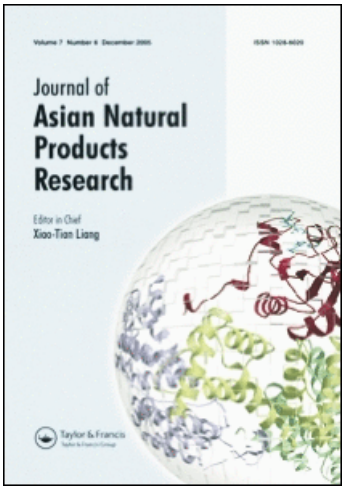

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new steroidal saponins from the seeds of *Allium cepa* L.

Ling Yuan[a]; Teng-Fei Ji[a]; Chuang-Jun Li[a]; Ai-Guo Wang[a]; Jian-Bo Yang[a]; Ya-Lun Su[a]
[a] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

To cite this Article Yuan, Ling , Ji, Teng-Fei , Li, Chuang-Jun , Wang, Ai-Guo , Yang, Jian-Bo and Su, Ya-Lun(2009) 'Two new steroidal saponins from the seeds of *Allium cepa* L.', Journal of Asian Natural Products Research, 11: 3, 213 — 218
To link to this Article: DOI: 10.1080/10286020802696411
URL: http://dx.doi.org/10.1080/10286020802696411

Journal of Asian Natural Products Research
Vol. 11, No. 3, March 2009, 213–218

Two new steroidal saponins from the seeds of *Allium cepa* L.

Ling Yuan, Teng-Fei Ji, Chuang-Jun Li, Ai-Guo Wang, Jian-Bo Yang and Ya-Lun Su*

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

(*Received 11 August 2008; final version received 20 November 2008*)

Two new steroidal saponins, ceparosides C (**1**) and D (**2**), have been isolated from the seeds of *Allium cepa* L. Their structures have been established as 26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5,20(22)-dien-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside (**1**) and 26-*O*-(β-D-glucopyranosyl)-(25*S*)-furost-5,20(22)-dien-3β, 26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside (**2**) by means of spectral and chemical methods.

Keywords: steroidal saponins; *Allium cepa*; ceparocide C; ceparocide D

1. Introduction

The onion seeds (the seeds of *Allium cepa* L.) are used as a traditionally herbal medicine of the Uygur (Uighur) nationality in Xinjiang, which can improve the functions of internal organs, treat diarrhea, fervescence, calenture, and puffiness of face and eye, promote blood flow, and prevent ectroma. There are few reports about the constituents and pharmacological action of the onion seeds. In the search for biologically active constituents from the natural products, we investigated the constituents of the ethanolic extract of the seeds, and have isolated four steroidal saponins [1] and seven other kinds of known compounds [2]. Further study on the constituents of the onion seeds led to the isolation of two new steroidal saponins, named ceparosides C (**1**) and D (**2**). Their structures were elucidated as 26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5,20(22)-dien-3β, 26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside (**1**) and 26-*O*-(β-D-glucopyranosyl)-(25*S*)-furost-5,20(22)-dien-3β, 26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside (**2**) by means of spectral and chemical methods (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous solid and was deduced to possess a furostanol structure based on TLC examination using Ehrlich's reagent [3]. The IR spectrum exhibited absorption bands at 3346 and 1026 cm^{-1}, suggestive of the oligoglycosidic structure. In the negative- and positive-ion ESI-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 1069 $[M+Na]^+$ and 1045 $[M-H]^-$, respectively, and high-resolution FAB-MS analysis revealed the molecular formula of **1** to be $C_{51}H_{82}O_{22}$. Acid hydrolysis of **1** with 2 N hydrochloric acid-1,4-dioxane (1:1, v/v) furnished D-glucose, L-rhamnose, and D-galactose in the ratio of 2:1:1, which were identified by gas-liquid chromatography analysis of the thiazolidine

*Corresponding author. Email: suyalun@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802696411
http://www.informaworld.com

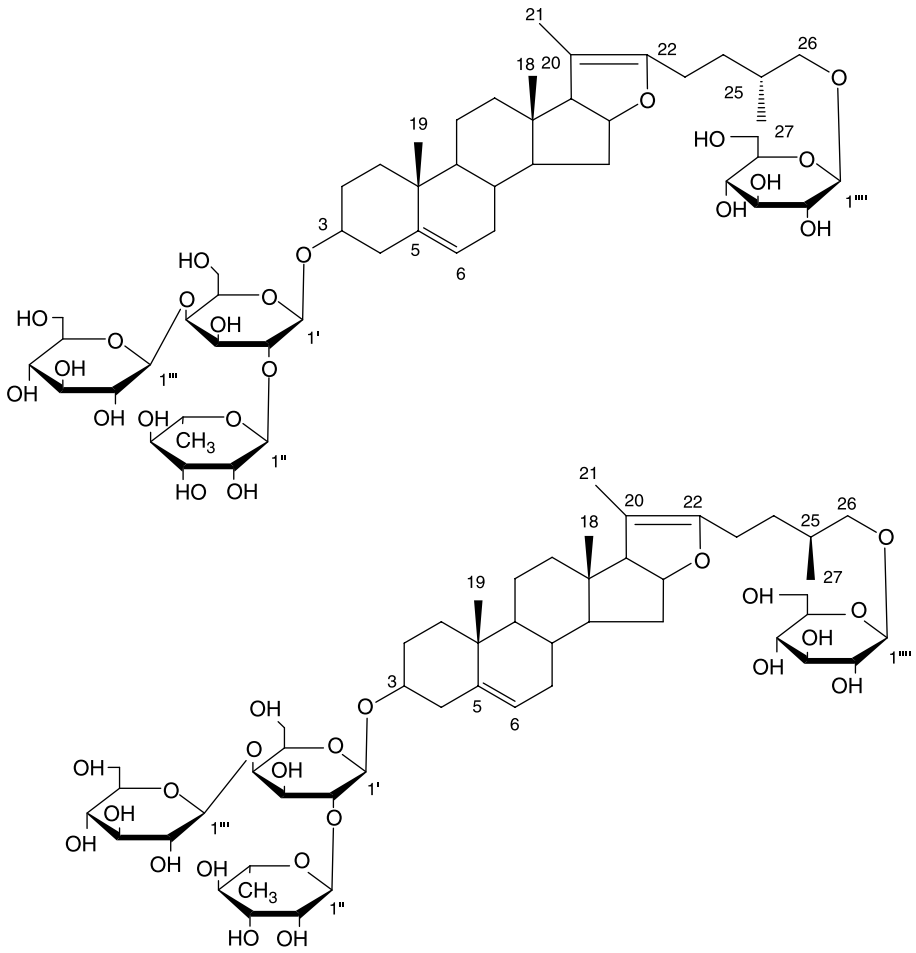

Figure 1. Structures of compounds **1** and **2**.

derivatives. The ^{1}H NMR spectrum of **1** revealed the presence of five methyl proton signals at δ_H 0.71 (s, Me-18), 1.06 (s, Me-19), 1.63 (s, Me-21), 1.01 (d, $J = 6.5$ Hz, Me-27), 1.71 (d, $J = 6.0$ Hz, Me-Rha), as well as an olefinic proton at δ_H 5.31 (d, $J = 4.0$ Hz, H-6). The signals at δ_C 140.9, 121.7 and 103.6, 152.4 in the ^{13}C NMR spectrum were assigned for C-5(6) and C-20(22) by comparison with the literature values [4]. Comparison of the ^{1}H and ^{13}C NMR spectral data of compound **1** with those of pseudoprotodioscin [5] indicated the same furostanol aglycone of furost-5, 20(22)-dien-3β, 26-diol, and the small difference between the chemical shifts of two protons of C-26 (δ_{H1}, 3.62; δ_{H2}, 3.94; $26_{H1} - 26_{H2} < 0.48$ ppm) provided the evidence for C-25*R* configuration in **1** [6]. The HMBC spectrum indicated long-range correlations between CH_3-21 and C-20, C-22, and C-17, between angular methyl protons CH_3-19 and the carbon signals C-5, C-10, and C-1, and between the olefinic proton H-6 and the carbon signal C-10 (see Figure 2). These data convinced the presence of two double bonds at C-5(6) and C-20(22).

The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by the analysis of ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and TOCSY spectra. The ^{13}C NMR spectroscopic data for the sugar moieties indicated that all

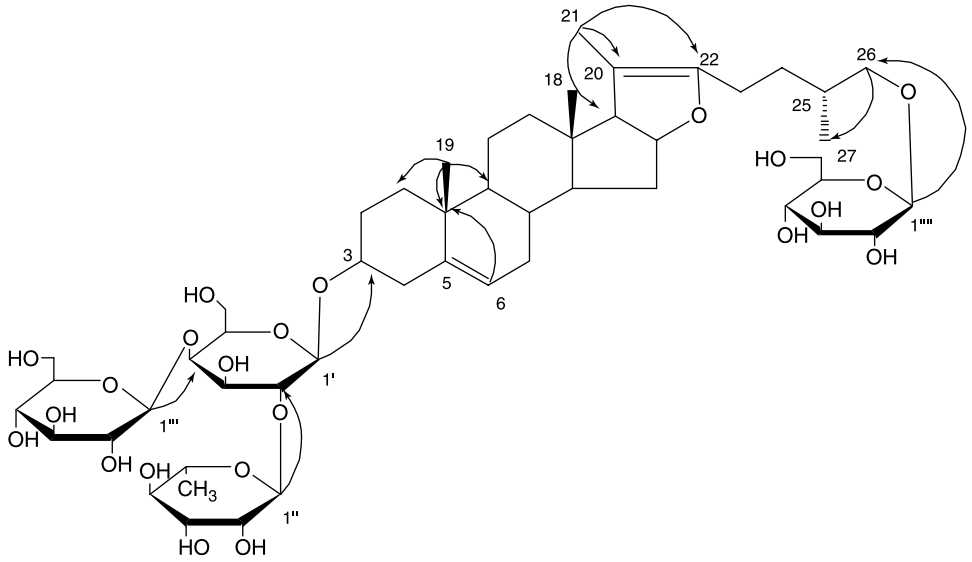

Figure 2. Key HMBC correlations of compound **1**.

the monosaccharides were in pyranose forms. The α-anomeric configuration for rhamnose was determined by its C_5 data (δ_C 69.5) [7]. The β-anomeric configurations for the two glucoses and galactose were determined from their large $^3J_{1,2}$ coupling constants ($J = 8.5$, 7.5, 8.0 Hz). In the HMBC spectrum, the anomeric proton signals at δ_H 4.89 (H-1′), 6.25 (H-1″), 5.17 (H-1‴), and 4.82 (H-1⁗) showed cross-peaks with the carbon signals at δ_C 77.8 (C-3), 76.7 (C-2′), 81.2 (C-4′), and 74.9 (C-26), respectively (Figure 2, Table 1). These signals provide ample evidence to determine the linkages between the sugars and the sugar and the aglycone. On the basis of the foregoing evidence, the structure of **1** was determined as 26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5, 20(22)-dien-3β, 26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-galactopyranoside, named ceparoside C.

Compound **2** was obtained as a white amorphous solid with $[\alpha]_D^{20} - 41$ ($c = 0.01$, MeOH) and mp 177.4–178.0°C, and gave positive reaction with Ehrlich's reagent on TLC, which indicated **2** was a furostanol saponin. The IR spectrum exhibited absorption bands at 3354, 1070, and 1050 cm^{-1}, suggestive of the oligoglycosidic structure. HR-FAB-MS showed $[M + Na]^+$ at *m/z* 1069.5231, corresponding to the molecular formula $C_{51}H_{82}O_{22}$. The ^1H and ^{13}C NMR spectral data of **2** were quite similar to those of **1**, except for the chemical shifts of the two protons at C-26 (δ_{H1}, 4.06; δ_{H2}, 3.48; $26_{H1} - 26_{H2} > 0.57$ ppm); thus, compound **2** was deduced to be the 25*S* isomer of **1**. The structure of compound **2** was elucidated as 26-*O*-(β-D-glucopyranosyl)-(25*S*)-furost-5,20(22)-dien-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-galactopyranoside, named ceparoside D.

3. Experimental

3.1 General experimental procedures

Melting points were determined at an XT_4-100_x micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. The IR spectra were obtained on Nicolet 5700 IR spectrometer. The NMR spectra were recorded on a Inova 500 (^1H, 500 MHz; ^{13}C, 125 MHz) spectrometer. ESI-MS was performed with Angilent 1100 LC/MSD. For column chromatography, silica gel (200–300 mesh, Qingdao

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **1** and **2** in pyridine-d_5 (δ, ppm).

	1		**2**	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	37.6	0.98 m, 1.72 m	37.6	0.98 m, 1.71 m
2	30.2	2.12 m, 2.14 m	30.2	2.14 m, 2.15 m
3	77.8	3.88 m	77.8	3.90 m
4	38.9	2.72 m, 2.78 m	38.9	2.73 m, 2.78 m
5	140.9	–	140.9	–
6	121.7	5.31 d (4.0)	121.7	5.30 d (4.0)
7	34.5	1.50[a], 2.10 m	34.5	1.51[a], 2.11 m
8	31.4	1.50[a]	31.4	1.49[a]
9	50.3	0.92 m	50.3	0.93 m
10	37.1	–	37.1	–
11	21.2	1.32 m, 1.46 m	21.2	1.32 m, 1.46 m
12	39.6	1.15 m, 1.72[a]	39.6	1.13 m, 1.71[a]
13	43.4	–	43.4	–
14	54.9	0.88 m	54.9	0.88 m
15	32.4	1.50 m, 1.88 m	32.4	1.50 m, 1.87 m
16	84.5	4.79[a]	84.5	4.80[a]
17	64.5	2.44 d (10.5)	64.5	2.43 d (10.0)
18	14.1	0.71 s	14.1	0.69 s
19	19.4	1.06 s	19.4	1.06 s
20	103.6	–	103.5	–
21	11.8	1.63 s	11.8	1.61 s
22	152.4	–	152.4	–
23	23.7	2.22 m, 2.23 m	23.7	2.22 m, 2.23 m
24	31.4	1.46[a], 1.82[a]	31.4	1.43[a], 1.84[a]
25	33.5	1.94 m	33.5	1.94 m
26	74.9	3.62 dd (6.0, 9.5), 3.94[a]	74.9	3.48 dd (7.0, 9.5), 4.06[a]
27	17.3	1.01 d (6.5)	17.2	1.00 d (6.0)
Gal(inner)				
1′	100.3	4.89 d (8.0)	100.3	4.89 d (8.0)
2′	76.7	4.54[a]	76.7	4.52[a]
3′	76.4	4.26[a]	76.4	4.26[a]
4′	81.2	4.58[a]	81.2	4.57[a]
5′	78.5	4.24[a]	78.5	4.24[a]
6′	63.1	4.18[a], 4.56[a]	62.9	4.17[a], 4.56[a]
Rha				
1″	102.2	6.25 s	102.2	6.24 s
2″	72.5	4.76 m	72.6	4.76 m
3″	72.8	4.60 m	72.8	4.60 m
4″	74.1	4.32 t (9.5)	74.1	4.32 t (9.5)
5″	69.5	4.96 m	69.5	4.96 m
6″	18.7	1.71 d (6.0)	18.7	1.71 d (6.0)
Glu				
1‴	107.2	5.17 d (8.5)	107.2	5.16 d (8.5)
2‴	75.6	4.08 m	75.6	4.07 m
3‴	78.6	4.19 m	78.6	4.19 m
4‴	72.2	4.02 m	72.2	4.03 m
5‴	78.5	3.95 m	78.5	3.95 m
6‴	60.9	4.40 m, 4.60 m	60.9	4.38 m, 4.59 m
26-glu				
1⁗	104.9	4.82 d (7.5)	104.9	4.82 d (7.5)
2⁗	75.2	4.02 m	75.2	4.03 m

Table 1 – *continued*

Position	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
3‴′	78.6	4.24 m	78.6	4.24 m
4‴′	71.7	4.24 m	71.7	4.24 m
5‴′	78.5	3.96 m	78.5	3.96 m
6‴′	62.9	4.40 m, 4.57 m	62.9	4.39 m, 4.55 m

[a] Overlap with other signal.

Marine Chemistry Company, Qingdao, China), ODS (40–60 μm, Alltech, Deerfield, IL, USA), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used.

3.2 Plant material

The seeds of *A. cepa* L. were purchased in XinJiang (Autonomous Region), China and identified by Prof. Zhang-Yanfu, Institute of XinJiang Materia Medica. The voucher specimen (No. 050219) has been deposited at the Department of Phytochemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

The seeds of *A. cepa* (14.75 kg) were powered and extracted with petroleum ether (3 × 3 l) to remove the oil, then the dried residue was extracted with 95% EtOH under reflux (3 × 3 l), and the combined extracts were concentrated, then the resulting extract (2100 g) was suspended in water and successively extracted with petroleum ether, ethyl acetate, and *n*-butanol was saturated with water to give the respective extracts after solvent removal. The *n*-butanol portion (100 g) was subjected to column chromatography on macroporous resin with gradient EtOH (0–95%) to give five fractions (Fractions I–V). Fraction IV (eluted with 70% EtOH) was separated on a C-18 medium pressure column, eluted with gradient MeOH (30–100%) and then purified with preparative HPLC (eluted with 65% MeOH) repeatedly to give compounds **1** (36 mg) and **2** (28 mg), successively.

3.3.1 Ceparoside C (*1*)

White amorphous solid, $[\alpha]_D^{20} - 47$ ($c = 0.01$, MeOH); mp 179.0–180.5 °C; IR ν_{max} (cm^{-1}): 3346, 2934, 2902, 1640, 1434, 1365, 1026, 914, and 633; ^{1}H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data: see Table 1; ESI-MS *m/z*: 1069.6 $[M + Na]^+$ and 1045.5 $[M - H]^-$; HR-FAB-MS *m/z*: 1069.5261 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{22}Na$, 1069.5195).

3.3.2 Ceparoside D (*2*)

White amorphous solid, $[\alpha]_D^{20} - 41$ ($c = 0.01$, MeOH), mp 177.4–178.0°C. IR ν_{max} (cm^{-1}): 3354, 2934, 2904, 1691, 1647, 1450, 1435, 1377, 1367, 1070, 1050, 914, and 637; ^{1}H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data: see Table 1; ESI-MS *m/z*: 1069.6 $[M + Na]^+$, 1045.5 $[M - H]^-$; HR-FAB-MS *m/z*: 1069.5261 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{22}Na$, 1069.5195).

3.4 Acid hydrolysis and determination of the absolute configuration of the monosaccharides

Compound **1** (20 mg) was dissolved in 2 M HCl–H_2O (5 ml) and heated at 80°C for 4 h. After filtration of the reaction mixture, the precipitate was extracted with $CHCl_3$ to afford sapogenin. The H_2O layer was evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was

dissolved in pyridine (1 ml), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60°C for 2 h and evaporated under N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the *n*-hexane extract was analyzed by GC under the following conditions: capillary column, DB-5 (30 m × 0.25 mm × 0.25 μm); detection, FID; detector temperature, 280°C; injection temperature, 250°C; initial temperature was maintained at 100°C for 2 min and then raised to 280°C at the rate of 10°C/min, and final temperature was maintained for 5 min; carrier, N_2 gas. In the acid hydrolysate of **1**, L-rhamnose, D-galactose, and D-glucose were confirmed by comparison of the retention times of their derivatives with those of L-rhamnose, D-galactose, and D-glucose derivatives prepared in a similar way, which showed retention times of 20.76, 22.53, and 22.27 min, respectively. The sugars of compounds **2** were also identified by the same method.

Acknowledgements

The authors are grateful to the staff of the analytical group at the Institute of Materia Medica for measuring the spectral data.

References

[1] L. Yuan, T.F. Ji, A.G. Wang, J.B. Yang, and Y.L. Su, *Chin. Chem. Lett.* **19**, 461 (2008).
[2] L. Yuan, T.F. Ji, A.G. Wang, J.B. Yang, and Y.L. Su, *J. Chin. Med. Mater.* **2**, 222 (2008).
[3] S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* **16**, 1162 (1968).
[4] H.F. Chen, N.L. Wang, and X.S. Yao, *Chin. J. Med. Chem.* **15** (31), 142 (2005).
[5] M. Dong, X.Z. Feng, B.X. Wang, L.J. Wu, and T. Ikejima, *Tetrahedron* **57**, 501 (2001).
[6] P.K. Agrawal, *Steroids* **70**, 715 (2005).
[7] S.M. Sang, S.L. Mao, A.N. Lao, Z.L. Chen, and C.T. Ho, *J. Agric. Food Chem.* **49**, 1475 (2001).