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SHORT COMMUNICATION

# THE ISOLATION AND IDENTIFICATION OF CYASTERONE FROM *AJUGA CHIA* (LABIATAE)

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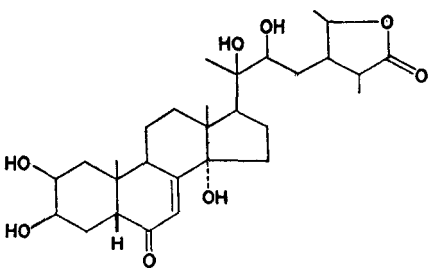
Abstract-Cyasterone, an insect molting hormone, has been isolated from *Ajuga chia* and identified by means of chromatographic (column, TLC) and spectroscopic (u.v., ix., NMR, m.s.) methods.

## INTRODUCTION

FOLLOWING the elucidation of the nature of the ecdysones which are responsible for the metamorphoses in insects,' it was found that such substances are widely distributed also in the plant kingdom ('phytoecdysones').[2–6] In fact, plants are richer in ecdysones than insects and crustaceae.[6]




Cyastarone

Cyasterone, a $C_{29}$ ecdysone has been isolated by Takemoto and coworkers from *Cyathula capitata* (Amaranthaceae)[8] and more recently[9] from *Ajuga decumbens* (Labiatae). It is of interest that, whereas most insect molting substances are $C_{27}$ compounds, biogenetically related to cholesterol, cyasterone as a $C_{29}$ compound is related to $\beta$-sitosterol.

In the present communication we report the isolation and identification of cyasterone from *Ajugu chia* (Labiatae).

## RESULTS AND DISCUSSION

The i.r. spectra of cyasterone shows bands at 1650 $cm^{-1}$ (cyclohexenone), 1750 $cm^{-1}$ ($\gamma$-lactone), and 3450 cm-' (OH). The u.v. $\lambda_{max}$ in ethanol was at 243 nm (log $\epsilon$ 4·1). The NMR and mass spectrum are shown in Tables 1 and 2.

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TABLE 1. NMR OF CYASTERONE*

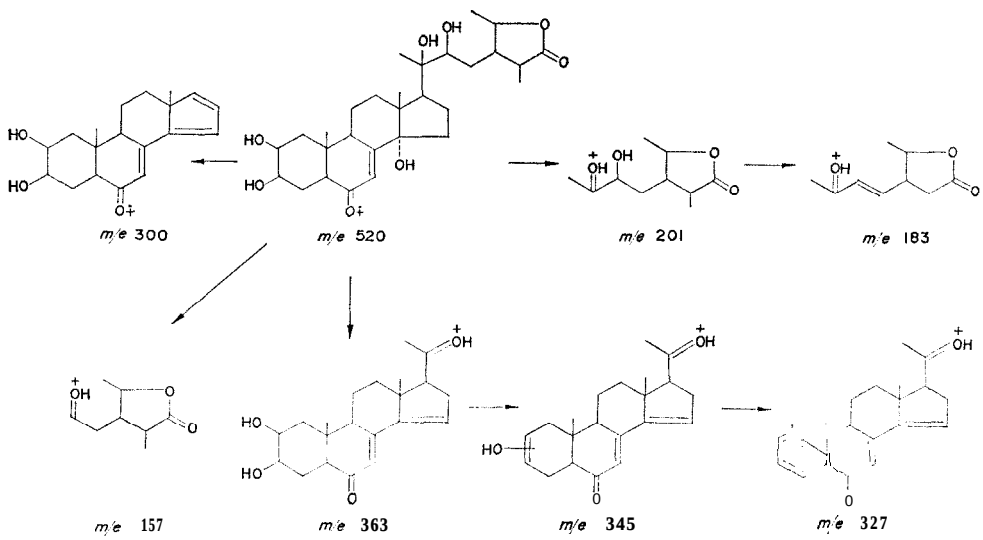
| δ (ppm) | Characteristic of |
|---|---|
| 1·00 | 3H,s-C-19 |
| 1.25 | 3H,s-C-18 |
| 1.32 | 6H,d-C-27; C-29 |
| 1·46 | 3H,s-C-21 |

* In deutero-pyridine.

TABLE 2. MASS SPECTRUM OF CYASTERONE

| *m/e* | Characteristic of | *m/e* | Characteristic of |
|---|---|---|---|
| 520 | Parent peak, weak | 451 | M-15-54 ($M^+—CH_3—3H_2O$) |
| 502 | M-18 ($M^+—H_2O$) | 433 | M-15-72 ($M^+—CH_3—4H_2O$) |
| 484 | M-36 ($M^+—2H_2O$) | 415 | M-15-90 ($M^+—CH_3—5H_2O$) |
| 466 | M-54 ($M^+—3H_2O$) | 363 | M-157 |
| 448 | M-72 ($M^+—4H_2O$) | 345 | M-157-18 |
| 505 | M-15 ($M^+—CH_3$) | 327 | M-157-36 |
| | | 300 | M-220 |
| | | 201 | M-319 |
| | | 183 | M-319-18 |
| | | 157 | M-363 |
| | | 43 | Base peak |

The principal fragmentation pathways are rationalized in the following scheme:[8]



## EXPERIMENTAL

### Source *of Plant Material*

***The*** plant material was collected in May in Eitanim area, near Jerusalem, Israel.

### *Extraction of Phytoecdysones*

***The*** leaves and stems of the fresh plant (1·6 kg) were soaked in 9 1. MeOH, and homogenized. The filtered extract was concentrated *in vacuo* and treated with $H_2O$ to give 30% aq. MeOH. This solution was extracted with *n*-hexane and the hexane discarded. The aqueous MeOH was concentrated to about half volume and extracted 4 *x* EtOAc. The yellowish-green EtOAc extract was evaporated by dryness and subjected to chromatography on a silica gel column (2 x 40 cm). Elution with $CHCl_3$–MeOH (9: 1) furnished crude cyasterone in the fractions 5-9 (each fraction, 100 ml), as indicated by TLC. These fractions were pooled, and concentrated, and the residue was recrystallized from MeOH (in presence of charcoal), to give 30 mg (0·002%) of colorless needles of pure cyasterone of m.p. 160–162°. $[\alpha]_D^{27}$ + 61·3° (c, 1·0, $H_2O$). The m.p. was not depressed by admixture of an authentic sample.

### *Thin-layer Chromatography*

The crude extract, the pure isolated compound and an authentic specimen were applied to silica gel GF-254 plates (0.25 mm; 20 x 20 cm) and developed with $CHCl_3$–EtOH–acetone (6:2:1). Observation under u.v. (360 nm) revealed cyasterone as dark spots on a yellow-green fluorescent background; spraying the plate with 50% $H_2SO_4$ and heating gave pink spots. The $R_f$ value is 0.54.

The following apparatus were used for the spectral measurements: i.r.—Perkin Elmer 337 i.r.-spectrophotometer; u.v.-Unicam SP-800 spectrophotometer; NMR-Varian HA-100; m.s.—Hitachi–Perkin Elmer RMU-6D mass spectrometer; rotation-Perkin-Elmer 141 polarimeter.

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