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Application of High-Performance Liquid Chromatography Using Water-Containing Silica Gel Columns for Large-Scale Preparation of Cerebroside

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The efficient large-scale preparation of cerebroside, which has interesting pharmacological effects, from crude soya lecithin (7.29 g crude and 6.8 g pure cerebroside from 1 kg of soya lecithin), was achieved by the application of high-performance liquid chromatography using water-containing silica gel.

Keywords——cerebroside; water-containing silica gel; high-performance liquid chromatography; anti-ulcerogenic activity; soya lecithin

Cerebrosides, a kind of sphingoglycolipids, are widely distributed in nature and have been extensively investigated in the biochemical field. Some interesting pharmacological effects of cerebrosides, *e.g.* anti-ulcerogenic activity,[1] sedative effect,[2] *etc.*, have also been recognized recently. Cerebrosides are present mainly in the animal kingdom, but some plants are also important sources of cerebrosides.[1,3] The plant cerebrosides have usually been isolated by repeated chromatography after mild hydrolysis, and finally purified by silica gel column chromatography with increasing concentrations of methanol or acetone in chloroform, or by preparative thin layer chromatography (TLC).[1,3] However, the separation systems hitherto used for the isolation of plant cerebrosides are time-consuming and unfavorable for large-scale preparation, because the cerebrosides are contained in small amounts in plants and are usually present with various other compounds having similar properties, such as sterylglycosides. The ordinary method of adsorption column chromatography on silica gel has some problems such as tailing and the need for a large volume of eluents, even if the high-performance liquid chromatography (HPLC) is applied.

We would like to present here an efficient method for large-scale preparation of cerebroside from crude soya lecithin by HPLC with a water-containing silica gel column system.

Experimental

Materials——Commercial crude soya lecithin (SLP-White, True Lecithin Industry) was used as a source of plant cerebroside because it contains a reasonable amount of cerebroside as well as lecithin, and the cerebroside from soybean has recently been shown to have interesting pharmacological effects.[1]

All preparative steps were monitored by TLC (Kieselgel 60 F_{254} or HPTLC Kieselgel 60 F_{254}, Merck; chloroform–methanol–water (40 : 10 : 1); 10% H_2SO_4 spray-heat) together with standard cerebrosides and sterylglycosides (from *Tetragonia tetragonoides*).[4]

Cerebroside obtained was checked by TLC and the infrared (IR) and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) specrtra were taken.

Small-Scale Preparation——Crude lecithin (13.3 g) dissolved in a small amount of chloroform was added to methanol (350 ml) with stirring to give precipitates. After removal of the precipitates, the methanol-soluble frac-

tion (8.72 g) was chromatographed roughly by a modification of the method of Rouser *et al.*[5] on silica gel (Wakogel C-200, Wako; 4 times the amount of the sample). The column was eluted with chloroform (200 ml)→chloroform–acetone (2 : 1) (3445 ml)→chloroform–acetone (1 : 1) (780 ml)→acetone (1150 ml)→methanol (4510 ml). The fraction (294 mg) containing cerebroside was obtained in the eluates with chloroform–acetone (1 : 1) and acetone. This fraction was further treated by low-pressure liquid chromatography (Packed Column NQ-2 (24 × 360 cm), Fujigelhambai) to purify the cerebroside. A solution of chloroform–methanol–water (20 : 2 : 0.1 or 80 : 10 : 0.8) (2328 or 3196 ml) was used for the chromatography.

Large-Scale Preparation——Treatment of the crude soya lecithin (1 kg) with methanol gave methanol-soluble (598 g) and insoluble (458 g) parts. The former was sujbected to silica gel column chromatography (column size 10.3 × 57 cm) with the following solvent systems: chloroform (1400 ml)→chloroform–acetone (2 : 1) (66420 ml)→chloroform–acetone (1 : 1) (13000 ml)→acetone (29850 ml)→methanol (15000 ml). Two fractions containing cerebroside were obtained: fraction A (7.9 g) eluted with chloroform–acetone (1 : 1) and fraction B (9.3 g) eluted with chloroform–acetone (1 : 1)→acetone.

The further fractionation of fraction A was achieved by the use of HPLC under the following conditions—Waters system 500A; column, PrepPAK-500/silica (Waters); eluent, chloroform–methanol–water (80 : 10 : 0.8); flow rate, 250 ml/min; detection, RI. The sample was filtered through a SEP-PAK Silica Cartridge (Waters) and injected after stabilization of the column by treatment with chloroform–methanol–water (80 : 10 : 0.8). After elution of the cerebroside, the column was washed with chloroform–methanol (2 : 1) and stabilized with chloroform–methanol–water (80 : 10 : 0.8) to use again for the fractionation of fraction B. A combined cerebroside major fraction (7.29 g) was obtained from fractions A and B.

HPLC using a water-containing silica gel column (eluent, chloroform–methanol–water (20 : 2 : 0.1); flow rate, 200 ml/min) was finally employed to purify the cerebroside (6.80 g, white powder) from the cerebroside major fraction (7.29 g).

Results and Discussion

It has recently been found that HPLC using water-containing silica gel columns and water-containing solvent systems is useful for the separation of water-soluble glycosides including saponins.[6] The water-containing columns were expected to be adaptable for the preparation of cerebroside, because the properties of cerebroside are similar to those of saponins, although cerebroside is insoluble in most aqueous and organic solvents except chloroform–methanol mixture and pyridine. Pure cerebroside (6.8 g) was obtained from crude soya lecithin (1 kg) through HPLC on water-containing silica gel columns in our experiment. Thus, water-containing silica gel chromatography is indeed well suited to the purification of cerebrosides, and the problems of tailing and high consumption of eluents in other methods were overcome. This method using water-containing silica gel columns may be extended to the purification of cerebrosides and analogous lipids such as diglycosylceramides from other organisms as well as plants. Cerebrosides have interesting pharmacological effects, *e.g.* antiulcerogenic activity,[1] sedative effect,[2] *etc.*, so that the efficient large-scale preparation of cerebrosides should make possible further progress in pharmacological studies.

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References

1) E. Okuyama and M. Yamazaki, *Chem. Pharm. Bull.*, **31**, 2209 (1983).
2) M. Yamazaki, unpublished data.
3) H. E. Carter, R. A. Hendry, S. Nojima, N. Z. Stanacev, and K. Ohno, *J. Biol. Chem.*, **236**, 1912 (1961); R. Laine and O. Renkonen, *Biochemistry*, **12**, 1106 (1973); Y. Fujino and M. Ohnishi, *Chem. Phys. Lipids*, **17**, 275 (1976); M. D. Choudhury and P. Chakrabart, *Phytochemistry*, **19**, 519 (1980); B. Bris, C. Delbart, D. Coustant, and R. Linder, *ibid.*, **20**, 1255 (1981); M. Ohnishi, S. Ito, and Y. Fujino, *Biochim. Biophys. Acta*, **752**, 416 (1983).
4) E. Okuyama and M. Yamazaki, *Yakugaku Zasshi*, **103**, 43 (1983).
5) G. Rouser, G. Kritchevsky, G. Simon, and G. J. Nelson, *Lipids*, **2**, 37 (1967).
6) H. Kaizuka and K. Takahashi, *J. Chromatogr.*, **258**, 135 (1983).