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PURIFICATION OF SAIKOSAPONINS a, c AND d

APPLICATION OF LARGE-SCALE REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The preparative separation of saikosaponins a, c, and d from *Bupleurum falcatum* was performed by high-performance liquid chromatography on a 100 × 11 cm I.D. axially compressed column, packed with 20-μm ODS silica gel using a step gradient with mixtures of acetonitrile and water as the mobile phase. Gram quantities of pure compounds were obtained in a single run.

INTRODUCTION

Bupleuri Radix (root of *Bupleurum* spp., Umbelliferae, Japanese name: Saiko) is a well known and important crude drug in oriental medicine, which has anti-inflammatory and hepatobiliary activity. From this crude drug, three major oleanane saponins, saikosaponins a, c, and d (Fig. 1) and many other minor saponins have been isolated[1,2] by normal-phase column chromatography. This paper describes the preparative separation of the three major saponins from *B. falcatum* by reversed-phase high-performance liquid chromatography (HPLC) on an axially compressed column of large diameter.

EXPERIMENTAL

Apparatus

The apparatus for analytical HPLC was a LC-6A liquid chromatograph (Shimadzu, Tokyo, Japan), equipped with a SPD-6A UV detector (Shimadzu) and a computing integrator, Chromatopac C-R3A (Shimadzu). Analyses were carried out on an ODS silica gel column, YMC-Pack A-312 (150 × 6 mm I.D., YMC, Kyoto, Japan) with mixtures of acetonitrile and water at a flow-rate of 1 ml min^{-1} and a detection wavelength of 210 nm.

Preparative chromatography was carried out with a Kurita-LC 110 system (Kurita, Tokyo, Japan). The 100 × 11 cm I.D. column was packed with 5 kg of ODS silica gel (average particle size 20 μm, YMC). After axial compression at 80

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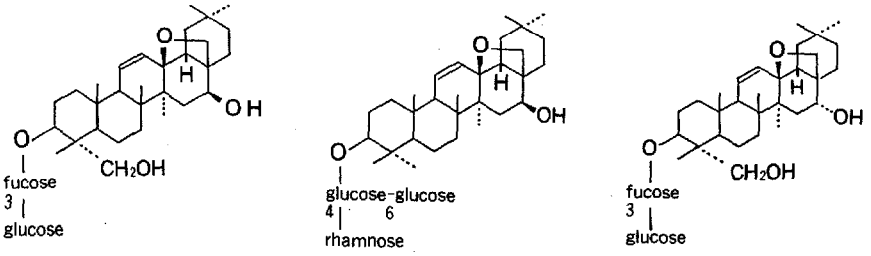

Fig. 1. Structures of the three major saponins in *B. falcatum*.

kg/cm^2, the dimensions of the adsorbent bed were 92 × 11 cm I.D. The column temperature was 22°C. The eluent inlet pressure was about 60–70 kg/cm^2, which gave a flow-rate of *ca.* 210 ml min^{-1}.

Sample preparation from the crude drug

Roots of the cultivated *B. falcatum* (517 g) were extracted with methanol containing 2% sodium hydroxide[3]. The extract was diluted with water and then extracted with diethyl ether. The aqueous layer was extracted with 1-butanol, and the extract was evaporated to dryness. The residue was dissolved in water and chromatographed on an adsorbent bed of 13 × 7 cm I.D. containing a highly porous polymer matrix (Diaion HP-20; Mitsubishi, Tokyo, Japan) with 20% methanol (3 l) followed by 85% methanol (3 l) as the eluents. The 85% methanol fractions was concentrated to give 10 g of a crude saponin mixture, which contained 3.4 g of saikosaponins a, c, and d.

RESULTS AND DISCUSSION

Analytical HPLC

In preliminary studies on preparative HPLC, various packing conditions, mobile phases, and sample capacities were established from data obtained on an analytical HPLC system. The sample capacity was examined with the objective to obtain a high recovery (>90%) and high purity (>98%) of each saikosaponin in a single run.

The capacity was 2 mg of the crude saponin mixture per g of ODS silica gel. Effective baseline separation of the three major components (saikosaponins a, c, and d) could be obtained at the maximum load using a step-gradient programme with three mixtures of acetonitrile and water. The mobile phase compositions were 27% acetonitrile for saikosaponin c, 30% acetonitrile for saikosaponin a, and 35% acetonitrile for saikosaponin d.

Preparative HPLC

An attempt to isolate each of the saikosaponins by preparative HPLC was carried out using the solvent system described for analytical HPLC. The crude saponin was dissolved in an eluent consisting of acetonitrile and water (27:73), at a concentration of 10 g/200 ml and was injected into the HPLC by means of a pump. The chromatogram obtained by preparative HPLC is shown in Fig. 2. Elution with

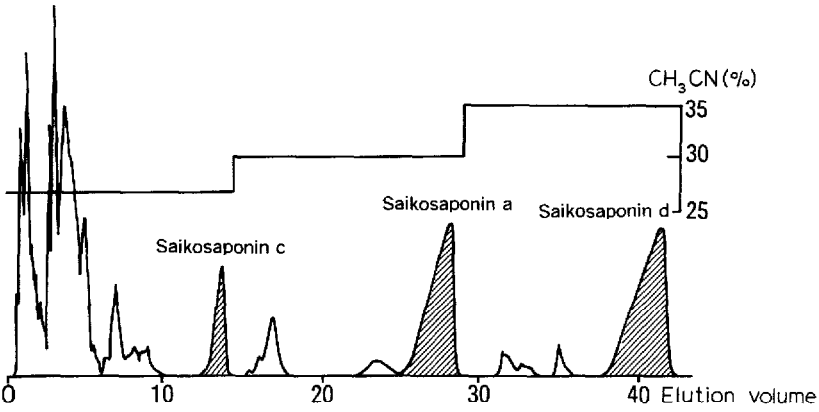

Fig. 2. Elution profile of the crude saponin fraction (10 g) extracted from *B. falcatum* root under preparative conditions. Column: Kurita-LC 110 (918 × 110 mm I.D.). Packing: ODS-silica gel (20 μm). Eluent: acetonitrile–water. Flow-rate: 210 ml/min. Detector: UV 210 nm. The elution volume is given in bed volumes (see *Apparatus*).

a three-step gradient yielded each of the pure compounds with a recovery of more than 90%. The purity of the fractions obtained by this method was tested by silica gel TLC (solvent system, ethyl acetate–ethanol–water, 18:2:1)[4] and by analytical HPLC. All three compounds (saikosaponin a, c, and d) gave a single spot on TLC. Analytical HPLC revealed that the impurities were less than 1%. From the crude saponin (10 g), 403 mg of saikosaponin c, 1210 mg of saikosaponin a and 1604 mg of saikosaponin d were obtained by preparative HPLC in a single run.

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