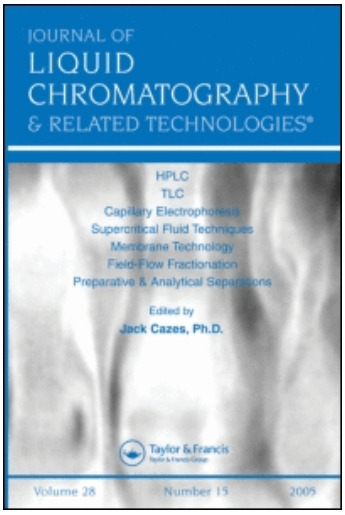

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APPLICATION OF CENTRIFUGAL PARTITION CHROMATOGRAPHY TO TANNINS AND RELATED POLYPHENOLS

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ABSTRACT

Centrifugal partition chromatography (CPC) has been applied to the separation and purification of bioactive polyphenols in extracts from an oriental crude drug, licorice, and also of oligomeric hydrolyzable tannins extracted from Heterocentron roseum. A tannin-like component, licochalcone B from Sinkiang licorice was separated from another component of related structure, licochalcone A, by normal-phase CPC using a solvent system, $CHCl_3$-MeOH-H_2O. A labile trimeric hydrolyzable tannin, nobotanin J which is readily hydrolyzed to a monomer and dimer(s) on a solid support upon gel-column chromatography, and a highly polar tetramer, nobotanin K were efficiently purified in the normal-phase and reversed-phase development, respectively.

INTRODUCTION

Recently found activities of tannins, such as antitumor,[1] antiperoxidation[2] and other activities,

which are largely dependent on the structure of each tannin, increased needs for isolation of each component in the tannin mixture obtained from plant, as tannins are frequently encoutered in our lives being contained in foods and drinks, and also in medicinal plants. As tannins are often present as mixtures of labile polyphenols of large molecule, which are easily adsorbed on various substances, column chromatography of tannins and related polyphenols using solid support often meets difficulties caused by adsorption, and sometimes by hydrolysis, on the solid support. We previously reported examples of preliminary fractionation of the tannin components by centrifugal partition chromatography (CPC) followed by further purification by CPC developed in reversed mode, or by gel-column chromatography.[3]

We now present results of application of CPC to polyphenols of other type, and also to purification of labile oligomeric hydrolyzable tannins which could not be purified by the column chromatography because of facile hydrolysis occurring during the development.

EXPERIMENTAL

Materials

Sinkiang licorice (licorice roots from Sinkiang region of China) was extracted with acetone-water (7:3). After filtration and concentration, the residual aqueous solution was extracted successively

with ether, ethyl acetate and n-butanol. The ether extract was subjected to CPC separation followed by column chromatography over silica gel developing with $CHCl_3$-MeOH (98:2).

The fresh leaves of Heterocentron roseum was homogenized in acetone-water (7:3) and filtered. The filtrate was concentrated in vacuo, and extracted with ether, ethyl acetate and n-butanol. The butanol extract was fractionated by column chromatography over Diaion HP-20 (Mitsubishi Chem. Ind.)($H_2O \rightarrow H_2O$-MeOH (8:2) $\rightarrow H_2O$-MeOH (6:4) $\rightarrow H_2O$-MeOH (4:6) $\rightarrow$ MeOH), and over Toyopearl HW-40 (coarse)(Toyosoda) using a solvent mixture, H_2O-MeOH-acetone as eluant. The fractions containing trimer [nobotanin J (**1**)] and tetramer [nobotanin K (**6**)] were subjected to CPC.

Centrifugal partition chromatography

CPC Model L-90 (Sanki Engineering, Nagaokakyo, Kyoto, Japan), comprising a centrifuge which has twelve column cartridges (total volume 180 ml), each containing a polyfluoroethylene resin block (150 x 40 x 40 mm), was used. The sample solution and the solvent were pumped into the column rotating at 700 rpm, with a pump, Model CPC-LBP-II (Sanki). UV absorbance monitor, Model CPC-UVM-I, equipped with a cell of light path 0.2 mm (Sanki) was used at 254 nm. Fractions (each 7-10 ml) were collected with a fraction collector, Model SF-160K (Toyo

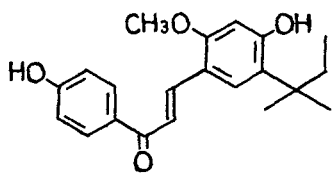
Kagaku Sangyo, Tokyo, Japan), and every two or three fraction was checked by high-performance liquid chromatography (HPLC). Normal-phase HPLC was conducted on a column of Develosil 60-5 using hexane-MeOH-THF-HCOOH (60:45:15:1, v/v/v) containing oxalic acid (500 mg/1.2 l) as eluant, and the reversed-phase HPLC on a column YMC A312 (ODS) developed with 0.05M H_3PO_4-0.05M KH_2PO_4-EtOAc (40:40:15:5, v/v/v).

Solvent

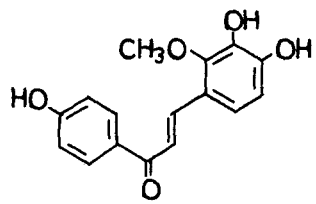
The solvent system used were as follows: solvent A, n-BuOH-n-PrOH-H_2O (4:1:5, v/v/v); solvent B, $CHCl_3$-MeOH-H_2O (7:13:8, v/v/v).

RESULTS AND DISCUSSION

(1) Bioactive polyphenols from Licorice: We have recently found that extracts of licorice roots have tannin-like activity (binding with hemoglobin[4]). The active fraction from Sinkiang licorice was separated from other structurally related components [e.g., licochalcone A (1)] in the ether extracts, by CPC with normal-phase development using solvent system B. Further separation by column chromatography gave a



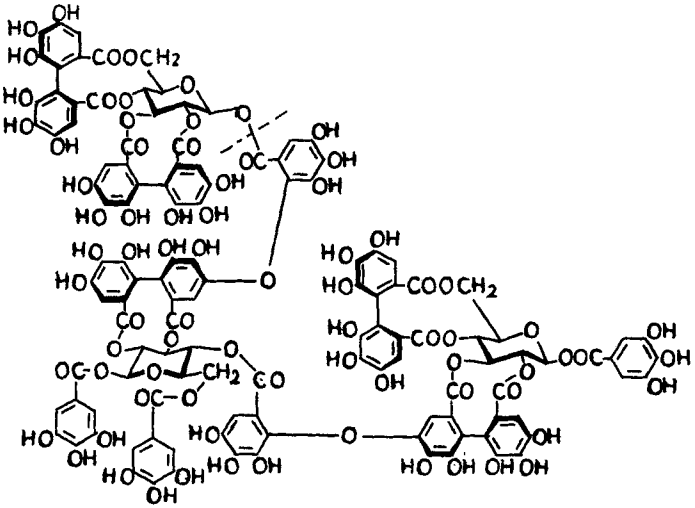
licochalcone A (1)



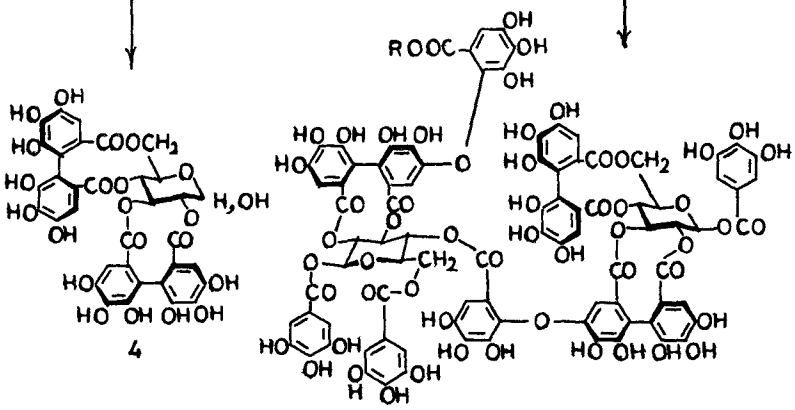
licochalcone B (2)

component exhibiting significant tannin-like activity, which was characteized as licochalcone B (2). Licochalcones A and B thus isolated showed inhibitory effects on the formation of 5-HETE, 5,12-diHETE, 12-HETE, TXB_2, LTB_4 and LTC_4 in arachidonate metabolism.[5] Licochalcone A (1) also showed inhibitory effects on the cytopathic activity of human immunodeficiency virus.[6]

(2) Oligomeric hydrolyzable tannins from Heterocentron roseum: H. roseum, a tropical plant belonging to Melastomataceae, is rich in oligomeric hydrolyzable tannins. Although five dimers (nobotanins B and F ~ I) and a trimer (nobotanin E) were isolated by column chromatography on a vinyl polymer resin (Toyopearl HW-40C),[7] another trimer, nobotanin J (3) which is more labile than the others, and is degraded into a monomer (4) and dimer (5) [or (6)] when kept in aqueous (or MeOH) solution, could not be purified even by repeated chromatography on the resin column, as it was always contaminated by the partial hydrolyzates 4 and 5 (and/or 6) which were produced on a solid support during development. This hydrolysis was particularly remarkable upon the column chromatography on Sephadex LH-20 (EtOH-H_2O-acetone), resulting in conversion of more than 70% of 3, into a monomer (4) and dimers including(5). This unfavorable hydrolysis was



nobotanin J (3)



4

5: R=H
6: R=Me

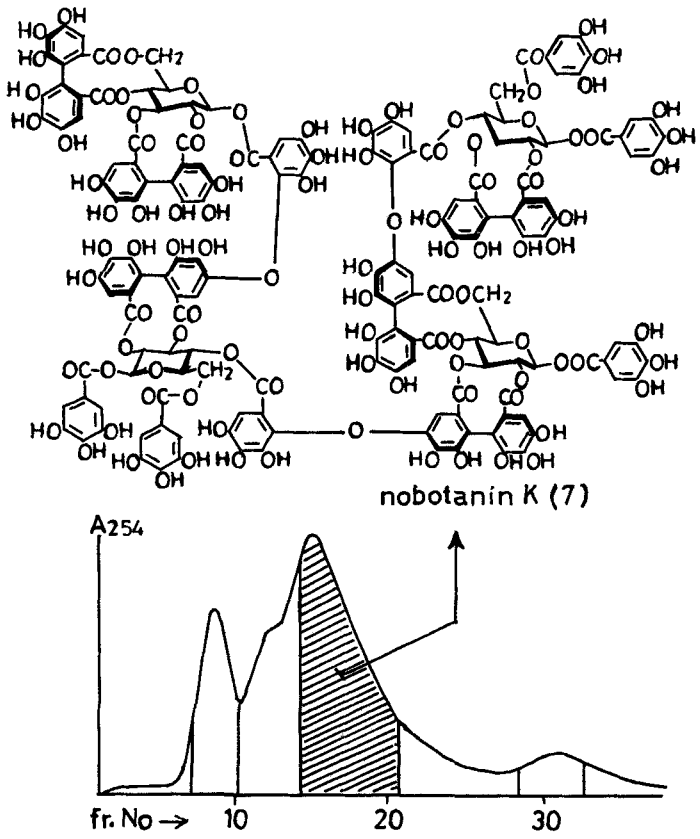


Fig.1 Centrifugal partition chromatogram of tetramer fraction

minimized by applying CPC, performed in a short time in the absence of the solid support. Thus the purification of crude nobotanin J was finally achieved by normal phase CPC using a solvent system, n-BuOH-n-PrOH-H_2O (4:1:5) to afford the trimer (3), which was shown to be pure by the ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra.

A tetramer, nobotanin K (**7**), which is much more polar than nobotanin J and others, and strongly adsorbed on the solid support, was also successfully separated from the other related tetramers in the mixture, by the reversed phase CPC developed with the same solvent system (Fig. 1).

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