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Note

# Separation and detection of α- and β-ecdysone using thin-layer chromatography

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It is now believed that most insect tissues convert the precusor hormone, α-ecdysone (2β,3β,14α,22*R*,25-pentahydroxy-5β-cholest-7-en-6-one) to β-ecdysone (ecdysterone: 2β,3β,14α,20*R*,22*R*,25-hexahydroxy-5β-cholest-7-en-6-one) which is now considered to be the more active hormone[1]. To determine whether a tissue binds or utilizes α- or β-ecdysone, these hormones need to be separated and detected. Most investigators have used paper chromatography[2–4] or gas chromatography[5,6] to separate ecdysteroids. We wish to report a simple and rapid method to separate the two hormones using silica gel thin-layer chromatography (TLC). The sensitivity of various detection methods is also reported.

## MATERIALS AND METHODS

β-Ecdysone was obtained from Rhoto (Osaka, Japan) and α-ecdysone was a gift from John B. Siddall, Zoecon Research Corporation, Palo Alto, Calif., U.S.A. The solvents used were reagent grade (Fisher Scientific, St. Louis, Mo., U.S.A.).

α- and β-Ecdysone were spotted together or alone in amounts of 0.5–100 μg on Kontes Quantum 20 × 20 cm LQDF silica gel-coated glass plates (Kontes Glass, Vineland, N.J., U.S.A.) containing a pre-absorbent. The thin-layer chromatograms were run in 5 different solvent systems for 1 h at 22–24°. The Camag Vario-KS-chamber (Camag, New Berlin, Wisc., U.S.A.) was used. The different solvents were run simultaneously on one chromatogram in order to maximize uniformity of conditions among solvent runs. The plates were air dried and three previously described detection methods[7] were employed: (1) short wavelength UV, (2) vanillin–sulfuric acid spray and (3) anisaldehyde spray.

## RESULTS AND DISCUSSION

The $R_F$ values for α- and β-ecdysone obtained using various solvent systems are given in Table I. The use of the Kontes plates with a pre-absorbent allows for good separation since the samples run as a discrete narrow band and not as a circle or spot. The best separation of α- and β-ecdysone was achieved using chloroform–methanol (4:1) which gave a separation distance of 7 mm between the inner edges of the bands.

TABLE I

$R_F$ VALUES OBTAINED FOR α- AND β-ECDYSONE USING VARIOUS SOLVENT SYSTEMS
Sample size, 50 μg of α- and β-ecdysone each; detection, UV (254 nm).

| *Solvent system* | $R_F$ | |
|---|---|---|
| | *α-Ecdysone* | *β-Ecdysone* |
| Chloroform–methanol (3:2) | No separation | |
| Chloroform–methanol (4:1) | 0.68 | 0.60 |
| Chloroform–methanol (9:2) | 0.29 | 0.22 |
| Chloroform–methanol–water (75:24:4) | 0.78 | 0.73 |
| Chloroform–ethyl acetate (1:1) | No separation | |

TABLE II

SENSITIVITY OF VARIOUS DETECTION METHODS FOR α- AND β-ECDYSONE
Solvent system, chloroform–methanol (4:1). − = Negative; +, ++, +++, ++++ = positive on a relative scale.

| *Detection method* | *Amount spotted (μg)* | | | |
|---|---|---|---|---|
| | *50* | *10* | *1* | *0.5* |
| UV | ++++ | ++++ | ++++ | +++ |
| Vanillin–sulfuric acid spray | ++++ | +++ | − | − |
| Anisaldehyde spray | +++± | ++++ | + | − |

Table II gives the sensitivity of three detection methods using chloroform–methanol (4:1) as the solvent system. Amounts of 0.5 μg of ecdysteroids are clearly visible under UV light but 1.0-μg amounts are only slightly visible using the anisaldehyde spray and are not visible using the vanillin–sulfuric acid spray. We prefer UV detection for TLC, as it is more sensitive and more rapid than other methods.

The advantages of the TLC system described are (1) the use of only one solvent run for good separation, (2) the presence of narrow, discrete bands of steroid, and (3) rapid, sensitive detection. We feel that this method is the most rapid and best resolving and separating method that has been reported in detail to date.

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