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Preparation of Pharmaceutical Grade Sodium Aescinate and Aescin Polysulphate Salt from *Aesculus indica* Seeds

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Efficient methods for the preparation of pharmaceutical grade aescin, sodium aescinate and aescin polysulphate sodium salt from the carefully dried seeds of *Aesculus indica* Wallex Camb, HkF (Hippocastanaceae) have been described. The identity and purity of products was achieved through comparison with standards using potentiometric assay, HPLC, UV, IR, NMR spectroscopy and mass spectrometry.

Key words: *Aesculus indica*, β Aescin, Sodium aescinate, Aescin polysulphate salt.

Introduction

Aescin, an oedema mobilising anti-inflammatory principle of natural origin, has been the subject of many investigations. It is a mixture of triterpenoid glycosides, occuring in the genus *Aesculus* (Wullf and Tschesche 1969). Different methods for preparation of aescin and its polysulphate salt have been reported in literature (Madaus 1977; Rucman and Jankovic 1978 a) however, the information available is mainly confined to patent literature. So far we know, the preparation of pharmaceutical grade sodium aescinate and aescin polysulphate sodium salt from *Aesculus indica* Wallex Camb, HkF (Hippocastanaceae) has not been attempted previously. The present paper describes economically viable procedures for preparation of these pharmaceuticals from the indigenous raw materials.

Materials and Methods

Purification of β-aescin. Crude aescin was extracted and precipitated from the seeds of *Aesculus indica* using water-alcohol mixture . Fresh seeds (1 kg) were peeled, pulverized and carefully dried at 50-60°C in an electric dryer. Dried seeds (600 g) were powdered and extracted with (4 litres) 5:5 EtOH - H_2O mixture for 6 h (Khan *et al* 1995).

The crude aescin 0.8 g was suspended in 40 ml water and titrated against 0.1N NaOH using potentiometer (Bonati 1980). After estimation of aescin content, the crude compound was then evaluated by qualitative methods (Turner 1986) for starch, proteins, mucilage, sugars and coloring principles. Afterwards crude aescin 1 kg was suspended in 1500 ml alcohol, the mixture was heated at 70 °C for 30 min in a reflux condenser, the solution cooled to 10 °C, the clear extract decanted and amorphous residue discarded. Concentration and drying of clarified extract yielded 880 g anhydrous purified aescin. Potentiometric assay and qualitative evaluation of purified aescin was performed and the product was dissolved in 1200 ml anhydrous ethanol with stirring. The solution was cooled to 5-7 °C and the suspended matter was removed by filtration. The filtrate was treated with one litre water in small portions with intensive stirring till the alcohol water mixture appeared turbid. At this stage the turbid solution was heated at 60-65 °C for 20 min and the clear liquid was allowed to stand at room temperature for crystallizing out of β-aescin. The final yield after three successive crystallizations from the mother liquor was 810 g (81%).

High performance liquid chromatography of crude and crystalline aescin was conducted according to the method of Wagner *et al* (1985). The instrument used was LC - 6A Schimadzu linked 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. The UV spectra in methanol were recorded on a varian DMS-200 instrument and showed absorption maxima at 210 nm. Infrared spectra were obtained from KBr discs using a Pye Unicam SP3-100 spectrophotometer. The compound showed absorptions at 3500, 2875, 1720, 1380, 1260, 1075 and 1040 cm^{-1}. ^1H,^{13}C NMR spectra were recorded with the help of Bruker 400 MHz spectrophotometer using tetramethylsilane (TMS) as internal standard. Deuterated methanol was used as solvent. ^1H NMR spectrum exhibited signals at δ 0.84 (s,C-26[H_3]), 0.86 (s,C-24[H_3]), 0.89(s,C-29[H_3]), 0.92(s, C-30[H_3]), 1.07 (s,C-25[H_3]), 1.21 (s, C-27[H_3]), 1.48 (s, C-23[H_3]), 1.80 (s,C-2[H_3]), 1.85 (t,C-3 [H_3] tigloyl), 1.92(s, C-2 [H_3] angeloyl), 1.95 (s, C-22 [H_3]), 1.98 (d, C-3 [H_3]), 3.23(m, α-H), 3.36 (d, C-21 [H_2]), 4.01 (s, C-28 [H_2]), 4.68 (s, C-16 [β-H]), 5.36 (s, C-12 [H]), 5.46 (d, C-22 [β-

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H]), 5.48 (d, C-21 [α-H]), 6.08-6.11 (qq, C-3 [H] angeloyl), 6.81 -6.83 (qq, C-3 [H] tigloyl). Mass spectra was obtained using JMS-Hx 110 Jeol Spectrometer.

Preparation of sodium aescinate. Crystalline aescin (15g) was added to water (100 ml), the suspension was treated with aqueous NaOH (0.1N) until pH 6.9 was attained. The solution was filtered, concentrated and freeze dried. The yield was 14 g (93 %), mp 250-252°.

Preparation of aescin polysulphate salt. Freshly distilled (100 ml) pyridine was placed in one litre three-neck round bottom flask contained in an ice bath and provided with a reflux condenser, mercury sealed mechanical stirrer and dropping funnel. Whilst stirring chlorosulfonic acid (30 ml) was gradually added with cooling. After mixing, dried crystalline (10 g) β-aescin dissolved in (15 ml) pyridine was added and the contents heated at 70°C for 5 min. The reaction mixture was poured into (500 ml) absolute ethanol, stirred and cooled. The syrupy product layered at the bottom was separated and the supernatant liquid was decanted. After washing with ethanol, the product was dissolved in (30 ml) water and pH adjusted to 7.2 with 2N sodium hydroxide. The solution was filtered, concentrated and the salt was recovered from ethanol.The yield was 4.4 g. The product contained 9 % bound sulfur. Mass spectra of the polysulphate salt was obtained.

Results and Discussion

Crystallisation of saponin glycosides from crude products is often achieved through multistage partition and purification techniques (Meilroy 1951). During extraction, neutral solvents are often used because saponin glycosides tend to hydrolyse under acidic conditions. For precipitation of non-glycosidic constituents from the residual extract, lead acetate is commonly used. After precipitation of impurities, excess lead is removed from the clarified extract by H_2S. Removal of non glycosidic constituents from saponin glycosides can also be accomplished by partitioning between immiscible solvents such as butanol and water (Rucman and Jankovic 1978 b). In the present work, potentiometric assay of crude aescin showed presence of 80% free acid estimated as aescin; the remaining portion was treated as non-glycosidic constituent. Qualitative tests on non-glycosidic components showed presence of starch, carbohydrates, proteins and coloring principles. These glycosidic and the non-glycosidic constituents were found to be completely dispersed in alcoholic phase at room temperature and were not separable by simple sedimentation. Therefore, the alcoholic solution was heated to denature proteins, coagulate colouring principles and precipitate gelatinised starch particles. As a result of heating, the large and denser non glycosidic constituents adsorbed on the surface of aescin

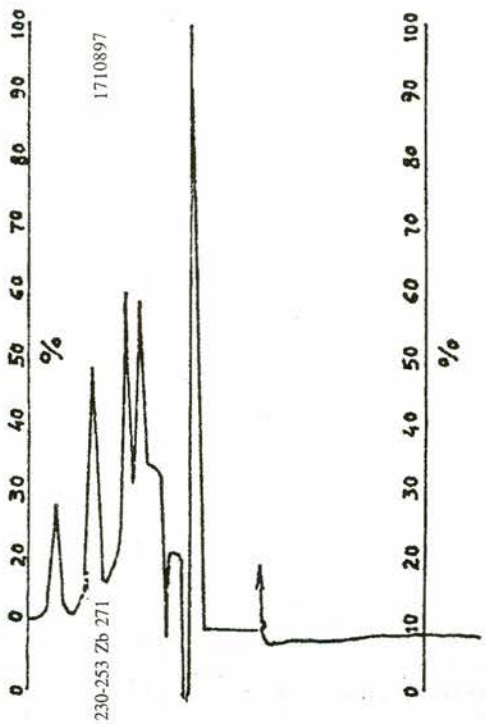

Fig 1. HPLC separation of the components from crude aescin.

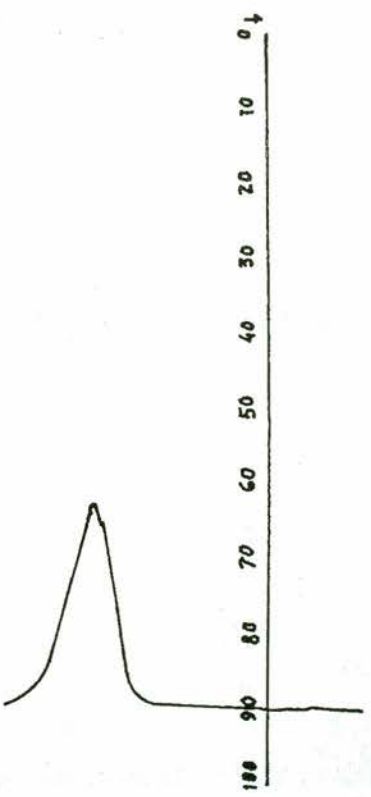

Fig 2. HPLC of crystalline aescin.

molecules, were released into the solvent phase as insoluble residue and separated by filtration. The purified aescin on potentiometric assay showed presence of 92% free acid. The result suggested that complete removal of foreign matter was not achieved during the previous purification attempt. Further evaluation of purified aescin showed continued presence of microfine starch. As starch grains absorb water, swell and gelatinise to form mucilage, therefore, for its removal from the purified aescin, anhydrous ethanol was used. Cooling the solution to 10 °C facilitated coagulation from the solvent phase. With the removal of adhering non glycosidic impurities, crystallisation of aescin occurred easily. The crystals obtained showed presence of 97% free acid. It can be concluded from the results that the mild experimental conditions chosen for purification and crystallisation prevented the far reaching decomposition of non-glycosidic constituents, sugar moiety and glycosidic linkage. As compared to multistage extraction purification procedures (Rucman and Jankovic 1978 a,b; Voticky *et al* 1986; Proksa *et al* 1987) the method described is simple efficient and economically viable for commercial preparation of pharmaceutical grade β-aescin. The crystalline aescin has HPLC, (Fig 1, 2) IR, (Fig 3) UV (Fig 4), ^{1}H, ^{13}C NMR and MS (Fig 5) characteristics identical to pure aescin (Wullf and Tschesche 1969). Transformation of the crystalline aescin into sodium salt appeared straight forward and the yield was close to the estimated value.

Partially derivatised glycoside polysulfates are usually prepared under anhydrous conditions using sulfating agents and electron donating solvents. For sulfation of the sugar moieties, chlorosulphonic acid ,thionylchloride, sulfuric acid and pyridine sulfur trioxide complex have been reported.(Whistler *et al* 1963) Due to the complex structure of aescin molecule and vigorous nature of sulfating agents, direct sulfation procedures were not attempted.Sulfation was achieved under anhydrous conditions in the presence of excess sulfur triox-

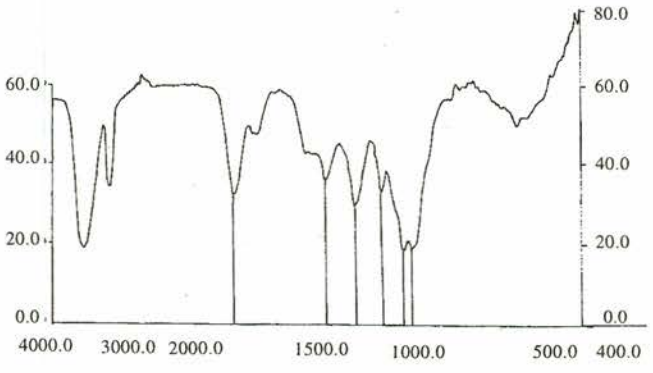

Fig 3. IR-spectra of crystalline aescin.

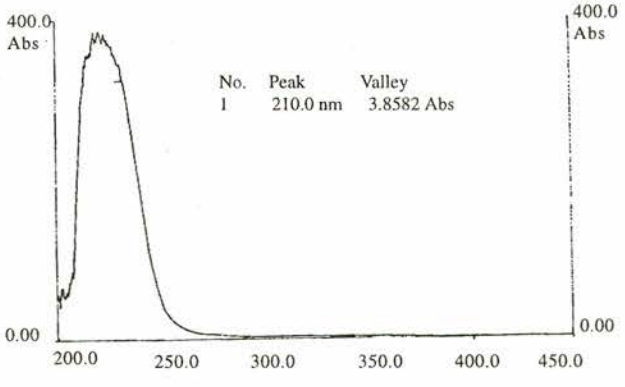

Fig 4. UV spectra of crystalline aescin.

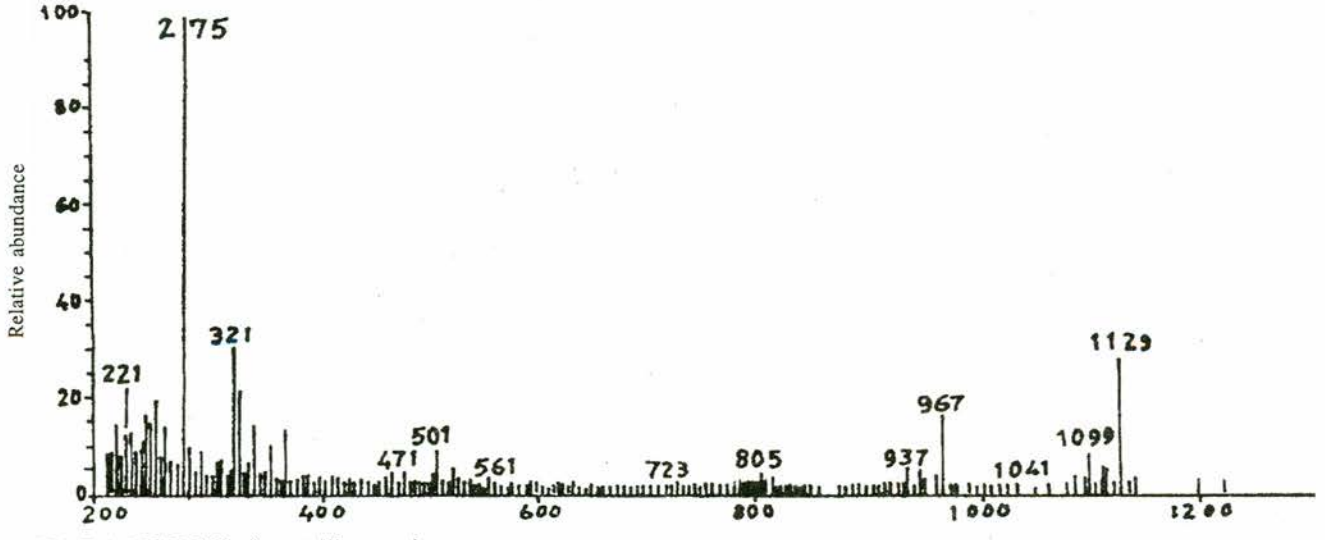

Fig 5. (--) FAB-MS of crystalline aescin.

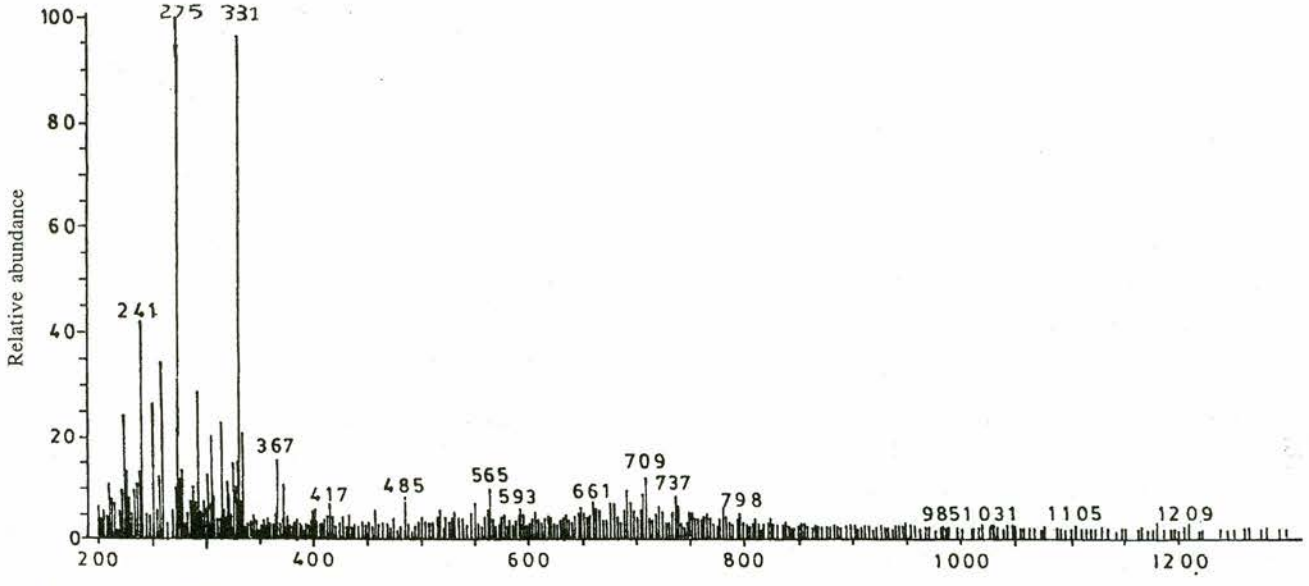

Fig 6. (--) FAB-MS of Aescin polysulphate.

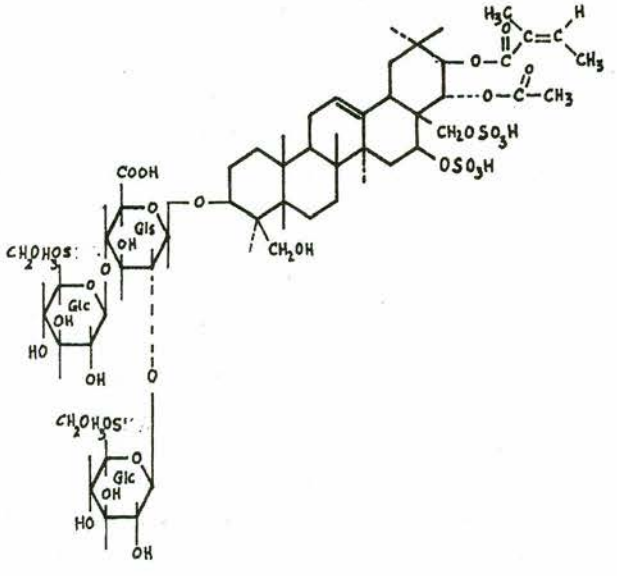

Fig 7. Structure of aescin polysuphate.

ide complex and pyridine. The reaction mixture did not appear too acidic and therefore decomposition of aescin molecule and subsequent formation of mixture of products were not observed. On account of mild sulfating conditions, separation of the crude aescin polysulfate from the reaction mixture was achieved using anhydrous ethanol. The by-products and intermediates formed during the reaction were retained in the alcoholic solution. Negative fast atom bombardment mass spectral breakdown pattern (Fig 6) of the polysulphate (Fig 7) with m/e 241, 485, 565 and 661 strongly suggested binding of sulphate moieties.

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