Cardiac Glycosides from *Erysimum cheiranthoides*

Zhen-Huan LEI,*[,a] Hitoshi NAKAYAMA,[a] Akihiko KUNIYASU,[a] Bao-Shan TAI,[b] and Toshihiro NOHARA[a]

[a] *Faculty of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan: and* [b] *Department of Traditional Chinese Medicine, Heilongjang Institute of Commerce; 138 Tongda St., Daoli, Harbin, China.*

Received January 7, 2002; accepted February 26, 2002

Three new cardiac glycosides named cheiranthoside VIII (1), cheiranthoside IX (2) and cheiranthoside X (3) were isolated from the seeds of *Erysimum cheiranthoides*. Based on spectroscopic data, the structures of 1—3 were characterized as strophanthidin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-antiaropyranoside, cheiranthidin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-boiviopyranoside and cheiranthidin 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-digitoxopyranoside, respectively. The aglycone moiety possessing a carboxyl group at C-10 of 2 and 3 was regarded to be determined for the first time.

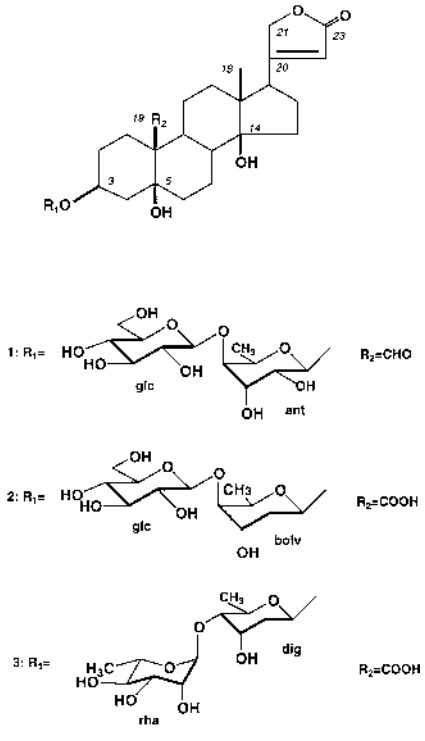
Key words *Erysimum cheiranthoides*; Cruciferae; 19-carboxylated cardiac glycoside; cheiranthoside; strophanthidin

Erysimum cheiranthoides L. (Cruciferae) is a Chinese crude drug used for treating cardiac diseases, weak cardiopalmus, edema, dyspepsia, *etc.*[1] In previous papers,[2—4] we reported seven new cardenolides. As a continuing study on the constituents in the seeds, three additional new glycosides named cheiranthosides VIII, IX and X were isolated, and their chemical structures are described here.

Cheiranthoside VIII (**1**), obtained as a white powder, $[\alpha]_D$ −5.2° (MeOH), showed a quasimolecular ion at *m/z* 711 $[M-H]^-$ in the negative FAB-MS. Its ^{1}H-NMR spectrum displayed signals due to H_3-18 (3H, s) at δ 1.00, H-17 (1H, br d, J=8.5 Hz) at δ 2.78, H_2-21 (each 1H, br d, J=18.3 Hz) at δ 4.98 and 5.28, H-22 (1H, s) at δ 6.12, CHO-19 at δ 10.37 on the cardiac steroidal framework, and two anomeric protons at δ 4.57 (1H, d, J=7.3 Hz) and 5.38 (1H, d, J=8.5 Hz), and H_3-6 at δ 1.52 (3H, d, J=6.1 Hz) representing of one 6-deoxysugar in the sugar moiety. The ^{13}C-NMR spectrum showed signals due to a total of 35 carbons, among which 23 carbon signals were originated from the steroidal aglycone part and were coincident with those of strophanthidin; the remaining 12 carbons should be assigned to a sugar moiety. Comparative studies of the ^{1}H- and ^{13}C-NMR spectra with those of the glycosidic linkages of cheiranthosides already obtained from the seeds of the title plant resulted in the identification of **1** with that of the sugar linkage, β-D-glucopyranosyl-(1→4)-β-D-antiaropyranosyl[5—7] moiety, of cheiranthoside VII.[4] Heteronuclear multiplebonds correlation (HMBC) spectrum of **1** showed the connectivities between C-3 (δ_C 74.8) of the aglycone and the H-1 (δ_H 5.38) of antiarose, and between the H-1 (δ_H 4.57) of glucose and the C-4 (δ_C 79.1) of antiarose. Therefore, **1** was characterized as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-antiaropyranosyl strophanthidin, as shown in the Formulae.

Cheiranthoside IX (**2**), obtained as a white powder, $[\alpha]_D$ +1.8° (MeOH), showed a quasimolecular ion peak at *m/z* 711 $[M-H]^-$ in the negative FAB-MS. The ^{1}H-NMR spectrum showed signals due to H_3-18 (3H, s) at δ 1.03, H-17 (1H, br s) at δ 2.80, H_2-21 (each 1H, br d, J=18.3 Hz) at δ 5.05 and 5.33 , H-22 (1H, s) at δ 6.12 in the aglycone part, and two anomeric protons at δ 4.92 (1H, d, J=7.3 Hz) and 5.46 (1H, br d, J=9.8 Hz) and a methyl group at δ 1.54 (3H, d, J=6.7 Hz) of a 6-deoxyhexosyl moiety in the sugar part. The ^{13}C-NMR spectrum of **2** showed a total of 35 carbon signals, among which 23 were assigned to an aglycone, in which a carboxyl carbon signal at δ 176.1 was replaced by an aldehyde carbon signal at δ 208.8 in **1**. This cardiac aglycone could be deduced to have a carboxyl group instead of the aldehyde group substituted at C-10 of strophanthidin. As far as is known, the cardiac aglycone carrying the carboxylic group at C-10 was obtained for the first time and named cheiranthidin. Concerning the sugar linkage, the presence of a terminal glucopyranosyl moiety was easily revealed, and the remaining six carbon signals (δ 98.2, 35.2, 66.3, 76.5, 69.5, 17.7) were attributable to a 4-*O*-substituted boiviopyranosyl moiety, by comparison with those of a β-D-glucopyranosyl-(1→4)-β-D-boiviopyranosyl moiety in cheiranthoside I.[2] Therefore, the chemical structure of **2** was characterized as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-boiviopyranosyl cheiranthidin, as shown in the Formulae.

Cheiranthoside X (**3**) obtained as a white powder, $[\alpha]_D$ +16.3° (MeOH), showed a quasimolecular ion peak at *m/z* 695 $[M-H]^-$ in the negative FAB-MS. The ^{1}H-NMR spectrum showed signals due to H_3-18 at δ 1.04, H-17 (1H, br d,



* To whom correspondence should be addressed. e-mail: none@gpo.kumamoto-u.ac.jp

J=8.5 Hz) at δ 2.81, H_2-21 (each 1H, br d, J=18.3 Hz) at δ 5.06 and 5.33, and H-22 (1H, s) at δ 6.13, as well as two anomeric protons at δ 5.45 (1H, br d, J=9.8 Hz) and 5.48 (1H, s). Moreover, the respective proton signals at δ 5.45 (1H, d, J=9.8 Hz), 4.37 (1H, d, J=6.7 Hz), 1.82, 2.24 (each 1H, d, J=15.4 Hz), 1.38 (3H, d, J=6.7 Hz), 5.48 (1H, s), 4.38 (1H, d, J=6.1 Hz), and 1.38 (3H, d, J=6.1 Hz) could be assigned to the H-1, H-5 and H_2-2, H_3-6 of β-D-digitoxopyranosyl, and the H-1, H-5 and H_3-6 of α-L-rhamnopyranosyl residues. The ^{13}C-NMR spectrum of **2** showed a total of 35 carbon signals, among which 23 were assigned to the aglycone, cheiranthidin, 6 to a terminal rhamnopyranosyl moiety and the remaining 6 (δ 104.1, 72.5, 73.8, 79.0, 70.4, 18.6) to a 4-*O*-substituted-β-D-digitoxopyranosyl moiety, by comparison with those of cheiranthoside II.[2] Therefore, the chemical structure of **3** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-digitoxopyranosyl cheiranthidin, as shown in the Formulae.

Although much attention has focused on the change in Na^+, K^+-ATPase inhibiting activity, the introduction of carboxylic acid at C-10 resulted in a decrease of their activities in **2** and **3**.[8]

It is worth noting that the cardiac glycosides having a novel aglycone, cheiranthidin, were isolated from *Cruciferae* plant.

Experimental

General Optical rotations were determined on a JASCO DIP-1000 digital polarimeter. FAB-MS were obtained in a glycerol matrix in the negative ion mode using JEOL JMS-DX 300 and JMS-DX 303 HF instruments. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer, and chemical shifts were relative to tetramethylsilane (TMS). TLC was performed on precoated silica gel 60F_{254} (Merck), and detection was achieved by spraying with 10% H_2SO_4, followed by heating. Column chromatography was carried out on silica gel 60 (230—400 mesh, Merck), MCI gel CHP-20P (75—150 μm, Mitsubishi Chemical Ind.), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals) and Chromatorex ODS (30—5 μm, Fuji Silysia Chemical Ltd.).

Plant Material The seeds of *E. cheiranthoides* were harvested at Harbin, Heilongjiang Province, in China.

Extraction and Isolation The seeds (2.5 kg) of *Erysimum cheiranthoides* L. were extracted with MeOH, and the extract (189 g) was partitioned between hexane and water. The aqueous layer (123 g) was subjected to MCI gel CHP 20P column chromatography, and eluted with water, 40% MeOH, 60% MeOH, 80% MeOH and MeOH, gradiently increasing the MeOH. The 40% eluate (10 g) was subjected to Sephadex LH-20, Chromatorex ODS and silica gel column chromatographies to provide cheiranthosides VIII (**1**, 4.2 mg), IX (**2**, 13.2 mg) and X (**3**, 8.8 mg).

Cheiranthoside VIII (**1**): A white powder, $[\alpha]_D^{31}$ −5.2° (c=0.42, MeOH), negative FAB-MS (m/z): 711 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 1.00 (3H, s, H_3-18), 1.52 (3H, d, J=6.1 Hz, ant H_3-6), 2.78 (1H, br d, J=8.5 Hz, H-17), 4.19 (overlapped, glc H-4), 4.31 (1H, br s, ant H-4), 4.57 (1H, d, J=7.3 Hz, glc H-1), 4.60 (1H, dd, J=3.0, 8.2 Hz, ant H-2), 4.98, 5.28 (each 1H, br d, J=18.3 Hz, H_2-21), 5.38 (1H, d, J=8.2 Hz, ant H-1), 6.12 (1H, s, H-22), 10.37 (1H, s, H-19). ^{13}C-NMR (pyridine-d_5) δ: 18.7, 26.0, 74.8, 35.0, 73.6, 37.8, 24.9, 41.9, 39.5, 55.5, 22.7, 39.6, 49.9, 84.4, 32.2, 27.2, 51.1, 16.0, 208.8, 175.7, 73.7, 117.8, 174.5 (C-1—23, respectively). 99.3, 69.3, 70.1, 79.1, 69.2, 17.1 (ant C-1—6, respectively), 103.3, 72.9, 78.5, 72.1, 78.5, 63.0 (glc C-1—6, respectively). *Anal.* Calcd for $C_{35}H_{52}O_{15}$: C, 58.97; H, 7.35. Found: C, 59.12; H, 7.33.

Cheiranthoside IX (**2**): A white powder, $[\alpha]_D^{18}$ +1.8° (c=0.45, MeOH), negative FAB-MS (m/z): 711 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 1.03 (3H, s, H_3-18), 1.54 (3H, d, J=6.7 Hz, boiv H_3-6), 2.80 (1H, br s, H-17), 4.92 (1H, d, J=7.3 Hz, glc H-1), 5.05, 5.33 (each 1H, br d, J=18.3 Hz, H_2-21), 5.46 (1H, br d, J=9.8 Hz, boiv H-1), 6.12 (1H, s, H-22). ^{13}C-NMR (pyridine-d_5) δ: 24.3, 26.8, 74.8, 36.4, 74.8, 37.0, 24.2, 40.6, 39.4, 55.0, 21.6, 39.9, 50.3, 84.9, 32.6, 27.5, 51.3, 16.5, 176.1, 176.1, 73.8, 117.7, 174.6 (C-1—23, respectively), 98.2, 35.2, 66.3, 76.6, 69.5, 17.7 (boiv C-1—6, respectively), 103.4, 74.8, 78.4, 71.9, 78.5, 63.1 (glc C-1—6, respectively). *Anal.* Calcd for $C_{35}H_{52}O_{15}$: C, 58.97; H, 7.35. Found: C, 59.03; H, 7.37.

Cheiranthoside X (**3**): A white powder, $[\alpha]_D^{18}$ +16.3° (c=0.53, MeOH), negative FAB-MS (m/z): 695 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 1.04 (3H, s, H_3-18), 1.38 (3H, d, J=6.7 Hz, dig H_3-6), 1.57 (3H, d, J=6.1 Hz, rha H_3-6), 1.82, 2.24 (each 1H, d, J=15.4 Hz, dig H_2-2), 1.92, 2.47 (each 1H, br d, J=10.4 Hz, H_2-7), 2.81 (1H, br d, J=8.5 Hz, H-17), 3.62 (1H, dd, J=2.4, 9.2 Hz, dig H-4), 4.60 (1H, br q, J=6.1 Hz, dig H-5), 4.38 (1H, d, J=6.1 Hz, rha H-5), 5.06, 5.33 (each 1H, br d, J=18.3 Hz, H_2-21), 5.45 (1H, br d, J=9.8 Hz, dig H-1), 5.48 (1H, s, rha H-1), 6.13 (1H, s, H-22). ^{13}C-NMR (pyridine-d_5) δ: 24.2, 26.7, 75.3, 36.4, 73.8, 37.2, 24.2, 40.5, 39.4, 55.1, 21.5, 40.1, 50.3, 84.8, 32.5, 27.5, 51.2, 16.5, 177.0, 176.0, 73.8, 117.7, 174.6 (C-1—23, respectively), 97.7, 39.8, 68.9, 82.5, 67.5, 18.5 (dig C-1—6, respectively), 104.1, 72.4, 72.5, 73.8, 70.4, 18.6 (rha C-1—6, respectively). *Anal.* Calcd for $C_{35}H_{52}O_{14}$: C, 60.33; H,7.52. Found: C, 60.35; H, 7.49.

References

1) Zhu Y. C., "Planate Medicinales Chinae Boreali-Orientalis," Heilongjiang Science and Technology Publishing House, 1989, p. 463.
2) Lei Z. H., Yahara S., Nohara T., Shan T. B., Xiong J. Z., *Phytochemistry*, **41**, 1187—1189 (1996).
3) Lei Z. H., Jin Z. X., Ma Y. L., Tai B. S., Kong T. Q., Yahara S., Nohara T., *Phytochemistry*, **49**, 1801—1803 (1998).
4) Lei Z. H., Yahara S., Nohara T., Tai B. S., Xiong J. Z., Ma Y. L., *Chem. Pharm. Bull.*, **48**, 290—292 (2000).
5) Makarevich I. F., Kolesnikov D. G., *Khim. Prirodn. Soedin.*, **5**, 363—367 (1965).
6) Mahato S. B., Sahu N. P., Roy S. K., *J. Chem. Soc.*, *Perkin Trans. I*, **1989**, 2065—2068 (1989).
7) Carter C. A., Gray E. A., Schneider T. L., *Tetrahedron*, **53**, 16959—16968 (1997).
8) Lei Z. H., Kuniyasu A., Tai B. S., Nakayama H., Nohara T., *Planta Medica*, **67**, 369—370 (2001).