Large Scale Crystallisation of Aescin

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Summary: A two step process for the crystallisation of β-aescin from Aesculus indica seeds has been discussed.

Introduction

Aescin an oedema mobilising anti-inflammatory principal of natural origin has been the subject of many investigations. It is a mixture of triterpenoid glycosides, which occurs in the Genus Aesculus. Different methods for the extraction of aescin are reported in the literature [1,2] however the information available is mainly confined to patent literature. To our knowledge bulk scale crystallisation of aescin from Aesculus indica has not been attempted previously. The present paper describes an economically viable procedure which involves removal of coprecipitated impurities, followed by crystallisation from an ethanol water mixture.

Results and Discussion

Commercial scale crystallisation of natural products often presents several problems. When it appears that a plant derived product may contain several adhering constituents, the choice of obtaining the major constituent by crystallisation becomes very significant. Potentiometric assay of commercial aescin showed presence of 95% free acid, estimated as aescin whereas under similar conditions crude aescin yielded 80% free acid and the remaining portion was treated as impurities. HPLC spectra of crude aescin (Fig. 1) showed several peaks due to the impurities. Peak at 210 nm was due to the aescin. For selective crystallisation of aescin mild experimental conditions were chosen

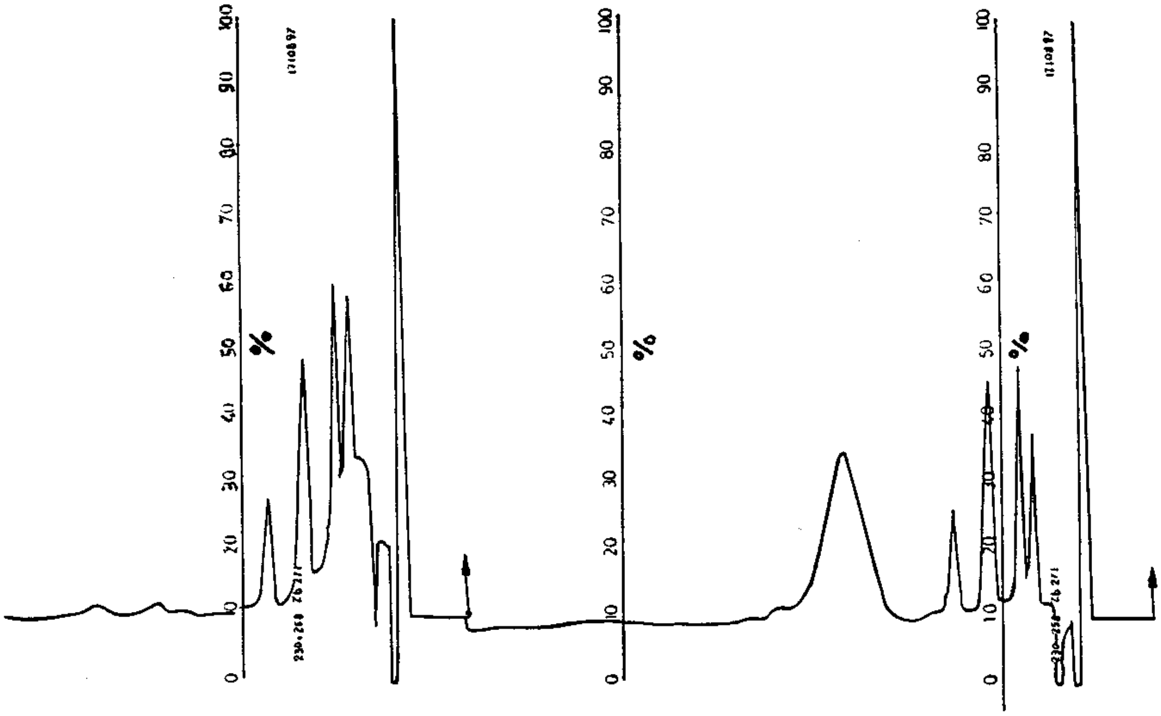

Fig. 1: HPLC Separation of the Components from Crude Aescin.

that prevented the decomposition of the adhering impurities such as starch and non glycosidic components.Initial separation of these macro impurities was achieved by solubilisation and sedimentation. It was thought that if particles of varying size were allowed to suspend in alcohol, then the force of gravity would appear to be the decisive factor for their sedimentation. As a result the large and denser molecules quickly settled on the bottom and were removed by filtration. Microfine particles appeared completely dispersed in the solvent phase. Lowering the temperature to 10° coagulated and facilitated their removal from the solvent phase. With the removal of adhering impurities crystallisation of aescin appeared very simple. Potentiometric assay of crystalline aescin showed presence of 97% free acid as aescin. The product proved as a single entity on HPLC examination. A single peak at 210 nm (Fig. 2) was a clear evidence of the purity of the crystalline material. Negative fast atom bombardment mass spectra (Fig. 3) of crystalline aescin was similar to reported data [3] obtained with aescin extracted from Aesculus hippocastanum. Compared to patent procedure [1,2,4] for the crystallisation of aescin, the method described is simple, efficient and economically feasible.

Experimental

Aescin was extracted from the seeds of Aesculus indica by the method of Khan *et al.*, [5]. Crude aescin (1 Kg) was dissolved in absolute alcohol (1500 ml) with stirring and shaking. The solution was filtered, the filtrate stoppered and cooled to 10°C in an ice cold water bath containing crushed ice. The turbid solution was carefully filtered and to the clear filtrate, water was added in small portions till evidence of turbidity reappeared. At this stage the mixture was agitated, heated to 40-45°C for 20 min and then allowed to stay at room temperature to allow the crystallizing out of

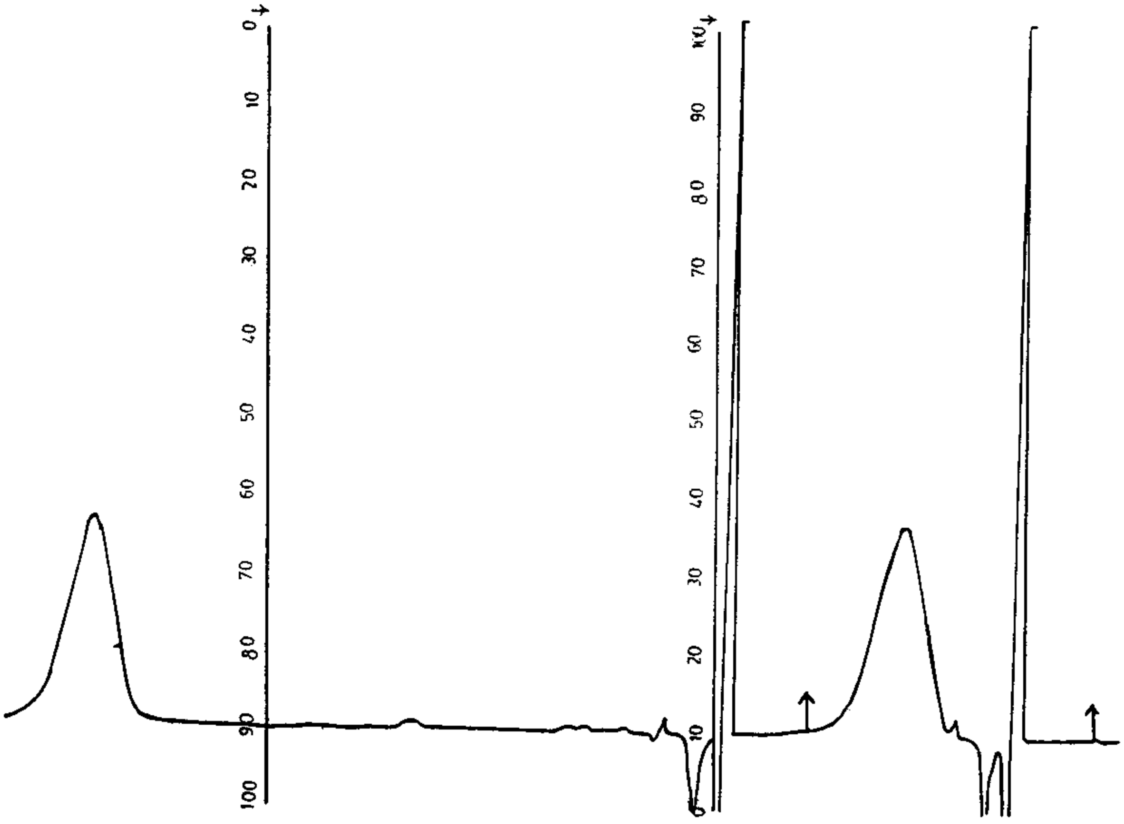

Fig. 2: HPLC of Crystalline Aescin

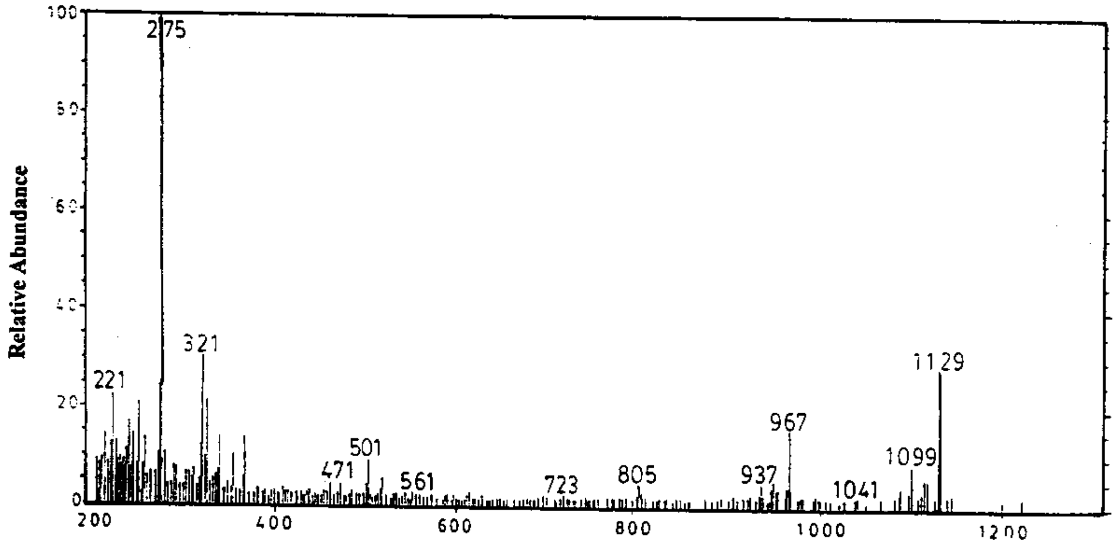

Fig. 3: (-) FAB-MS of Crystalline Aescin.

the aescin. The crystals were filtered dried under vaccum. The final yield was 900 gm.

Potentiometric assay

Potentiometric assay of commercial aescin obtained from Inverni della Beffa Italy; crude and crystalline aescins, was performed using Shott Gerate automatic piston burette. The sample 0.8 g were suspended in 40 ml water and titrated against 0.1 N NaOH [6].

High performance liquid chromatography

HPLC of crude and crystalline aescins was conducted according to the method of Wagner *et al.*, [7]. The equipment used was, LC-6A Schimadzu attached with a Z-Module Radial compression RPC-18 column and SPD-6 AV, UV-Vis wave length detector. The wave length detector was set at 210 nm.

Mass spectrometry

Mass pectra of crystalline aescin was obtained at the HEJ Research Institute of Chemistry, University of Karachi, using JMS-HX 110 Jeol instrument.

Acknowledgement

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