Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 481–484
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Maltol glucosides from the tuber of *Smilax bockii*

Hongzhu Guo[a,b], Kazuo Koike[a], Wei Li[a], Dean Guo[b], Tamotsu Nikaido[a,*]

[a]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan*
[b]*Division of Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China*

Received 8 July 2003; received in revised form 7 October 2003

Abstract

Two maltol glucosides, bockiosides A and B, along with 10 known compounds, were isolated from the tuber of *Smilax bockii* (Liliaceae), and their structures were elucidated by spectral experiments, chemical analysis and comparison with literature data.

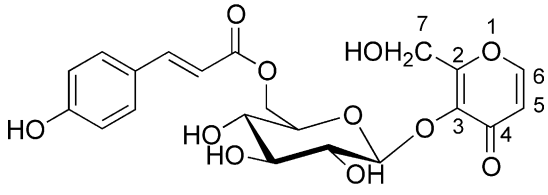
Keywords: *Smilax bockii*; Liliaceae; Bockioside A; Bockioside B; Maltol glucoside

1. Introduction

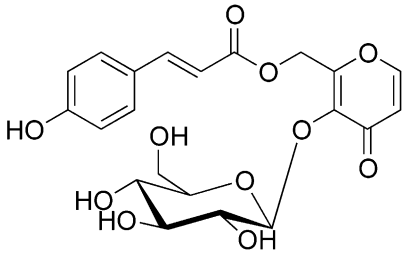
Many species of the genus *Smilax* (Liliaceae), known to be rich in steroid saponins, are used as herbs. An example is *S. glabra*, listed in Chinese Pharmacopoeia as having anti-inflammatory and antiviral properties (China Pharmacopoeia Committee, 2000). Extensive phytochemical investigations on the genus have led to isolation of various steroid saponins (Sashida et al., 1992), phenylpropanoids (Chen et al., 2000), phenolic compounds (Li et al., 2002), and flavonol glycosides (Chen et al., 1999). *S. bockii*, one specimen of the genus, is used as a folk herb in south of China to treat rheumatoid arthritis and woman ailments (China Materia Medica Dictionary, 1977), but to our best knowledge, there was no report on its chemical constituents. As part of our current interest in chemotaxonomy of the genus *Smilax*, we carried out a systematic phytochemical investigation on the tuber of *S. bockii*, which led to the isolation of 12 compounds (**1**–**12**), including two new maltol glucosides (**1**, **2**).

2. Result and discussion

An EtOH extract of *S. bockii* was suspended in water and then partitioned successively with EtOAc and *n*-BuOH. The EtOAc soluble part was subjected to silica gel, ODS open column chromatography (CC) and further purified with prep-HPLC to furnish astilbin (**6**) (De Britto et al., 1995), engeletin (**7**) (Gaffield et al., 1975), arthromerin B (**8**) (Yu et al., 1992), and rutin (**9**) (Harborne et al., 1982). The *n*-BuOH soluble part was subjected to Diaion HP-20, silica gel, ODS CC and further purified by prep-HPLC to furnish pseudoproto-Pb (**11**), pseudoproto-dioscin (**12**) (Hirai et al., 1986), maltol 3-*O*-β-D-glucoside (**3**) (Sala et al., 2001), hydroxymaltol 3-*O*-β-D-glucoside (**4**) (Murakami et al., 1978; Looker and Fisher, 1985), isoinnovanoside (**5**) (Yasue et al., 1970), 2-hydroxy-5-(2-hydroxyethyl) phenyl-β-D-glucopyranoside (**10**) (Sugiyama and Kikuchi, 1992), and two new maltol glucosides, bockiosides A (**1**) and B (**2**).



1



2

* Corresponding author. Tel.: +81-47-472-1391; fax: +81-47-472-1404.
E-mail address: nikaido@phar.toho-u.ac.jp (T. Nikaido).

0031-9422/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2003.10.020

Bockioside A (**1**) was obtained as a colorless amorphous solid and its molecular formula, $C_{21}H_{22}O_{11}$, was determined by positive ion ESI MS ($[M+K]^+$, *m/z* 489.4) and HR SI MS. The ^1H and ^{13}C NMR spectra of **1** showed signals due to a hydroxymaltol group [δ_H 7.88 (1H, *d*, *J* = 5.6 Hz), 6.36 (1H, *d*, *J* = 5.6 Hz), 4.71 (1H, *d*, *J* = 14.0 Hz) 4.57 (1H, *d*, *J* = 14.0 Hz); δ_c 161.5, 142.3, 177.3, 117.6, 157.4, 57.7], a *p*-coumaric acid group [δ_H 7.55 (1H, *d*, *J* = 16.0 Hz), 6.25 (1H, *d*, *J* = 16.0 Hz), 7.41 (2H, *d*, *J* = 8.8 Hz), 6.77 (2H, *d*, *J* = 8.8 Hz); δ_c 127.1, 131.3, 116.8, 161.4, 146.9, 114.8, 168.9], and a β-glucopyranose group, with an anomeric proton at δ 4.92 (1H, *d*, *J* = 7.8 Hz) (Table 1). Enzymatic hydrolysis of **1** furnished D-glucopyranose which was confirmed by co-HPLC analysis of its 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyglucitol acetate derivative with the same derivative of standard sugar. An obvious correlation signal between δ 4.92 and δ 142.3 has been observed in HMBC experiment, which suggested that the linkage was at C-3 (Fig. 1). In the ^{13}C NMR spectrum, C-6 (δ 64.2) of glucose was shifted downward about 1 ppm relative to that of **4** (Looker and Fisher, 1985), which indicated the *p*-coumaric acid group is located at C-6 of glucose. The correlations were also observed between δ 4.42 (H-6a), 4.34 (H-6b) and δ 168.9 in the HMBC spectrum, which confirmed that **1** is a *p*-coumaric acid ester of hydroxymaltol 3-*O*-β-D-glucoside (**4**) at C-6 of glucose. Thus, the structure of **1** was elucidated as hydroxymaltol 3-*O*-(6-*O*-*p*-coumaryl)-β-D-glucopyranoside.

Bockioside B (**2**) was isolated as a colorless solid, and its molecular formula, $C_{21}H_{22}O_{11}$, was determined by

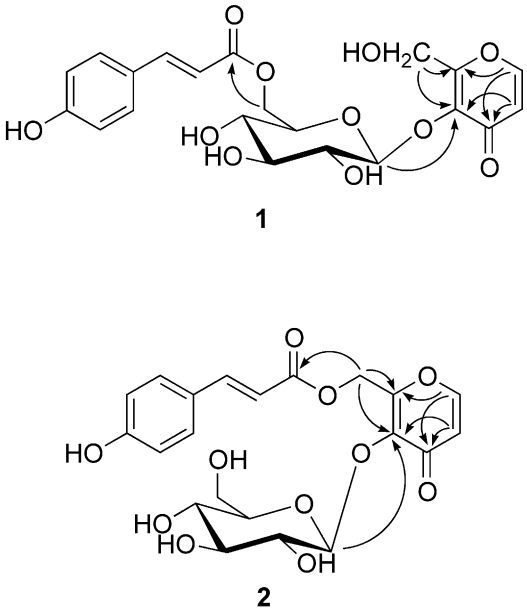


Fig. 1. Key ^1H–^{13}C long-range correlations by HMBC spectra of **1** and **2**.

Table 1
^1H and ^{13}C NMR spectroscopy data for bockiosides A (**1**) and B (**2**)

Position	**1**		**2**	
	^{13}C	^1H	^{13}C	^1H
Hydroxylmaltol				
2	161.5		161.7	
3	142.3		143.8	
4	177.3		177.2	
5	117.6	6.36 (*d*, *J* = 5.6 Hz)	117.9	6.50 (*d*, *J* = 6.0 Hz)
6	15.74	7.88 (*d*, *J* = 5.6 Hz)	157.5	8.08 (*d*, *J* = 6.0 Hz)
7	57.7	4.71 (*d*, *J* = 14.0 Hz)	59.2	5.38 (*s*)
		4.71 (*d*, *J* = 14.0 Hz)		
p-*Coumaric acid*				
1	127.1		126.9	
2 and 6	131.3	7.41 (*d*, *J* = 8.8 Hz)	131.4	7.46 (*d*, *J* = 8.0 Hz)
3 and 5	116.8	6.77 (*d*, *J* = 8.8 Hz)	116.9	6.80 (*d*, *J* = 8.0 Hz)
4	161.4		159.6	
α	146.9	7.55 (*d*, *J* = 16.0 Hz)	147.8	7.68 (*d*, *J* = 16.0 Hz)
β	114.8	6.25 (*d*, *J* = 16.0 Hz)	113.9	6.37 (*d*, *J* = 16.0 Hz)
C=O	168.9		168.4	
Glucose				
1	104.2	4.92 (*d*, *J* = 7.8 Hz)	104.6	4.95 (*d*, *J* = 8.0 Hz)
2	75.3	3.35 (*dd*, *J* = 9.0, 7.8 Hz)	75.4	
3	77.7	3.40 (*t*, *J* = 9.0 Hz)	77.9	3.24–3.41 (4H, overlapped signals, Glc-H-2-5)
4	71.5	3.32 (*t*, *J* = 9.0 Hz)	71.2	
5	76.1	4.92 (*ddd*, *J* = 9.0, 6.4, 2.4 Hz)	78.7	
6	64.2	4.42 (*dd*, *J* = 12.0, 2.4 Hz)	62.6	3.80 (*dd*, *J* = 12.0, 2.4 Hz
		4.34 (*dd*, *J* = 12.0, 6.4 Hz)		3.64 (*dd*, *J* = 12.0, 5.6 Hz)

positive ion ESI MS ($[M+Na]^+$, m/z 473.4) and HR SI MS, which is same as that of **1**. In the ^1H and ^{13}C NMR spectra, it was also suggested that there were three parts including a hydroxymaltol, a *p*-coumaric acid and a glucose in **2**. Comparing ^{13}C NMR of **2** with **1**, C-6 (δ 62.6) of glucose was shifted upfield by 1.5 ppm relative to that of **1**, and C-7 (δ 59.2) of hydroxymaltol was shifted downward about 1.5 ppm relative that of **1** (δ 57.7), which suggested that *p*-coumaric acid moiety is at C-7 position of hydroxymaltol in **2** instead of C-6 of glucose. In the HMBC spectrum, an obvious correlation was observed between δ 5.38 and δ 168.4, which also confirmed that **2** is a *p*-coumaric acid ester of **4** at C-7 of hydroxymaltol. Enzymatic hydrolysis of **2** also furnished D-glucopyranose which was confirmed by co-HPLC analysis of its 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyglucitol acetate derivative with the same derivative of standard sugar. Thus the structure of **2** was elucidated as 7-*O*-*p*-coumaroylhydroxymaltol 3-*O*-β-D-glucopyranoside.

3. Experimental

3.1. General

IR and UV were recorded on Perkin-Elmer 983 FTIR and Shimadzu UV260 spectrophotometers, respectively. Optical rotations were performed with a JASCO DIP-370 digital polarimeter. IR spectra were carried out on a JASCO D-300 FTIR spectrophotometer. HR SI MS was conducted using a Bruker APEX II FT-ICR mass spectrometer. ESI MS was conducted using a LCQ mass spectrometer. The NMR experiments were carried out at 500 MHz (JEOL ECP500) using a standard Jeol pulse sequences for 1D and 2D NMR experiments in methanol-d_4 solution.

Diaion HP-20 (Mitsubishi Chemical Co.), silica gel (silica gel 60, Merck), and ODS (Chromatorex, 100–200 mesh, Fujisylisia) were used for open CC. Analytical HPLC was performed on a Waters 600 pump system connected to a Shimadzu SPD M10AvP diode array detector, using Inertsil ODS-3 column (4.6 mm i.d×250 mm, 5 μm). Preparative HPLC was performed on a JASCO PU 980 pump connected with a JASCO UV 970 detector (at 254 nm), using a Senshu Pak PEGASIL ODS IIcolumn (20 mm i.d×250 mm, 5 μm).

3.2. Plant material

Tuber of *Smilax bockii*, was collected from Sichuan Province, China and identified by Professor Dean Guo. A voucher specimen was deposited in the Division of Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Peking University, China.

3.3. Extraction and isolation

The tuber of *S. bockii* (4 kg) was refluxed with 95% EtOH (3×10 l). The EtOH extract was concentrated (120 g), suspended in water and then partitioned successively with EtOAc and *n*-BuOH. The EtOAc soluble part (24.2 g) was subjected to silica gel CC (500 g) eluted by $CHCl_3$–MeOH–H_2O (40:10:1) to furnish fractions A–E. Fraction B was subjected to ODS open CC eluted with 40–60% MeOH and purified with prep-HPLC (50% MeOH) to furnish **6** (40 mg), **7** (80 mg), **8** (20 mg) and **9** (8 mg). The *n*-BuOH soluble part (120 g) was subjected to a column of Diaion HP-20 (2.5 l) and eluted with water and 20, 40, 60, 80 and 100% MeOH. The 80% MeOH fraction (16 g) was subjected to silica gel chromatography with $CHCl_3$–MeOH–H_2O (70:20:1), ODS CC with 30–80% MeOH and purified by prep-HPLC (40% MeCN) to furnish **11** (23 mg) and **12** (16 mg). The 100% MeOH eluate was applied to silica gel this being eluted with $CHCl_3$–MeOH (10:1–1:1) to give fractions A–D. Fr. B was subjected to ODS CC with 40–80% MeOH and separated by prep-HPLC (30% MeOH) to furnish **1** (10 mg) and **2** (6 mg). Fr. D was separated by prep-HPLC (30% MeOH) to furnish **3** (15 mg), **4** (13 mg) and **5** (10 mg). Fr. E was purified by Sephadex LH-20 with MeOH to furnish **10** (15 mg).

3.4. Bockioside A (1)

Colorless amorphous solid, $[\alpha]_D^{25}$ −73.8° (*c* 1.3, MeOH); UV λ_{max} (MeOH) (log ϵ): 310 nm (2.34); IR (KBr, cm^{-1}) ν_{max}: 3338 (OH), 1604 (CO); For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; ESI MS: m/z 489.4 $[M+K]^+$; HR SI MS: m/z 451.1236 (calc. for $C_{21}H_{22}O_{11}$ 451.1234).

3.5. Bockioside B (2)

Colorless amorphous solid, $[\alpha]_D^{25}$ −21.3° (*c* 0.3, MeOH); UV λ_{max} (MeOH) (log ϵ): 312 nm (2.32); IR (KBr, cm^{-1}) ν_{max}: 3367 (OH), 1606 (CO); For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; ESI MS: m/z 473.4 $[M+Na]^+$, 451.2 $[M+H]^+$; HR SI MS: m/z 451.1234 (calc. for $C_{21}H_{22}O_{11}$ 451.1234).

3.6. Enzymatic hydrolysis of 1, 2

The solution of compound **1** or **2** (4.0 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (Sigma Chemical Co., 6.3 mg) and the reaction mixture was stirred at 40 °C for 24 h. The reaction mixture was then passed through a Sep-Pak C_{18} cartridge (Waters) eluted with H_2O and MeOH, successively. The H_2O eluate was concentrated and the residue was dissolved in 1 ml H_2O, to which L-(−)-α-methyl-

benzylamine (5 mg) and $NaBH_3CN$ (8 mg) in EtOH (1 ml) was added. After being stirred at 40 °C for 4 h followed by addition of glacial HOAc (0.2 ml) and evaporated to dryness, the reaction mixture was acetylated with acetic anhydride (0.3 ml) in pyridine (0.3 ml) for 24 h at room temp. After evaporation, H_2O (1 ml) was added to the residue and the solution was passed through a Sep-Pak C_{18} cartridge washed with H_2O, H_2O–MeCN (4:1, 1:1, each 5 ml), successively. The H_2O–MeCN (1:1) eluate was analyzed and the l-[(*S*)-*N*-acetyl-α-methylbenzylamino]-l-deoxyglucitol acetate derivatives was identified by co-HPLC analysis with the derivative of standard sugar prepared under the same conditions (Oshima and Kumanotani, 1981; Oshima et al., 1982).

HPLC conditions: Inertsil ODS-3, 4.6×250 mm; solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml/min; detection, UV 230 nm. The derivative of D-glucose was detected with t_R of 24.63 min.

Acknowledgements

H. Guo is grateful for a visiting researcher fellowship provided by Faculty of Pharmaceutical Science, Toho University.

References

Chen, T., Li, J., Cao, J., Xu, Q., Komatsu, K., Namba, T., 1999. A new flavanone isolated from rhizoma *Smilacis glabrae* and the structural requirements of its derivatives for preventing immunological hepatocyte damage. Planta Medica 65, 56–59.

Chen, T., Li, J., Xu, Q., 2000. Phenylpropanoid glycosides from *Smilax glabra*. Phytochemistry 53, 1051–1053.

China Pharmacopoeia Committee, 2000. China Pharmacopoeia, 2000 ed. Part 1. Chemical Industry Publishing House, Beijing. p. 15.

De Britto, J., Manickam, V.S., Gopalakrishnan, S., Ushioda, T., Tanaka, N., 1995. Chemical and chemotaxonomical studies of ferns. LXXXV. Determination of aglycon chirality in dihydroflavonol 3-*O*-α-L-rhamnosides by ^1H NMR spectroscopy. Chemical and Pharmaceutical Bulletin 43, 338–339.

Gaffield, W., Waiss, A.C., Tominaga, T., 1975. Structural relations and interconversions of isomeric astilbins. Journal of Organic Chemistry 40, 1057–1061.

Harborne, J.B., Mabry, T.J., 1982. The Flavonoids: Advances in Research. Chapman and Hall Ltd, London. p. 92.

Hirai, Y., Sanada, S., Ida, Y., Shoji, J., 1986. Studies on the constituents of Palmae plants. III. The constituents of *Chamaerops humilis* L. and *Trachycarpus wagnerianus* BECC. Chemical and Pharmaceutical Bulletin 34, 82–87.

Li, S.Y., Fuchino, H., Kawahara, N., Sekita, S., Satake, M., 2002. New phenolic constituents from *Smilax bracteata*. Journal of Natural Products 65, 262–266.

Looker, J.H., Fisher, M.S., 1985. Synthesis and characterization of the naturally occurring monocyclic ω-pyrone glucosides. Journal of Carbohydrate Chemistry 4, 381–392.

Murakami, T., Kudo, M., Taguchi, S., Tanaka, N., Saiki, Y., Chen, C., 1978. Chemical and chemotaxonomic studies on the genus Pteris and related genera (Pteridaceae). XVII. Additional constituents of *Pteris inaequalis* var. *aequata* (Miq.) Tagawa. Chemical and Pharmaceutical Bulletin 26, 643–645.

Oshima, R., Kumanotani, J., 1981. Determination of the configuration of monosaccharides by HPLC on diastereoisomeric l-deoxy-l-(*N*-acetyl-α-mehtylbenzylamino) alditol acetates. Chemistry Letters 943–946.

Oshima, R., Yamauchi, Y., Kumanotani, J., 1982. Resolution of the enantiomers of aldose by liquid chromatography of diastereoisomeric l-(*N*-acetyl-α-methylbenzylamino)-l-deoxyalditol acetates. Carbohydrate Research 107, 169–176.

Sala, A., Carmen Recio, M., Giner, R.M., Manez, S., Rios, J.L., 2001. New acetophenone glucosides isolated from extracts of *Helichrysum italicum* with antiinflammatory activity. Journal of Natural Products 64, 1360–1362.

Sashida, Y., Kubo, S., Mimaki, Y., Nikaido, T., Ohmoto, T., 1992. Steroidal saponins from *Smilax riparia* and *S. china*. Phytochemistry 31, 2439–2443.

Sugiyama, M., Kikuchi, M., 1992. Studies on the constituents of *Osmanthus* species. X. Structures of phenolic glucosides from the leaves of *Osmanthus asiaticus* Nakai. Chemical and Pharmaceutical Bulletin 40, 325–326.

Yasue, M., Kawamura, N., Sakakibara, J., 1970. Structure of innovanoside. Chemical Pharmaceutical and Bulletin 8, 856–858.

Yu, W., Li, H., Chen, X., Yang, L., 1992. Two afzelechin glycosides from *Arthromeris mairei*. Phytochemistry 31, 4385–4386.