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High-Performance Liquid Chromatographic Determination of Steroidal Saponins, Aculeatisides A and B, in *Solanum aculeatissimum* JACQ.

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A convenient high-performance liquid chromatographic method has been developed for the determination of steroidal glycosides, named aculeatisides A and B, in *Solanum aculeatissimum* JACQ. (Solanaceae). Under the standard conditions, the calibration curves for both glycosides are linear up to 12 μg per injection (50 μl). The proposed method is simple and useful for the routine assay of aculeatisides in the plant.

Keywords——steroidal glycoside; aculeatiside; HPLC; assay; *Solanum aculeatissimum*

Solanum aculeatissimum (Solanaceae) has been used for the treatments of bronchitis and rheumatism in China. Recently, steroidal glycosides, named aculetisides A and B (Fig. 1), have been isolated from the roots of *S. aculeatissimum* and their chemical structures elucidated by Tomimatsu *et al.*[1]

The aglycone parts of these glycosides, nuatigenin and isonuatigenin, are known to be chemically convertible to 16-dehydropregnenolone.[2] This means that aculeatisides A and B are useful sources of pregnane as well as diosgenin, which is mainly produced from *Dioscorea composita* (barbasco).[3]

Several high-performance liquid chromatographic (HPLC) separations and estimations

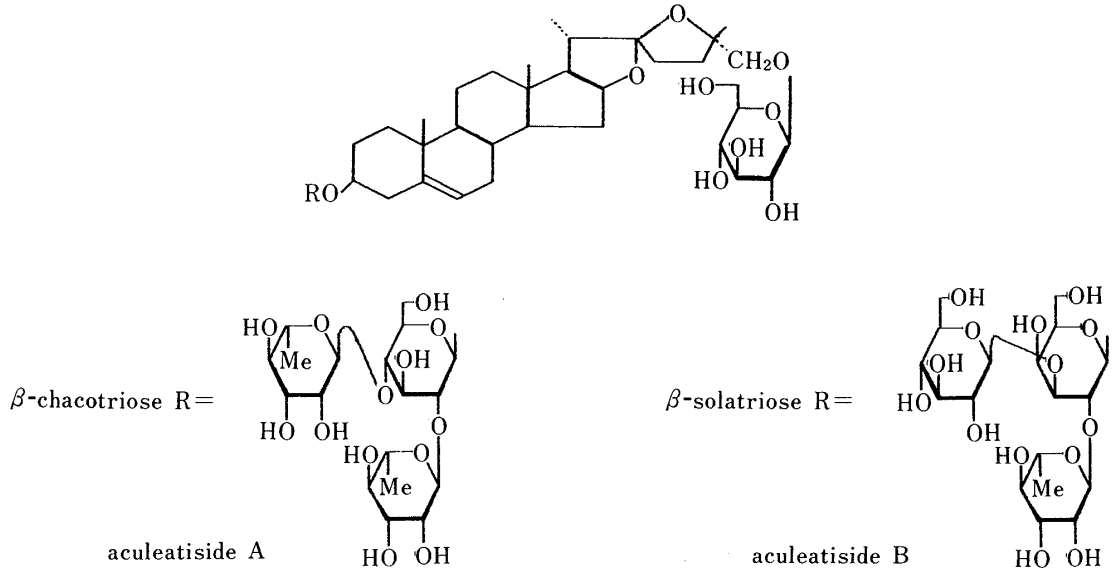

Fig. 1. Structures of Aculeatisides A and B

of saponins have been reported.[4] However, no HPLC method for the determination of aculeatisides A and B has been reported to our knowledge. In this paper, we describe a simple and convenient HPLC determination method for aculeatisides and its application for the assay of those in the roots of *S. aculeatissimum*.

Experimental

Apparatus——A Shimadzu LC-6A liquid chromatography pump was used with a Rheodyne model 17125 loop injector (50 μl) and a Shimadzu SPD-6A spectrophotometric detector. A Shimpack CLC-C8 column (150 × 6 mm i.d.) was used.

Chemicals and Materials——Aculetiasides A and B were kindly provided by Teikoku Seiyaku Co., Ltd, Kagawa, Japan. Diphenylcarbazone was purchased from Wako Pure Chemicals (Osaka, Japan) and used as an internal standard. Water was deionized and distilled. *Solanum aculeatissimum* was cultivated in fields at various locations. The plant was harvested in primal November and the next November after seeding in March.

Preparation of Samples——The root of *S. aculeatissimum* was washed with water and dried at 40 °C for 48 h. Then it was powdered mechanically and dried again at 40 °C for 2 h. One gram of the obtained root powder weighed and extracted with 100 ml of methanol at 40 °C for 2 h. After filtration, the residue was extracted once again in the same manner. The whole filtrate was evaporated to less than 10 ml, transferred into a 10 ml volumetric flask, and diluted with methanol to the mark. After filtration on a Millipore filter (0.22 μm), the filtrate was used as a sample for HPLC.

Chromatographic Conditions——For the separation of aculeatisides, 30% acetonitrile aqueous solution was used. The flow rate was set at 1.0 ml/min. The peaks of the analytes were monitored at 210 nm.

Assay Procedure——A known concentration of diphenylcarbazone in methanol (0.1 ml) was added to a sample solution (0.4 ml). After mixing, a portion of the mixture was subjected to HPLC.

Calibration graphs were prepared with standard methanol solutions of aculeatisides A and B. The peak height ratios of each aculeatiside and diphenylcarbazone were plotted against the concentration of aculeatisides.

Results and Discussion

In the preliminary experiments, several factors affecting the retention and separation of aculeatisides A and B were examined by reversed-phase HPLC. These steroidal glycosides show an absorption band from 195 to 230 nm, but have no absorption maximum in 30%

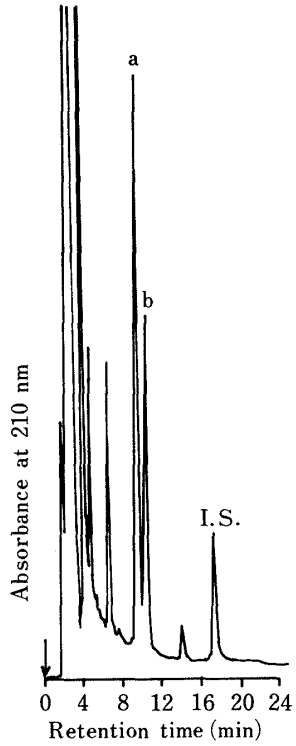

Fig. 2. Chromatogram of the Methanol Extractant of the Root of *S. aculeatissimum*

a, aculeatiside B; b, aculeatiside A; I.S., internal standard (diphenylcarbazone).

TABLE I. Content of Aculeatisides in *Solanum aculeatissimum* JACQ.

Place	Age (year)	Root dry wt. (g)	Content of aculeatiside (%)[a] A	B
a	1	62.3±12.7	2.61±0.15	3.56±0.23
	2	86.0±10.0	2.64±0.54	3.47±0.92
b	1	70.9±11.1	2.55±0.23	3.57±0.26
	2	115.1±28.1	2.70±0.48	3.55±0.35

a) Mean±S.E. ($n=5$).

acetonitrile aqueous solution. Therefore, the wavelength of 210 nm was selected for the detection. Among the eluates examined, 30% acetonitrile showed a favorable separation of aculeatisides A and B, and diphenylcarbazone. As shown in Fig. 1, the structures of aculeatisides A and B are the same in the aglycone parts and slightly different in the sugar parts. Therefore, the water content in the eluate affected the separation and retention of these saponins on the chromatogram. A typical chromatogram of the methanol extractant of the root is showed in Fig. 2. To confirm the purity of the peak on the chromatogram derived from each glycoside, a three-dimensional chromatogram was measured by the use of a photodiode-array detector (Shimadzu SPD-M6A). The values of purity were 99.98% for the peak of aculeatiside A and 99.99% for the peak of aculeatiside B.

The calibration plots obtained with standard aculeatisides were linear over the range of 1—12 μg/injection. The correlation coefficients for aculeatisides A and B were 0.998 and 0.999, respectively.

The free sterols and steroidal ketones in plant tissue cultures of *S. aculeatissimum* have been determined by gas chromatography and gas chromatography/mass spectrometry.[5] However, the HPLC determination of aculeatisides has not been reported. Therefore the proposed method was applied to the assay of aculeatisides in the root of *S. aculeatissium.* Table I shows the saponin content in roots of different ages and from two places. It was found that the contents of aculeatisides A and B were about 2.5% and 3.5%, respectively. The saponin content of the roots examined showed no difference with age or place of cultivation, though the growth of the roots was different. The proposed method is simple and convenient for the routine assay of aculeatisides in plants. Further applications of the method will be reported elsewhere.

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