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Isolation of dammarane saponins from *Panax notoginseng* by high-speed counter-current chromatography

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Abstract

The Chinese phytomedicinal formulation *Sanqi Zongdai Pian*, traditionally prepared from crude extracts from roots of *Panax notoginseng* (Araliaceae), contains highly polar dammarane saponins which were separated at a preparative scale using high-speed counter-current chromatography (HSCCC). In each operation, 283 mg methanolic extract of five tablets was separated and yielded pure 157, 17, 13 and 56 mg of ginsenoside-Rb_1, notoginsenoside-R_1, ginsenoside-Re and ginsenoside-Rg_1, respectively, *n*-hexane–*n*-butanol–water (3:4:7, v/v/v) was used for the two-phase solvent system of the HSCCC separation. The chemical structures of three ginsenosides and one notoginsenoside were elaborated by means of electrospray ionization MS–MS and NMR analysis.
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1. Introduction

Root material of *Panax notoginseng* (Burk.) F.H. Chen (Araliaceae), is a highly bioactive phytomedicinal remedy, which is widely used in traditional Chinese medicine. The active principle of *P. notoginseng* constituents is a series of bioactive dammarane saponins, such as ginsenosides and notoginsenosides. In ethnomedicine, *P. notoginseng* is used as a tonic, and also for hemostatic purposes. Preparations are e.g., "*Yunnan Baiyao*" used for treatment of trauma and bleeding caused by internal and external injury, "*Pian Zi Huang*" used against hepatitis, and also "*Sanqi Zongdai Pian*". The latter remedy which is known to be beneficial in case of blood circulation disorders and coronary heart diseases is the subject of this investigation [1–5].

Many Chinese herbal preparations consist of crude plant extracts of high complexity, and are not further purified prior to use. During phytochemical investigations, lead structures, which are responsible for bioactivity of decocts and crude extracts can be isolated and structurally characterized. Important scope of our analysis was to search for an efficient way to isolate pure dammarane saponins. These standards can be used for the quantification of active

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ingredients in fresh, and concentrated *P. notoginseng* extracts. It is generally accepted that ''modern'' phytomedicines must be standardized in order to prevent any risk of overdosing.

This paper describes the preparative isolation of pure dammarane saponins by high-speed counter-current chromatography (HSCCC). HSCCC is an all-liquid chromatographic system [16], working without solid support, and separation is based on fast partitioning effects of the analytes between two immiscible liquid phases [16]. Irreversible adsorbing effects and artefact formation is minimized. Application of HSCCC in natural product chemistry is steadily increasing, because of its superior separation abilities and excellent recovery rates [17–22].

2. Experimental

2.1. Reagents

The organic solvents, 1-butanol and *n*-hexane used for HSCCC separation were of analytical grade. Water was Nanopure quality.

2.2. Extraction of dammarane saponins

''*Sanqi Zongdai Pian*'' produced by Yunnan Daphne Pharmaceutical (China) is claimed to contain 50 mg of notoginsenosides from *P. notoginseng* in each tablet. For isolation, five tablets were powdered and extracted with methanol (3×50 ml). Extracts were combined and evaporated to dryness under reduced pressure to yield 283 mg of a light yellow powder, which was directly used for HSCCC separation.

2.3. High-speed counter-current chromatography

The HSCCC instrument was a multilayer coil counter-current chromatograph, manufactured by P.C. (Potomac, MD, USA), equipped with a 385-ml coil column made of polytetrafluoroethylene tubing (2.6 mm I.D.). The mobile phase was delivered by a Biotronik HPLC pump BT 3020 (Jasco, Gross-Umstadt, Germany). The HSCCC experiment was performed with a two-phase solvent system composed of *n*-hexane–*n*-butanol–water (3:4:7, v/v/v), which was selected by a partition experiment of the crude extract in a series of solvent systems composed of *n*-hexane–*n*-butanol–water at different volume ratios. After thorough equilibration of the solvents in a separatory funnel, the two resulting phases were separated shortly before use. The multilayer coil column was entirely filled with the upper organic phase as the stationary phase. Then the apparatus was rotated at 650 rpm. The total amount of dried saponin extract (283 mg, equivalent to five tablets of ''*Sanqi Zongdai Pian*'') was dissolved in 50 ml of mobile phase. Injection to the HSCCC system was done by a PTFE sample loop, followed by pumping of mobile phase at a flow-rate of 2.5 ml/min. Eluate was collected with a fraction collector Superfrac (Pharmacia, Uppsala, Sweden), 20 ml for each fraction. After the separation, the solvents in the column were pushed out and the retention of stationary phase, 62% was measured. Analysis of CCC fractions was done by TLC. Final purity of saponin fractions **1**–**4** (see Fig. 1) was verified by HPLC–electrospray ionization (ESI) MS analyses.

2.4. Analytical controls

2.4.1. TLC analysis

Evaluation of the HSCCC fractions was done by thin-layer-chromatography on normal-phase silica gel plates 60 F_{254} Merck (Darmstadt, Germany), and

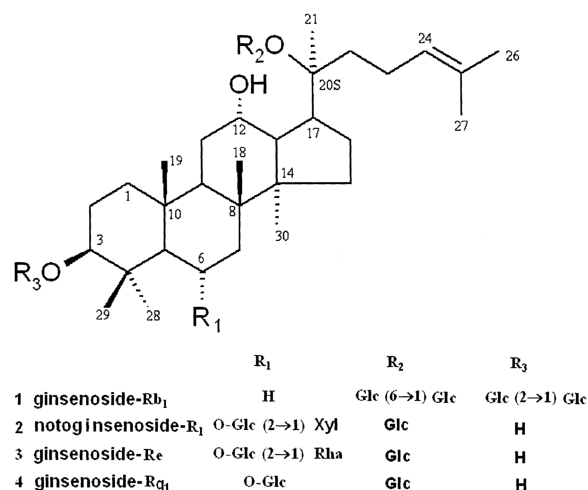

Fig. 1. Structure of dammarane saponins **1**–**4** isolated from the Chinese phytomedicine ''*Sanqi Zondai Pian*''.

chloroform–methanol–water (13:7:2, v/v/v) as developing system. The fractions containing the saponins **1–4** were finally run on reversed-phase plates RP-18W from Macherey-Nagel (Düren, Germany). Ginsenosides, and notoginsenosides were visualized by spraying 5% conc. sulfuric acid in ethanol, and subsequent heating to 110 °C on a hot plate.

2.4.2. ESI-MS–MS (syringe-pump), and HPLC–ESI-MS–MS

All ESI-MS–MS experiments were performed on a Bruker Esquire LC–MS ion trap multiple mass spectrometer (Bremen, Germany) in positive and negative ionization mode analyzing ions up to m/z 2200.

During ESI-MS, and MS–MS fragmentation studies, the purified samples were introduced via a syringe pump at a flow-rate of 240 μl/min. Drying gas was nitrogen (flow 7.0 l/min, 330 °C), and nebulizer pressure was set to 5 p.s.i. (1 p.s.i.= 6894.76 Pa). ESI-MS parameters (negative mode): capillary +4500 V, end plate +4000 V, capillary exit −90 V, capillary exit offset −60 V, skim 1 −30 V, skim 2 −10 V; ESI-MS parameters (positive mode): capillary −4500 V, end plate −4000 V, capillary exit +90 V, capillary exit offset +60 V, skim 1 +30 V, skim 2 +10 V. MS–MS experiments afforded fragmentation amplitude values between 0.8 and 1.2.

For HPLC–ESI-MS–MS analysis, a binary gradient pump G1312A, series 1100 from Hewlett-Packard (Waldbronn, Germany) was coupled to the Bruker Esquire LC–ESI-MS system. Drying gas 9.0 l/min, and nebulizer pressure was set to 40 p.s.i. ESI-MS parameters (negative mode): capillary +3500 V, end plate +3000 V, capillary exit −95 V, skim 1 −25 V, skim 2 −10 V.

Column material for HPLC–MS was a Prontosil C_{18} Aqua, 5 μm, 250×2.0 mm (Bischoff, Leonberg, Germany), flow-rate was 0.25 ml/min, and eluents were Nanopure water (solvent A), and acetonitrile (solvent B). Initial conditions of the gradient were 97% A and 3% B, hold over 10 min, starting a linear gradient in 30 min to 40% A and 60% B, in 15 min to 0% A and 100% B, and hold for 10 min.

2.4.3. NMR analysis

^{1}H, ^{13}C and DEPT 90/135 NMR spectra were recorded in [$^{2}H_5$] pyridine on a Bruker AMX 300 (Karlsruhe, Germany) with 300 MHz for ^{1}H, and 75.5 MHz for ^{13}C measurements, respectively. Two-dimensional NMR experiments (HMQC, HMBC) were run on a Bruker AM 360 spectrometer.

3. Results and discussion

3.1. HSCCC separation

The methanolic extract of the *P. notoginseng* formulation "*Sanqi Zongdai Pian*" was separated by high-speed counter-current chromatography (HSCCC) using a single solvent system composed of *n*-hexane–*n*-butanol–water (3:4:7, v/v/v). The solvent system possessed a good retention character at the operation condition, i.e., 62% of retention rate of the stationary phase after the separation was finished. The analytical results monitored by TLC to yield five components. Component **0**, yellow spots**,** elution volume 151–190 ml, could be deduced as starch, a typical tablet auxiliary material. Component **1**, dark blue spots, elution volume 211–290 ml; component **2**, blue spots, elution volume 311–430 ml; component **3**, blues pots, elution volume 451–570 ml; and component **4**, blue spots, elution volume 511–790 ml showed the coloration reaction of dammarane-type triterpene oligoglycosides, such as ginsenosides and notoginsenosides. All fractions corresponding to components **1**, **2**, **3** and **4** were combined, and then lyophilized to yield 157, 17, 13 and 56 mg, respectively, of colorless, powdery material. Purity analysis on reversed-phase C_{18}-TLC material gave single spots with R_f 0.67 for component **1**, 0.60 for components **2** and **3**, and 0.25 for component **4**. The freeze-dried samples were directly used for ESI-MS–MS and NMR analyses.

The order of elution in HSCCC separation reflects the polarity of separated saponin structures **1**–**4**, starting with ginsenoside-Rb_1 (**1**) carrying two diglucoside moieties followed by notoginsenoside-R_1 (**2**) with only two glucose and one xylose unit. Interestingly, the applied solvent system separated saponin **2** from the very similar ginsenoside-Re (**3**) which carries two glucose, and instead of xylose, the less polar 6-desoxy sugar rhamnose (Fig. 1). Ginsenoside-Rg_1 (**4**) having only two glucose units

eluted at the end, due to stronger affinity to organic stationary phase.

In previous investigations on the roots of *Panax notoginseng* by Masayuki et al. [3], 28 components were reported, which belong to the class of ginsenosides and notoginsenosides in which Rb_1, Rg_1, Rd, Re and R_1 were about 50.0, 19.5, 9.0, 8.5 and 4.5% of total ginsenoside and notoginsenoside content. The amount of minor constituents is below 1.5%. Our HSCCC results confirm that ginsenoside-Rb_1 (**1**), ginsenoside-Rg_1 (**2**), ginsenoside-Re (**3**), and notoginsenoside-R_1 (**4**) are four main dammarane saponins in the Chinese medicine "*Sanqi Zondai Pian*", while Rd was not obtained.

Several analytical investigations concern about the chromatographic separation of dammarane saponins from root extracts of *Panax notoginseng*. Shoji et al. [6], as well as Wu [7], isolated four dammarane saponins, known as ginsenoside-Rb_1 (**1**), Rd, Re (**3**), and Rg_1 (**4**). Wei et al. [1] detected six saponins, in which two of them were Rb_1 (**1**) and Rg_1 (**4**), the others remained unidentified. Zhou et al. [8] dialyzed an aqueous solution of a crude saponin fraction of the methanolic root extract, further silica-gel chromatography resulted in Rg_1, and a mixture of Re and R_1. The non-dialyzed fraction yielded ginsenoside Rb_1 and Rd. Later, mainly preparative LC and HPLC were adopted to yield pure ginsenosides and notoginsenosides [9–15]. Comparing to those separation, HSCCC has apparent advantages such as high purity (Fig. 2), good recovery and less solvent consumption.

3.2. Confirmation of chemical structures

Structures of components **1**, **2**, **3** and **4** were confirmed as the saponins **1–4** shown in Fig. 1 by means of modern spectroscopic techniques, including ^{1}H, ^{13}C, DEPT-NMR, HMQC, HMBC, and electrospray ionization ion trap multiple mass spectrometry (ESI-MS–MS). All ^{13}C-NMR data (Tables 1 and 2) are in very good accordance to previously published reference data [5,6,8–13,15]. To verify the (20*S*)-protopanaxatriol partial structure which is the aglycone for saponins **2–4**, and having *O*-glycosyl linkages at its C-6 and C-20 positions, heteronuclear correlation experiments such as HMQC and HMBC were performed on saponin **3**, and in case of saponin **4** after peracetylation. The long-range H–C correlation cross-peaks from the anomeric sugar protons clearly indicated the two glucosidic linkages to position C-6 and C-20 to the sapogenol moiety. Peracetylation of saponin **4** revealed strong ^{13}C NMR acetylation shifts at C-3, and C-12 verifying that sugar moieties are not linked to these positions. The ^{13}C resonances of the protopanaxatriol part in saponins **2–4** were nearly superimposeable, and all saponins **1–4** are not glycosylated at hydroxyl function C-12 due to identical resonances between δ 70.0–70.2 ppm for this carbon atom.

Saponin **1** possesses a protopanaxadiol aglycone without hydroxylation at C-6 which was recognized by a characteristic methylene resonance at δ 18.3 ppm. Two disaccharide moieties, such as a β-gentiobiosyl part, and a β-sophorosyl part are linked to C-20 and C-3 of the (20*S*)-protopanaxadiol moiety, respectively. Due to glycosidation, the oxymethin ^{13}C resonance of C-3 remarkably shifted downfield to δ 89.0 ppm, instead of carbinol carbon resonances between δ 78.0, and δ 78.7 ppm, respectively, observed in structures **2–4**.

The ESI-MS spectroscopic data of the four dammarane saponins are given as below:

Ginsenoside Rb_1 (**1**): colorless powder, ESI-MS (pos) *m/z*: 1131 $[M+Na]^+$, 577 $[M+2Na]^{2+}$, 365, MS–MS fragmentation of *m/z* 1131: 790, 365; ESI-MS (neg) *m/z*: 1107 $[M-H]^-$, MS/MS fragmentation of *m/z* 1107: 945 $[M\text{-glc-H}]^-$, 783 $[M\text{-2glc-H}]^-$, 621 $[M\text{-3glc-H}]^-$, 459 $[M\text{-4glc-H}]^-$; LC–MS: t_R 35.0 min; ^{13}C NMR ($[^2H_5]$pyridine) (Tables 1 and 2).

Notoginsenoside R_1 (**2**): colorless powder, ESI-MS (pos) *m/z*: 955 $[M+Na]^+$, 489 $[M+2Na]^{2+}$, MS–MS fragmentation of *m/z* 955: 776, 643, 335; ESI-MS (neg) *m/z*: 977 $[M+2Na-H]^-$, MS–MS fragmentation of *m/z* 977: 970, 931 $[M-H]^-$, 799 $[M\text{-xyl-H}]^-$, 769 $[M\text{-glc-H}]^-$, 739, 637 $[M\text{-glc-xyl-H}]^-$, 475 $[M\text{-2glc-xyl-H}]^-$; LC–MS: t_R 30.4 min; ^{13}C NMR ($[^2H_5]$pyridine) (Tables 1 and 2).

Ginsenoside Re (3): colorless powder, ESI-MS (pos) *m/z*: 969 $[M+Na]^+$, 496 $[M+2Na]^{2+}$, 413, 304, MS–MS fragmentation of *m/z* 969: 789, 643, 463, 349; ESI-MS (neg) *m/z*: 991 $[M+2Na-H]^-$, MS–MS fragmentation of *m/z* 991: 945 $[M-H]^-$, 783 $[M\text{-glc-H}]^-$, 637 $[M\text{-glc-rha-H}]^-$, 475 [M-2glc-

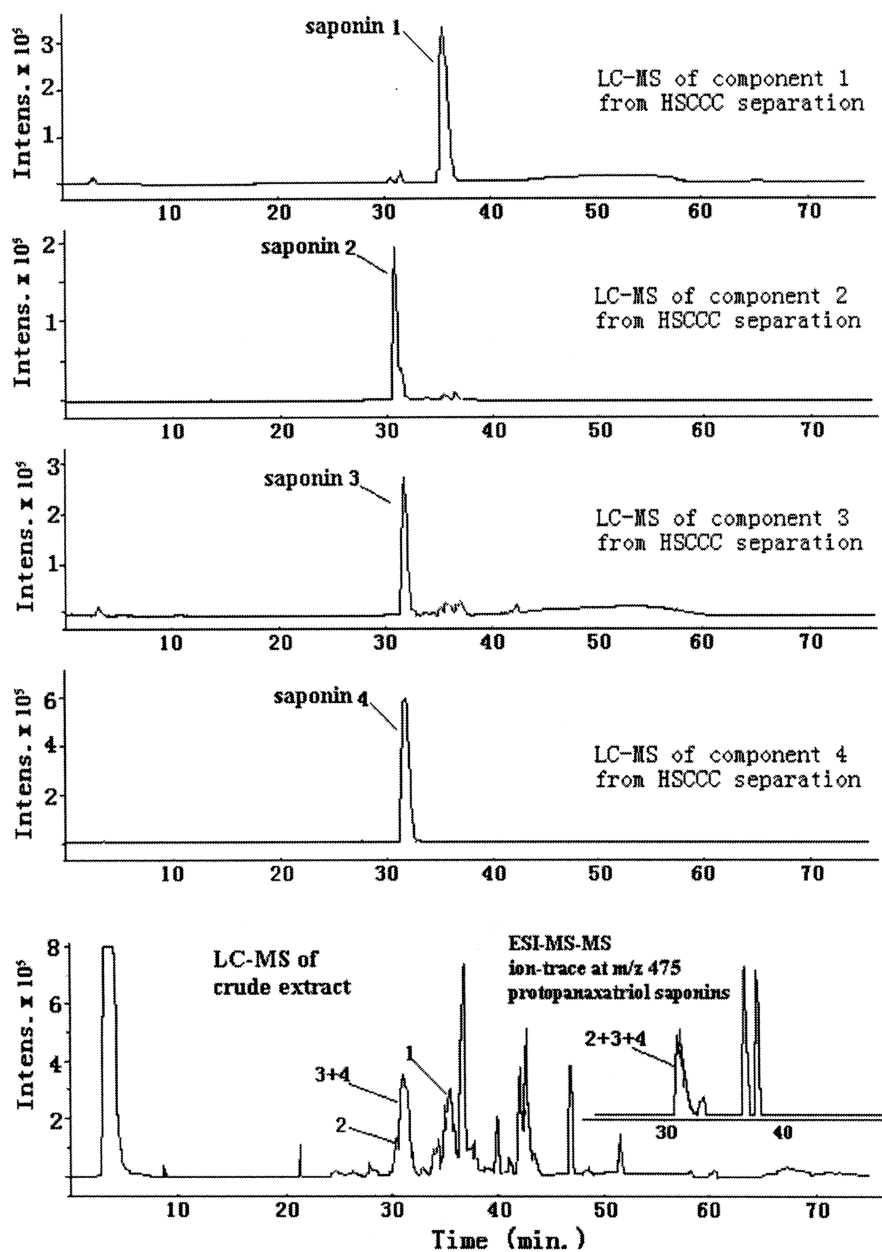

Fig. 2. HPLC–MS detection (negative ionization) of components **1**–**4** from HSCCC separation and the crude extract of "*Sanqi Zongdai Pian*"; MS–MS fragmentation and extraction of ion-signal *m*/*z* 475 reflects the selective ion-trace for saponins containing a protopanaxatriol aglycone **2**–**4**. HPLC condition: Column, Prontosil C_{18} Aqua, 5 μm, 250×2.0 mm; Elution flow-rate, 0.25 ml/min; Gradient elution, from water–acetonitrile (97:3) for 10 min, to water–acetonitrile (40:60) for 30 min, to water–acetonitrile (0:100) for 25 min. ESI-MS conditions: drying gas, 9.0 l/min; nebulizer pressure, 40 p.s.i.; capillary, +3500 V; end plate, +3000 V; capillary exit; −95 V; skim 1, −25 V; skim 2, −10 V.

Table 1
^{13}C NMR and DEPT 135/90 spectral data of saponins **1**–**4**: aglycone moiety (δ [ppm] measured in [$^2H_5$]pyridine)

Carbon	Saponin **1**, ginsenoside Rb_1		Saponin **2**, notoginsenoside R_1		Saponin **3**, ginsenoside Re		Saponin **4**, ginsenoside Rg_1	
	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT	^{13}C	DEPT
1	39.1	CH_2	39.4	CH_2	39.7	CH_2	39.7	CH_2
2	26.6	CH_2	27.6	CH_2	27.8	CH_2	27.9	CH_2
3	89.0	CH	78.0	CH	78.8	CH	78.7	CH
4	39.6	C	40.0	C	40.0	C	40.3	C
5	56.3	CH	61.2	CH	60.9	CH	61.4	CH
6	18.3	CH_2	79.4	CH	74.8	CH	79.6	CH
7	35.0	CH_2	44.8	CH_2	45.9	CH_2	45.2	CH_2
8	39.9	C	41.0	C	41.3	C	41.2	C
9	50.1	CH	49.8	CH	49.6	CH	50.0	CH
10	36.8	C	39.4	C	39.5	C	39.5	C
11	30.6[a]	CH_2	30.8[a]	CH_2	31.0[a]	CH_2	31.0[a]	CH_2
12	70.1	CH	70.2	CH	70.2	CH	70.2	CH
13	49.3	CH	49.0	CH	49.2	CH	49.2	CH
14	51.3	C	51.3	C	51.5	C	51.4	C
15	30.6[a]	CH_2	30.6[a]	CH_2	30.8[a]	CH_2	30.7[a]	CH_2
16	26.5	CH_2	26.5	CH_2	26.7	CH_2	26.6	CH_2
17	51.6	CH	51.6	CH	51.8	CH	51.6	CH
18	16.1[b]	CH_3	17.6[b]	CH_3	17.6[b]	CH_3	17.6[b]	CH_3
19	15.9[b]	CH_3	17.0[b]	CH_3	17.3[b]	CH_3	17.6[b]	CH_3
20	83.4	C	83.2	C	83.3	C	83.3	C
21	22.3	CH_3	22.3	CH_3	22.4	CH_3	22.3	CH_3
22	36.1	CH_2	35.9	CH_2	36.1	CH_2	36.2	CH_2
23	23.1	CH_2	23.2	CH_2	23.3	CH_2	23.2	CH_2
24	125.8	CH	125.8	CH	126.0	CH	126.0	CH
25	130.9	C	130.9	C	130.9	C	130.9	C
26	25.6	CH_3	25.6	CH_3	25.7	CH_3	25.7	CH_3
27	17.8[b]	CH_3	17.4[b]	CH_3	17.5[b]	CH_3	17.8[b]	CH_3
28	28.0	CH_3	31.6	CH_3	32.2	CH_3	31.7	CH_3
29	16.5[b]	CH_3	17.4[b]	CH_3	17.3[b]	CH_3	16.4[b]	CH_3
30	17.3[b]	CH_3	16.5[b]	CH_3	17.8[b]	CH_3	17.2[b]	CH_3

[a,b] The assignments in the vertical column with the same sign might be alternated.

rha-H]$^-$; LC–MS: t_R 31.6 min; ^{13}C NMR ([$^2H_5$]pyridine) (Tables 1 and 2).

Ginsenoside Rg_1 (**4**): colorless powder, ESI-MS (pos) m/z: 823 [M+Na]$^+$, 423 [M+2Na]$^{2+}$, 304, MS–MS fragmentation of m/z 823: 645, 463, 203; ESI-MS (neg) m/z: 845 [M+2Na-H]$^-$; MS–MS fragmentation of m/z 845: 799 [M-H]$^-$, 637 [M-glc-H]$^-$, 475 [M-2glc-H]$^-$; LC–MS: t_R 31.0 min; ^{13}C-NMR ([$^2H_5$]pyridine) (Tables 1 and 2). Ginsenoside Rg_1–deca acetate (**4a**): ESI-MS (pos) m/z: 1243 [M+Na]$^+$, 633 [M+2Na]$^{2+}$, MS–MS fragmentation of m/z 1243: 1184, 1123, 895, 835, 775, 715, 371.

The full-scan ESI mass spectrum in positive ionization mode displayed for all saponins **1**–**4** prominent sodium cationized quasimolecular ions of the nature [M+Na]$^+$, and also [M+2Na]$^{2+}$, but subsequent ion-isolation and MS fragmentation resulted in no further structural information. Molecular ion peaks [M-H]$^-$, and sodium adducts [M+2Na-H]$^-$ in negative ESI-MS determined the molecular masses of **1**–**4**. MS–MS fragmentation of [M+2Na-H]$^-$ revealed several new diagnostic ion peaks, including [M-H]$^-$, and indicating the cleavages of single sugars units. Ginsenoside Rb_1 (**1**), with a quasimolecular ion [M-H]$^-$ at m/z 1107.5, gave four fragmentation signals at m/z 945, m/z 783, m/z 621, and m/z 459 due to a subsequent loss of all attached glucose units ($\Delta m/z$ 162). Ion fragment m/z 459

Table 2
^{13}C NMR chemical shifts of sugar moieties of **1**–**4** (δ [ppm] measured in pyridine-d_5)

Saponin **1**, ginsenoside Rb_1		Saponin **2**, notoginsenosides R_1		Saponin **3**, ginsenoside Re		Saponin **4**, ginsenoside Rg_1	
Carbon	^{13}C	Carbon	^{13}C	Carbon	^{13}C	Carbon	^{13}C
3-Glc		**6-Glc**		**6-Glc**		**3-Glc**	
1	104.9	1	103.4	1	101.8	1	106.0
2	83.1	2	79.4	2	79.4	2	75.5
3	79.0	3	78.9[a]	3	78.2[a]	3	78.2
4	71.7	4	71.4[b]	4	72.7	4	72.0
5	77.9[a]	5	79.6	5	78.2[a]	5	80.1
6	64.3	6	62.8[c]	6	63.2[b]	6	63.0
(2→1)Glc		**(2→1)Xyl**		**(2→1)Rha**			
1	105.7	1	104.6	1	101.9		
2	76.8	2	75.6	2	72.3[c]		
3	77.9[a]	3	78.8[a]	3	72.4[c]		
4	71.6	4	71.6[b]	4	74.2		
5	78.2[a]	5	67.0[c]	5	69.5		
6	62.7			6	18.7		
20-Glc		**20-Glc**		**20-Glc**		**20-Glc**	
1	97.9	1	98.1	1	98.3	1	98.2
2	74.9	2	75.0	2	75.2	2	75.1
3	78.8[a]	3	78.6[a]	3	79.2	3	78.1
4	71.5	4	71.1[b]	4	71.8	4	71.8
5	76.8[a]	5	80.0	5	78.5[a]	5	79.2
6	70.0	6	62.7[c]	6	63.0[b]	6	63.2
(6→1)Glc							
1	105.1						
2	75.1						
3	78.1[a]						
4	71.4						
5	78.1[a]						
6	62.8						

[a,b,c] The assignments in the vertical column with the same sign might be alternated.

corroborates the protopanaxadiol partial structure of **1** which is not hydroxylated at C-6. In comparison to **1**, the intense fragmentation ion peak at m/z 475 is related to a protopanaxatriol moiety in the structures **2**–**4**. In more detail, the characteristic MS fragmentation signals of saponin **2** at m/z 931 and m/z 799 are related to the cleaveage of a xylose ($\Delta m/z$ 132), substance **3** yielded fragments at m/z 783, and m/z 637 with a typical mass difference of $\Delta m/z$ 146 explaining the loss of a rhamnose unit.

Investigation of ''*Sanqi Zongdai Pian*'' by HPLC–ESI-MS of the methanolic extract (Fig. 2) clearly indicated that phytomedicinal crude extracts prepared from *Panax notoginseng* contain various natural products beside bioactive saponins. ESI-MS detection was done in both polarities, but interestingly, only negative ionization was efficient to yield good results for saponin detection under HPLC conditions.

Predominantly, dammarane saponins of *P. notoginseng* are composed of a protopanaxatriol aglycone. Applying MS–MS fragmentation, and extraction of the target ion m/z 475 displayed the selective ion-trace for the HSCCC-isolated saponins **2**–**4** with this characteristic backbone (cf. Fig. 2). Additional protopanaxatriol saponin components appeared in this ion-trace which could be scope of further CCC separation studies.

The HPLC separation of the highly polar dammarane saponins required a stationary phase with polar-endcapped C_{18} stationary phase material, such

as Prontosil C_{18} Aqua, resistant to aqueous solvent systems. Unexpectedly, separation on this phase resulted that the most polar saponin **1** with four attached glucose units eluted much later (t_R 35.0 min) than the less polar components **2–4** (t_R 30.0–31.6 min) which might be explained by stronger interactions of the sugar moieties to the polar endcapping groups. The phase, however, did not allow a baseline separation of the substances **2–4** (Fig. 2). This also shows that in the case of polar dammarane saponins, preparative HSCCC has a higher separation efficiency compared to HPLC.

4. Conclusion

The present study demonstrates that HSCCC is a fast and effective methodology suitable for a highly selective preparation of larger amounts of dammarane saponins from ''*Sanqi Zongdai Pian*'' or crude extracts from roots of *Panax notoginseng* in a complex matrix since the one-step separation yielded the pure saponins ginsenoside-Rb_1 (**1**), notoginsenoside-R_1 (**2**), ginsenoside-Re (**3**), and ginsenoside-Rg_1 (**4**).

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References

[1] J. Wei, J. Wang, L. Chang, Y. Du, Acta Pharmacol. Sinica 15 (1980) 359.
[2] Z. Zhang, Z. Wang, J. Chen, L. Jiang, L. Zou, Q. Wang, Y. Song, Q. Li, Acta Pharmacol. Sinica 15 (1980) 385.
[3] M. Yoshikawa, T. Murakami, T. Ueno, K. Yashiro, N. Hirokawa, N. Murakami, J. Yamahara, H. Matsuda, R. Saijoh, O. Tanaka, Chem. Pharm. Bull. 45 (1997) 1039.
[4] C.Y. Kwan, T.K. Kwan, Acta Pharmacol. Sin. 21 (2000) 1101.
[5] M. Yoshikawa, T. Morikawa, K. Yashiro, T. Murakami, H. Matsuda, Chem. Pharm. Bull. 49 (2001) 1452.
[6] S. Sanada, J. Shoji, Shoyakugaka Zasshi 32 (1978) 96.
[7] M. Wu, Acta Bot. Yunnanica 1 (1979) 11.
[8] J. Zhou, M.-Z. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari, T. Fuwa, Chem. Pharm. Bull. 29 (1981) 2844.
[9] T.-R. Yang, R. Kasai, J. Zhou, O. Tanaka, Phytochemistry 22 (1983) 1473.
[10] H. Matsuura, R. Kasai, O. Tanaka, Y. Saruwatari, T. Fuwa, J. Zhou, Chem. Pharm. Bull. 31 (1983) 2281.
[11] T. Namba, K. Matsushige, T. Morita, O. Tanaka, Chem. Pharm. Bull. 34 (1986) 730.
[12] H. Yamaguchi, R. Kasai, H. Matsuura, O. Tanaka, T. Fuwa, Chem. Pharm. Bull. 36 (1988) 3468.
[13] W.G. Ma, M. Mizutani, K.E. Malterud, S.L. Lu, B. Ducrey, S. Tahara, Phytochemistry 52 (1999) 1133.
[14] P. Zhao, Y.-Q. Liu, C.-R. Yang, Phytochemistry 41 (1996) 1419.
[15] O. Tanaka, S. Yahara, Phytochemistry 17 (1978) 1353.
[16] Y. Ito, W.D. Conway (Eds.), High-speed Countercurrent Chromatography, Wiley-Interscience, New York, 1996.
[17] Q. Du, H. Cheng, Y. Ito, J. Chromatogr. A 921 (2001) 331.
[18] Q. Du, Z. Li, Y. Ito, J. Chromatogr. A 921 (2001) 271.
[19] Q. Du, W. Cai, Y. Ito, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 2515.
[20] Q. Du, M. Xia, Y. Ito, J. Chromatogr. A 962 (2002) 239.
[21] Q. Du, P. Wu, Y. Ito, Anal. Chem. 72 (2000) 3363.
[22] Q. Du, S. Che, P. Winterhalter, G.I.T. Lab. J. 6 (2002) 220.