October-December 2005 Volume 1 Issue 1-2

Trade Science Inc.

Natural Products

An Indian Journal

Short Communication

NPAIJ, 1(1-2), 2005 [1-4]

Isolation Of 20 -Hydroxyecdysone From Indian Medicinal Plant *Achyranthes Aspera* And Development Of Simple HPLC Analysis

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Received: 16th October, 2005
Accepted: 2nd December, 2005

Web Publication Date : 21st January, 2006

ABSTRACT

Develop a simple procedure for extraction and isolation of 20-hydroxyecdysone from *Achyranthes aspera* and characterized by DSC, UV, IR, CD, ^{1}H and ^{13}C NMR, MS and quantified by HPLC.

KEYWORDS

Achyranthes aspera;
HPLC analysis;
Circular dichorism(CD);
20-hydroxyecdysone.

INTRODUCTION

Ecdysteroids are hormones controlling cell proliferation, growth and the developmental cycles of insects and other invertebrates. They are occasionally present in various unrelated plants for no apparent reason; no phytohormonal function has yet been identified. In certain cases, ecdysteroids are accumulated to high levels in leaves, roots or seeds. Some ecdysteroid containing plants have been known as medicinal plants for centuries. It has been clearly established that most of the common phytoecdyste roids with 20-hydroxyecdysone or ecdystrone like active affect insect growth and development on ingestion[1]. There are many types of ecdysteroids in plants, the most common being 20-hydroxyecdysone (20E), the amount of which varies among plant species[2,3]. *Achyranthes aspera* (syn: snake's tail, aghada or devil's horsewhip etc) has a considerable importance in indigenous system of medicine. It has commonly used in dropsy, pile boils and colic in children, cure of cough, astringent, paste applied to clear opacity of cornea, wounds as haemostatic; reported useful in cancer[4]. Presences of ecdysterone and oleanolic acid are common in this plant[5].

The yield range of phytoecdysteroids are varies from plant species to species and isolation procedures are quite tedious and several steps involve from extraction, purification to characterization[6]. The reported gravimetric yield not reflects the actual lev-

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els at which these compound occur[7,8]. So, we have simplified the extraction process and isolate and characterized the compound and later quantify the amount present in plant through HPLC.

EXPERIMENTAL

Achyranthes aspera plant material were collected from Bombay local market and identified by the plant botanist. The voucher specimen is preserved in our laboratory (MNPRL), UICT.

Extraction and Isolation

We have followed the basic principal of extraction:

1. Crushing, milling and extraction of the plant sample and filtration of the extract.
2. Concentration, solvent-solvent partition.
3. Solid phase extraction

The plant material, dried root and stem (500g) was defatted with pet ether and exhaustively extracted with methanol by soxhlet apparatus, the methanol extract concentrate to $1/4^{th}$ then partitioned with n-hexane, chloroform and butanol, each fraction subjected to HPLC analysis to find out where the quantity of 20 hydroxyecdysone is more. We have used chloroform extract for further purification, steps involved repeated column chromatography with silica gel (#60-120) and fractions are eluted with stepwise gradient: chloroform-ethanol and ethyl acetate-ethanol. Highly purified fractions pool together and further chromatographic process performed with silica gel (#100-200) and sepadex LH-20 to obtain maximum purer form followed by crystallization from methanol-ethyl acetate mixture (1:2). A cluster of transparent orthorhombic crystal of 20-hydroxyecdysone was deposited in the residual solution after keeping overnight at low temperature.

Characterization of the isolated compound

Melting point is not match with the literature (242^o), found 246^o, through Differential Scanning Calorimetric (DSC) 249^o, IR spectra were determined with Perkin-Elmer 1760-X infrared Fourier transforms spectrometer with an ordinate scale for the region 4000-450 cm^{-1}.

IR (KBr) cm^{-1}:3392, 2969 (OH), strong absorption at 1651(enone), UV spectra were recorded on JascoV-530: spectra were taken through spectra manager for window 95/NT, version 1.50.00(Build2): λ_{max} (MeOH) 242nm. A circular Dicrorisom (CD) spectrum was recorded on Jasco J-810 spectrometer 0-400 n points. ^1H and ^{13}NMR spectra were recorded on Bruker AV 500MHZ NMR spectrometer with CD_3OD as solvent. ^{1}HNMR was measured with TMS as an internal standard. MS fragmentations were recorded by: Quadrapol-TOF micro spectrometer: Micromass (YA-05) instrument.

Quantifications by HPLC

A M/s Jasco HPLC system with Rheodyne 7725 loop injector, jasco PU-1580 intelligent pump, jasco UV-1575 intelligent detector, jasco MX 2080-31 solvent mixing module and Borwin software version 1.50 was used. Lichrospher 100RP-18e (250x4.6, 5μ) column, mobile phase (25:75) Acetonitrile: water (0.01%TFA), flow rate-1ml/min followed the isocratic elution at wavelength 246 nm. Peak was assigned by spiking with authentic sample. The concentration determined from a calibration curve, which was in conformity with Lambert-Beer's Law

Standard and sample preparation

1mg/ml solution of standard ecdystrone was prepared using methanol, fixed the concentration range 1,2,3,4,5 μg /ml and for sample preparation; defatted the plant material with pet ether in hot condition (soxhlet) and then continue it with hot methanol until the extraction was completed. The clear filtered solution was preferred for injection.

RESULTS AND DISCUSSION

CD spectra of 20-hydroxyecdysone have positive cotton effect (CE) in the $\pi \rightarrow \pi^*$ transition. Molar ellipticity ([θ]) of 20-hydroxyecdysone is 12.5837 at 329.5nm. Negative CE can be observed at 249 nm corresponding to $n \rightarrow \pi^*$ in the UV spectrum [θ]: (-) 30.7307. Positive CE can be seen at 218 nm, which has no corresponding UV maximum (θ): 20.0922
^{1}HNMR δ ppm: 3.831(2-H, m, J=1.5,5 Hz), 3.945(3-H, br s), 2.387(5-H, 17-H, dd, J=8.5,12.5,), 5.803(7-

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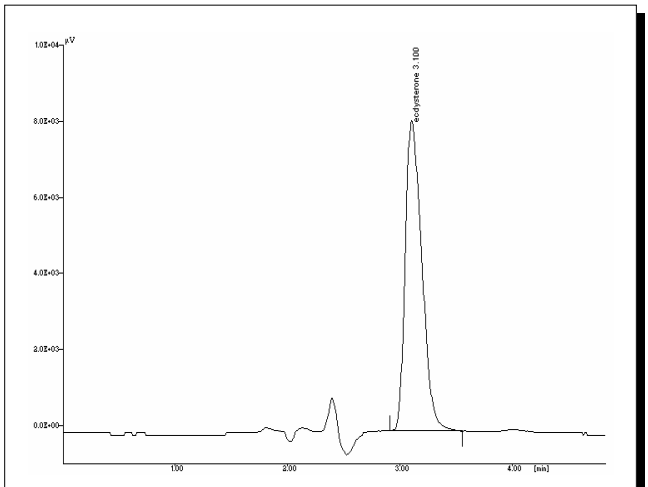
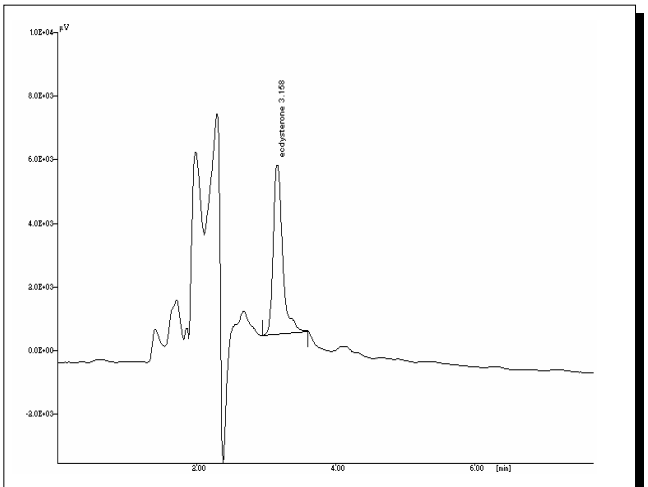
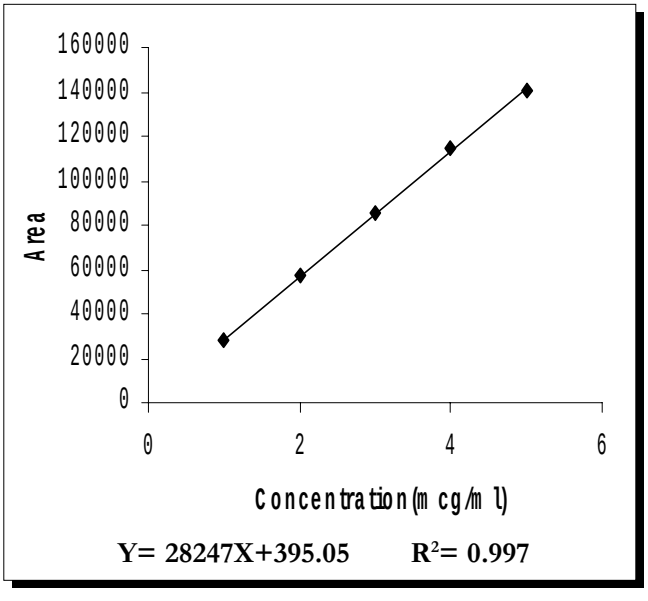


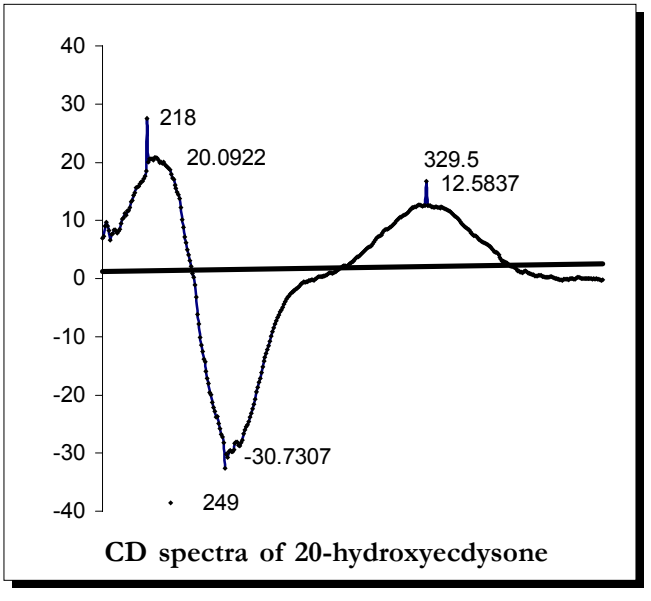
Standard chromatogram of ecdysrone or 20-hydroxyecdysone



Spike: *Achyranthus aspera* and 20 hydroxyecdys one



Y= 28247X+395.05 R^2= 0.997



CD spectra of 20-hydroxyecdysone

H), 3.148(9-H, t, J=8.5,), 2.129(12-H, ddd, J=2.5, 10.5, 15,), 0.886(18-H_3, s), 0.962 (19-H_3, s), 1.186(21-H_3, s), 3.322(22-H, d, J=5.5,), 1.194(26-H_3, s), 1.199(27-H_3, s)

^{13}CNMR: 37.84(C-1), 69.16(C-2), 68.97(C-3), 32.97 (C-4), 50.99(C-5), 206.90(C-6), 122.60(C-7), 168.43 (C-8), 35.55(C-9), 39.73(C-10), 21.53(C-11), 32.25 (C-12), 48.97(C-13), 85.69(C-14), 30.18(C-15), 21.53(C-16), 50.99(C-17). 18.53(C-18), 24.89(C-19), 78.37(C-20), 21.53(C-21), 78.37(C-22), 42.85(C-24), 71.76(C-25), 29.45(C-26), 29.45(C-27).

MS m/z, 481 (M+1), 463(M+1-18), 445(M+1-2x18), 427(M+1-3x18), 409(M+1-4x18), 303,

Different concentration of standards and sample solutions were injected which was in compliance with Beer's- Lambert's law and followed the guideline for limit of detection (LOD), limit of quantification (LOQ), The equation for 20 hydroxyecdysone was Y= 28247x +395.050 with a correlation coefficient (R^2) = 0.9997 and 96% recovery studies respectively. Relative standard deviation (0.25 and 0.23) respectively for intra day and inter day variation is in acceptable range. The presence of 20-hydroxyecdysone in plant is 0.18 % on dry weight basis. The proposed HPLC analysis that is precise, simple, and less time consuming method of analysis.

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