION OF ECDYSTERONE FROM INDIAN PLANTS

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Abstract—Ecdysterone has been isolated from the following plants: *Achyranthes aspera, Gomphrena celosioides, Trianthema portulacastrum* and *Sesuvium portulacastrum*, the latter being one of the best sources for ecdysterone.

ECDYSTERONE is the most widely occurring phytoecdysone. The compound and its analogues have potential use as chemosterilants,[1] and because they stimulate protein synthesis not only in insects[2] but also in mammals.[3] These actions prompted us to investigate 40 plant extracts for insect moulting hormone activity. We report here certain convenient sources for ecdysterone from common Indian plants.

Isolation of ecdysterone. The plant materials were extracted with methanol. The concentrates obtained after removal of the solvent were extracted successively with light petroleum, ether, chloroform and *n*-butanol. Ecdysterone was extracted by *n*-butanol. It was isolated by column chromatography followed by preparative TLC and crystallisations. Ecdysterone was characterized by its colour reactions, spectral and chemical properties. Identity was confirmed by comparison with an authentic sample.[4]

Ecdysterone content of various positive plants is indicated in Table 1. All the plants mentioned below grow abundantly in various parts of the country. *Achyranthes aspera* and *Trianthema portulacastrum* are of considerable importance in indigenous systems of medicine.[5] *Sesuvium portulacastrum*, a halophytic plant occurring along the coasts of

TABLE 1. ECDYSTERONE CONTENT OF INDIAN PLANTS

Name and part of the plant	Family	Yield (g/kg)
Achyranthes aspera Linn. (Seeds)	Amaranthaceae	0·25
A. aspera Linn. (stem and leaves)	Amaranthaceae	0·04
A. aspera Linn. (roots)	Amaranthaceae	0·09
Gomphrena celosioides Mart. (whole plant)	Amaranthaceae	0·25
Trianthema portulacastrum Linn. (whole plant)	Aizoaceae	0·01
Sesuvium portulacastrum Linn. (whole plant)	Aizoaceae	3·5

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Western India, is one of the best sources for ecdysterone. The only plant which is known to contain a higher percentage of ecdysterone is *Polypodium vulgare*.[6]

Bioassay. The bioassay for the moulting hormone activity was carried out by modifying the "Chilo dipping" technique used by Sato *et al.*[7] In the present work, *Musca domestica* (housefly) larvae were used which give quicker response compared to *Chilo*. The isolated abdomens of housefly larvae were dipped in the crude extracts of the plants. The moulting hormone activity was shown by the formation of a puparium. For quantitative experiments, the test solutions were injected into the isolated larval abdomens. A dose of 0·01 μg of ecdysterone gave a full pupation response.

EXPERIMENTAL

Preparation of the Extracts for Screening Tests

In a typical experiment, 5 g of the roots of *Achyranthes aspera* were extracted with hot MeOH. MeOH concentrate was taken up in 1 ml of 10% aqueous EtOH and tested for moulting hormone activity.

Isolation of ecdysterone. The plant material (500 g) was extracted in a soxhlet extractor with MeOH. MeOH concentrate was fractionated with light petroleum, Et_2O, $CHCl_3$, and *n*-BuOH. The BuOH concentrate was charged on silica gel or alumina (neutral) column. Fractions eluting with $CHCl_3$–MeOH mixture (100:25) contained ecdysterone. Further purification of ecdysterone could be achieved by preparative TLC (Silica gel GF_{254}; EtOAc, 100/MeOH, 15) and crystallization from MeOH–Et_2O. The sample was dried at 120° *in vacuo* for 8 hr. M.p. 240°. $[\alpha]_{589}^{25°}$, + 60·0 (c, 0·2 MeOH). Found: C, 67·1; H, 9·6; calculated for $C_{27}H_{44}O_7$, C, 67·5; H 9·2%. IR (KBr) ν_{max} 3360, 1642 cm^{-1}. UV λ_{max}^{MeOH} 242 nm (4·07). MS *m/e*, 462 (M-H_2O), 444 (M-2H_2O), 426 (M-3H_2O), 408 (M-4H_2O), 99 and 81. NMR: (pyridine, TMS = 00) 1·05 δ (3H), 1·17 δ (3H), 1·34 δ (6H) and 1·55 δ (3H) UV spectrum after treatment with HCl: λ_{max}^{HCl} 296 and 241 nm.

Bioassay. Mature *Musca domestica* larvae which had emptied their gut contents and were ready for pupation, were chosen for the assay. Ligatures were made at the anterior $\frac{1}{3}$ region of the larvae. The larvae which pupated only in the anterior portion were selected for the test. The anterior portions were cut off and the isolated larval abdomens were dipped in the test solution for 10 sec. Presence of moulting hormone activity was indicated by the formation of puparium within 12–15 hr at 30°.

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