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IDENTIFICATION AND QUANTIFICATION OF ECDYSTEROIDS FROM *AERVA TOMENTOSA* AND *PANDIAKA INVOLUCRATA*

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Ecdysteroids have been isolated and quantified from the roots of *Aerva tomentosa* (0.035%) and the aerial parts of *Pandiaka involucrata* (0.3%). The latter has been found as one of the best source of 20-hydroxyecdysone in plants, traces of 5,20-dihydroxyecdysone have also been detected from it.

Key words: Ecdysteroids, *Pandiaka, Aerva*, Amaranthaceae, 20-Hydroxyecdysone.

Introduction

Aerva tomentosa Forssk and *Pandiaka involucrata* Benth and Hook, both plants belong to the family Amaranthaceae.

Aerva tomentosa is widely distributed in Sindh, Punjab and Balochistan provinces in Pakistan. It is locally known as "Booh" in Sindh [1]. A decoction of the plant is used to remove swellings. *Pandiaka involucrata,* being unavailable in Pakistan, was obtained from Nigeria.

Ecdysteroids are of common occurrence in plant species and they have been found in some members of the family Amaranthaceae also, largely by bioassay method [2]. There is no report of their presence in the genera *Aerva* and *Pandiaka.*

In our previous communication [3], we have reported the presence of ecdysteroids in some of the genera of families Amaranthaceae and Chenopodiaceae. In this work, the estimation of 20-hydroxyecdysone in the species *Aerva tomentosa* and *Pandiaka involucrata* of the family Amaranthaceae is reported.

Experimental

Polyamide Woelm and silica gel 60 Pf 254 + 366 was used for column chromatography and TLC respectively. AEI MS12 instrument was used for mass spetrometry in which the sample was subjected by direct insertion probe at 240°C and ionization energies of 10 and 70 eV. For NMR spectroscopy a J. E.O.L. 100 MHz NMR Spectrophotometer was used. Infrared and UV spectra were recorded on SP 200 and SP1800 Pye Unicam spectrophotometers respectively. A Kofler hot bench apparatus was used for melting points and are uncorrected.

Aerva tomentosa was collected from Jamshoro (Sindh-Pakistan) and *Pandiaka involucrata* obtained from the University of Ife, Nigeria.

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Isolation and identification of 20-hydroxyecdysone and 5, 20 dihydroxyecdysone: Dried aerial parts of *Pandiaka involucrata* (500 g) and roots of *Aerva tomentosa* (500 g) were powdered separately and extracted with methanol in a Soxhlet extractor. The extracts were concentrated under vacuum and then adjusted to aqueous methanol 30% solution. The non-polar materials such as chlorophyll etc. was filtered off, the clear filtrate concentrated again and the extract was concentrated, mixed with Kieselguhr, dried and subjected to column chromatography (120 × 2.5 cm) using silica gel (Woelm, activity I, 200 g) with $CHCl_3$: MeOH, 15:1 (1 lit.) 9:1 (1 lit.) and 5:1 (2.5 lit.) as the solvent systems. Each fraction was checked on TLC (silica gel 60 pf 254+366, CH_2Cl_2: C_2H_5OH 95%, 5:1), visualised under UV at the absorption of 254 nm. The fractions found positive for ecdysteroids were mixed together, dried in vacuo at 60°C, dissolved in MeOH, subjected to preparative TLC on plates (20 × 40 cm) of silica gel 60 pf 254 +366, 1mm thick and developed CH_2Cl_2 : C_2H_5OH (95%), 5:1. One of the TLC plates in each case was sprayed with anisaldehyde-H_2SO_4 reagent [3] and heated in an oven at 110°C for 5-10 min. The lower band (leafy green) cochromatographed with authentic 20-hydroxyecdysone and the above one (blue) with 5,20-dihydroxyecdysone in case of *P. involucrata*, while in *A. tomentosa* only one band corresponding to 20-hydroxyecdysone was observed.

The bands of 20-hydroxyecdysone in each case, were scraped from the TLC plates (18 nos), mixed together and subjected to Soxhlet extraction with ethanol 95% for 24 hr. The extract was dried in vacuo, dissolved in a small volume of methanol, applied on a column of polyamide and eluted with water. The eluates were mixed together, dried and crystallised from acetone-methanol when needles of 20-hydroxyecdysone m.p. 234-235°C (literature [4] 237.5 239.5°C) separated. It showed UV max (MeOH) 242 nm, IR (KBr) 3400 and 1660 cm^{-1}, MS: *m/e* 462 (M^+ -H_2O), 444 (M^+ -$2H_2O$), 426 (M^+ -

$3H_2O$), 408 ($M^+ - 4H_2O$), 363 (M^+ - 117, side chain cleavage at C_{20} - C_{22}), 345 (M^+ - side chain, $-H_2O$), 327 (M^+ - side chain - $2H_2O$), 301 (M^+ - 161 - 18; M^+ - side chain cleavage at C_{17} - C_{20} $-H_2O$), 117 (cleavage at C_{20} - C_{22}), 99 (117, side chain cleavage at C_{20} - C_{22}, - H_2O), 81 (117 - 36, side chain cleavage at C_{20} - C_{22}, $-H_2O$), 43 (base peak). The ^1H NMR (DMSO d6) δ 0.78, 0.85 and 1.08 (each 3H, s, 18-Me, 19-Me, 21-Me), 1.10 (6H, s, 26-Me, 27-Me) and 5.65 (1H,s,7-H) [5].

The band of 5,20-dihydroxyecdysone (above 20-hydroxyecdysone zone) was worked out similarly but it failed to crystallise. It showed UV max (MeOH) 242 nm. MS: *m/e* 478 ($M^+ - H_2O$), 460, 442, 424, 379, 361, 343, 325, 99,81, and 43. IR max (KBr) 3400 and 1690 cm^{-1}.

Preparation of 20-hydroxyecdysone triacetate. It was prepared from 20-hydroxyecdysone isolated from *A. tomentosa* according to the method of Galbraith and Horn [6]. It showed m.p. 198 - 99°C, MS: *m/e* 606, 447, 429 and 385. ^1H NMR (DMSO-d6) δ 1.00 and 1.18 (each 3H,s, 18-Me, 19-Me, 21-Me), 1.20 (6H,s, 26-Me, 27-Me) and 5.86 (1H,s,7-H) [5]; IR (KBr) 3370 and 1640 cm^{-1}.

Preparation of 20-hydroxyecdysone diacetonide. The compound was isolated from *P. involucrata* and the derivative prepared as reported earlier [6]. It had m.p. 234-36°C and displayed identical mass and NMR signals as those of authentic derivative prepared similarly.

Quantitative determination of 20-hydroxyecdysone. The quantitative assay of ecdysteroids in *A. tomentosa* and *P. involucrata* was done by UV spectrophotometric technique used by Hardman *et al.* [7]. A standard curve was prepared with pure 20- hydroxyecdysone of different concentrations (10 - 100 µg/ml) against their absorption at 254 nm. A dry powdered plant material 20 g of each, was used for the determination of its 20-hydroxyecdysone content. The plant material was extracted with MeOH in a Soxhelt apparatus. The crude extract was subjected to polyamide coulumn chromatography eluting with water. The aq. eluates were tested on TLC and the spots absorbing at 254 nm were subjected to preparative TLC using the CH_2Cl_2: C_2H_5OH (95%), 5:1, as the solvent system. A distinct band absorbing at 254 nm was visible in case of *A. tomentosa* while a broad band was obtained for *P. involucrata.* These bands corresponding to 20-hydroxyecdysone were scraped from the plates, extracted with redistilled MeOH and made upto 100 ml. The solution of the former species was used as such but that from the latter diluted 10 times before 3 ml of each was used to take a reading. Three readings were taken with the solution from each species and the mean was taken as the average absorbance. The 20-hydroxyecdysone content was read off from the calibration curve and expressed as a percentage of 20-hydroxyecdysone on moisture free basis.

Results and Discussion

The aerial parts of *Pandiaka involucrata* Benth and Hook afforded two ecdysteroids i.e. 20-hydroxyecdysone and 5, 20-dihydroxyecdysone, the former being 0.3% (moisture free basis) and traces of the latter. The roots of *Aerva tomentosa* Forssk showed the presence of 20-hydroxyecdysone only to the extent of 0.03% (m.f.b). Comparison of the spectral data i.e. IR, UV, NMR and MS of 20-hydroxyecdysone with the authentic sample confirmed its identity. The very small quantity of 5, 20-dihydroxyecdysone obtained from *Pandiaka involucrata* did not crystallise but it showed close identity with authentic sample of 5,20-dihydroxyecdysone when subjected to IR, TLC, UV and MS. The two compounds had the same R_f (0.16) and colour reaction with the spray reagent [3] as the authentic samples. The diacetonide and triacetate derivatives also furnished similar MS and NMR peaks as that of the derivatives of authentic 20-hydroxyecdysone prepared in the similar way.

It was very difficult to isolate ecdysteroids in a single column chromatography operation. Thus silica gel column chromatography was supplemented by polyamide gel chromatography and preparative thin-layer chromatography. Preparative TLC has been used by Hoffmeister *et al.* [8] for the purification of ecdysteroid eluates from column chromatography to eliminate phenolic impurities which are considered to inhibit easy crystallization of ecdysteroids.

In this work, the *n*-butanol layer from *A. tomentosa* and *P. involucrata* was applied on silica gel column, the eluate after drying in vacuo was dissolved in MeOH and applied on silica gel Pf 254+366 plates. The plates were eluted with CH_2Cl_2 : C_2H_5OH (5:1) for twelve hours when clear separation of 20-hydroxyecdysone was achieved, which were visualized under UV at 254 nm. The band with lower R_f value was found to be due to 20-hydroxyecdysone from cochromatography and gave leafy green colour, same as the reference 20-hydroxyecdysone, spraying with anisaldehyde- sulphuric acid reagent. The band corresponding to 20-hydroxyecdysone from all plates was scraped and extracted with MeOH, concentrated, applied on a polyamide column and eluted with water. This step was necessary just to eliminate the impurities which were found absent after polyamide column chromatography. The dried mass was then crystallized from MeOH-acetone (5:1).

The quantitative estimation of ecdysteroids in biological material has long been undertaken by bioassay method [9]. Since it is very cumbersome and lengthy process, various spectrophotometric methods such as GLC, MS, HPLC, UV, NMR and TLC have been used for identification and quantitation of ecdysteroids [10-14].

In the present studies, the quantitative estimation of 20-hydroxyecdysone was carried out according to the method

used by Hardman *et al.* [7] where a standard curve was prepared by plotting different concentrations (10-100 µg/ml) of pure 20-hydroxyecdysone against their UV absorption at 254 nm. The content of 20-hydroxyecdysone in each plant was read off from the calibration curve which was found to be 0.03% in the roots of *Aerva tomentosa* and 0.3% in the aerial parts of *Pandiaka involucrata.* The latter has been found as one of the best sources for the presence of 20-hydroxyecdysone in plants.

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