Journal of Chromatography, 537 (1991) 469–474
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 763

Note

Preparative high-performance liquid chromatography on chemically modified porous glass

Isolation of acidic saponins from ginseng

HIDEKO KANAZAWA, YOSHIKO NAGATA, YOSHIKAZU MATSUSHIMA* and MASASHI TOMODA
Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105 (Japan)
and
NOBUHARU TAKAI
Institute of Industrial Science, University of Tokyo, Roppongi 7-22-1, Minato-ku, Tokyo 106 (Japan)
(First received June 5th, 1990; revised manuscript received August 14th, 1990)

Octadecylsilylated porous glass (MPG–ODS) has been demonstrated to be a useful packing material for reversed-phase high-performance liquid chromatography (HPLC) [1]. It is especially suitable for the determination of saponins of ginseng and bupleum root [2–5]. We recently reported a successful application of a large-scale column of MPG–ODS for the preparative high-performance liquid chromatography (HPLC) of ginsenoside-Rb_1, -Rc, -Rb_2, -Rd, -Rg_1 and -Re [6].

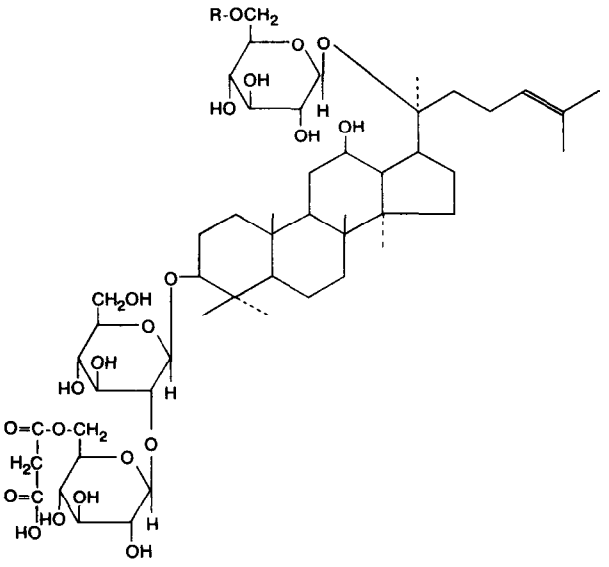
Kitagawa *et al.* [7] found that white ginseng contained a considerable amount of acidic malonate of the dammarane saponins malonyl ginsenoside-Rb_1, -Rb_2, -Rc and -Rd (Fig. 1). The malonyl ginsenosides are reported to be unstable and readily demalonylated on heating and hence are not present in red ginseng. We have shown that the malonyl ginsenosides could also be determined rapidly and accurately by HPLC on MPG–ODS [4]. These results prompted us to investigate the preparative HPLC of the malonyl ginsenosides, and the results are presented in this paper.

EXPERIMENTAL

Materials

Octadecylsilylated porous glass (MPG–ODS) was supplied by Ise Chemical Industries [packed columns of MPG–ODS are now commercially available from Wako (IPG-55-ODS-10H) and Hitachi (Hitachi Gel 3161)]. The particle size of the packing material was 10 μm for the analytical and 20 μm for the preparative columns. Acetonitrile used as the mobile phase in analytical HPLC was of HPLC grade (Wako, Tokyo, Japan). Water was deionized and distilled. Other chemicals were of analytical-reagent grade.

0021-9673/91/$03.50



malonyl ginsenoside-Rb_1 : R= β-D-glucopyranosyl

malonyl ginsenoside-Rb_2 : R= α-L-arabinopyranosyl

malonyl ginsenoside-Rc : R= α-L-arabinofuranosyl

malonyl ginsenoside-Rd : R=H

Fig. 1. Structure of malonyl ginsenosides.

Analytical HPLC

The analytical HPLC system was composed of a Tosoh Model CCPM multi-pump, a Rheodyne Model 7125 valve, a Tosoh Model UV-8000 monitor and a Hitachi Model 833A data processor. The system was operated at room temperature. A stainless-steel column (150 × 4 mm I.D.) packed with MPG-ODS was used. The mobile phases were mixtures of acetonitrile and 50 m*M* KH_2PO_4 solution at a flow-rate of 1 ml/min. The peaks were monitored at 203 nm unless stated otherwise. A Hitachi Model L-3000 system was used for multi-channel spectrophotometric detection.

Preparative HPLC

The preparative HPLC system consisted of a Tosoh Model CCPM prep pump, a Model UV-8010 monitor, a Model SC-8010 system controller and data processor, a Model FC-8000 fraction collector and a Model PP-8010 recorder. MPG–ODS was packed into stainless-steel tubing of 500 × 20 mm I.D. and 500 × 50 mm I.D. The peaks were monitored at 203 nm and the system was operated at room temperature.

Sample preparation from the crude drug

Roots of *Panax ginseng* were pulverized and extracted with 70% methanol at room temperature. The extract was evaporated and dissolved in water. The aqueous solution was passed through a solid phase, Mega Bond Elute (Analytichem International), pretreated with water and methanol. After the solid phase had been washed with water and 30% methanol, the sample was eluted with methanol and the eluate was evaporated to dryness under reduced pressure. The residue was dissolved in the eluent and injected into the preparative HPLC system.

Purification procedure

The chromatographic fractions were evaporated *in vacuo* to remove acetonitrile or in some instances diluted with water. The aqueous solutions were passed through a Sep-Pak C_{18} cartridge (Waters Assoc., Milford, MA, U.S.A.) pretreated with methanol and the cartridge was washed with water and 30% methanol. The saponins were eluted with methanol and the eluate was evaporatd to dryness under reduced pressure. The residue was dissolved in water and freeze-dried.

RESULTS

The determination of neutral ginsenosides by HPLC on an MPG–ODS column was successful with the use of mixtures of acetonitrile and water as the mobile phase [2,3,5]. For the determination of acidic saponins of ginseng, bupleum root and glycyrrhiza by the method, addition of inorganic phosphate to the mobile phases was required [4]. The mobile phases used in preparative HPLC were mixtures of acetonitrile and 50 m*M* KH_2PO_4 solution. A mobile phase containing 25% acetonitrile was used for the separation of the malonyl ginsenosides on the 500 × 20 mm I.D. column. The flow-rate was 15 ml/min and the pressure was 55 kg/cm^2. In a run, an extract from 0.5 g of ginseng root was injected in the HPLC system. The chromatogram is shown in Fig. 2.

The three malonyl ginsenosides were separated within 45 min and the average yields were 2.0 mg of purified malonyl ginsenoside-Rb_1, 1.1 mg of malonyl ginsenoside-Rc and 0.9 mg of malonyl ginsenoside-Rb_2 per gram of ginseng root.

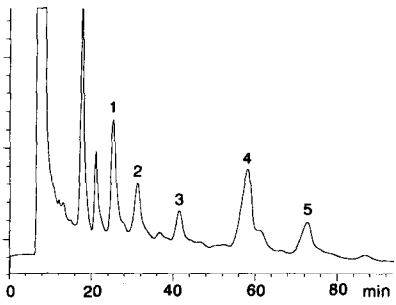


Fig. 2. Preparative chromatogram of a ginseng extract. Peaks: 1 = malonyl ginsenoside-Rb_1; 2 = malonyl ginsenoside-Rc; 3 = malonyl ginsenoside-Rb_2; 4 = ginsenoside-Rb_1; 5 = ginsenoside-Rc. Column, MPG–ODS (500 × 20 mm I.D., particle size 20 μm); an extract from 0.5 g of ginseng was injected; eluent, acetonitrile–50 m*M* KH_2PO_4 (25:75); flow-rate, 15.0 ml/min; detection, 203 nm.

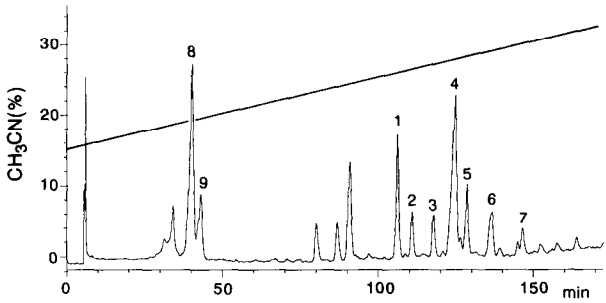


Fig. 3. Preparative chromatogram of a ginseng extract. Peaks: 1 = malonyl ginsenoside-Rb_1; 2 = malonyl ginsenoside-Rc; 3 = malonyl ginsenoside-Rb_2; 4 = ginsenoside-Rb_1; 5 = ginsenoside-Rc; 6 = ginsenoside-Rb_2; 7 = ginsenoside-Rd; 8 = ginsenoside-Rg_1; 9 = ginsenoside-Re. Column, MPG–ODS (500 × 20 mm I.D., particle size 20 μm); an extract from 0.4 g of ginseng was injected; eluent, acetonitrile–50 m*M* KH_2PO_4 with acetonitrile concentrations as plotted on the ordinate; flow-rate, 20.0 ml/min; detection, 203 nm.

The isolation of both the major acidic and neutral ginsenosides in a single run on the 500 × 20 mm I.D. column was achieved by linear gradient elution from 15% to 50% acetonitrile in 180 min at a flow-rate of 20 ml/min. As shown in Fig. 3, the peaks of six neutral saponins (peaks 4–9) and three acidic saponins (peaks 1–3) were well resolved. Under the conditions applied, ginsenoside-Rg_1, -Re, -Rb_1, -Rc, -Rb_2 and -Rd and malonyl ginsenoside-Rb_1, -Rb_2 and -Rc were isolated within 180 min.

Preparative HPLC on the 500 × 50 mm I.D. column was carried out using step gradient elution. The mobile phase compositions were acetonitrile 20% at 0–100 min and 25% at 100–220 min with a flow-rate of 39.9 ml/min and a pressure of 40 kg/cm^2. The chromatogram is shown Fig. 4. In a single run, 5.5 mg of pure malonyl ginsenoside-Rb_1, 3.5 mg of malonyl ginsenoside-Rb_2 and 2.9 mg of malonyl ginsenoside-Rc were separated from 8 g of ginseng roots.

The purity of the collected fractions was monitored by analytical HPLC. A multi-channel spectrophotometer was also employed for the detection of UV-absorbing impurities. Each fraction gave a single chromatographic peak and did not

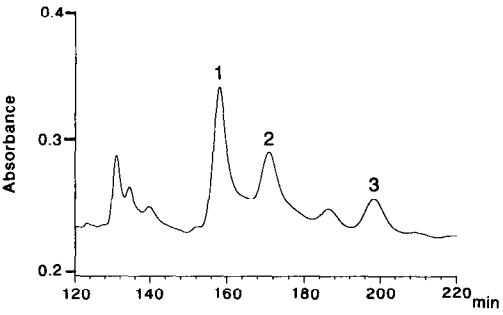


Fig. 4. Preparative chromatogram of a ginseng extract. Peaks: 1 = malonyl ginsenoside-Rb_1; 2 = malonyl ginsenoside-Rc; 3 = malonyl ginsenoside-Rb_2. Column, MPG–ODS (500 × 50 mm I.D., particle size 20 μm); an extract from 8.0 g of ginseng was injected; eluent, 0–100 min, acetonitrile–50 m*M* KH_2PO_4 (20:80), and 100–220 min, acetonitrile–50 m*M* KH_2PO_4 (25:75); flow-rate, 39.9 ml/min; detection, 203 nm.

contain any detectable UV- absorbing impurities. The retention times and UV spectra were the same as those for authentic samples of the assigned peaks. The fractions were also analysed by the thin-layer chromatography (TLC) with the lower layer of chloroform–methanol–water (13:7:2) as the solvent. A single TLC spot with the same R_F value as that for an authentic sample was observed for each fraction. The fractions were evaporated and freeze-dried as described under Experimental. White crystalline products were obtained.

DISCUSSION

There have been several reports [8–12] on the preparative-scale HPLC of neutral saponins from *Panax ginseng*. In a previous paper, we reported the preparative HPLC of neutral ginsenosides on MPG–ODS columns [6]. However, no report on the preparative HPLC of malonyl ginsenosides has appeared previously.

Malonyl ginsenosides were successfully separated by preparative HPLC on a reversed-phase column of MPG–ODS chemically modified porous glass as described above. The MPG–ODS column achieved the rapid analysis of neutral and acidic ginsenosides [2,4]. Retention times for the panaxatriol ginsenosides were almost half those on silica–ODS columns [13]. The rapid separation renders savings of time and solvent in the HPLC procedure. The proportion of organic solvent in the mobile phase was much smaller in HPLC with MPG–ODS than with silica–ODS columns. As we use large volumes of solvents in the preparative-scale HPLC, the savings in organic solvent are advantageous from both economical and environmental points of view.

A pore size of 550 Å seems to be favourable for the separations of ginsenosides and related saponins. The sharp separation of the peaks may be due to the narrow distribution range of the pore size [1,6]. The column was not suitable for the compounds of smaller molecular size [14].

As the ginsenosides and malonyl ginsenosides were well resolved on the preparative scale at room temperature, the method was suitable for the preparative separation of heat-unstable acidic saponins. The mobile phase for preparative HPLC contained inorganic phosphate, which must be removed from the separated fractions. It was easily removed by the solid-phase extraction technique.

In conclusion, the method is simple, rapid and convenient and is suitable for the purification of heat-unstable malonyl ginsenosides. It should be applicable to the isolation of other saponins of crude drugs. The MPG–ODS column has a number of advantages over conventional normal- and reversed-phase columns for the chromatography of saponins.

ACKNOWLEDGEMENTS

This work was supported in part by the Science Research Promotion Fund of the Japan Private School Promotion Foundation and by Ise Chemical Industries. Technical assistance by Emi Kurosaki, Fumiko Ishii and Tomomi Yoshinaga is gratefully acknowledged.

REFERENCES

1 Y. Matsushima, Y. Nagata, K. Takakusagi, M. Niyomura and N. Takai, *J. Chromatogr.*, 332 (1985) 265.
2 H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *Chromatographia*, 24 (1987) 517.
3 H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *Shoyakugaku Zasshi*, 43 (1989) 121.
4 N. Takai, H. Kanazawa, Y. Nagata, Y. Matsushima and M. Tomoda, *Seisan Kenkyu*, 41 (1989) 773.
5 H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *J. Chromatogr.*, 507 (1990) 327.
6 H. Kanazawa, Y. Nagata, Y. Matsushima, M. Tomoda and N. Takai, *Chem. Pharm. Bull.*, 38 (1990) 1630.
7 I. Kitagawa, T. Taniyama, T. Hayashi and M. Yosikawa, *Chem. Pharm. Bull.*, 31 (1983) 3353.
8 T. Nagasawa, T. Yokozawa, Y. Nishino and H. Oura, *Chem. Pharm. Bull.*, 28 (1980) 2059.
9 T. Nagasawa, T. Yokozawa, Y. Nishino and H. Oura, *Chem. Pharm. Bull.*, 28 (1980) 3701.
10 J. Zhou, M. Z. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari and T. Fuwa, *Chem. Pharm. Bull.*, 29 (1981) 2844.
11 H. Kaizuka and K. Takahashi, *J. Chromatogr.*, 258 (1983) 235.
12 H. Yamaguchi, H. Matsuura, R. Kasai, K. Mizutai, H. Fujino, K. Ohtani, T. Fuwa and O. Tanaka, *Chem. Pharm. Bull.*, 34 (1986) 2856.
13 T. G. Petersen and B. Palmqvist, *J. Chromatogr.*, 504 (1990) 139.
14 Y. Nagata, Y. Kim, Y. Nakamichi, Y. Matsushima and N. Takai, *Annu. Rep. Kyoritsu Coll. Pharm.*, 34 (1989) 39.