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Harpagometabolins I and II, Two New Metabolites from Harpagoside by Human Intestinal Bacteria

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Abstract: Harpagoside, which is one main iridoid constituent of the dried roots of *Scrophularia ningpoensis* Hemsl., was biotransformated by bacteria isolated from human fecal flora and three metabolites were obtained. The structures of the metabolites, including two new alkaloids, named harpagometabolins I (**1**) and II (**2**), and a known alkaloid acubinine B(**3**), were identified by chemi-cal methods and the spectroscopic evidences.

Keywords: *Scrophularia ningpoensis*, Harpagoside, harpagometabolin I, harpagometabolin II, acubinine B.

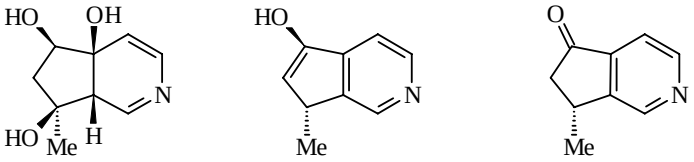
The dried roots of *Scrophularia ningpoensis* Hemsl. (radix scrophulariae) have been used in traditional chinese medicine for replendish the vital essence and relieve pyogenic inflammation[1]. Iridoids such as a harpagoside are main constituents of radix scrophulariae[2]. Until now, the pharmacological effects of these iridoids were not clearly understood. Biological transformation in the human intestinal tract can induce the conversion of the iridoids to more or less biologically active compounds. Hattori *et al*[3-5] have studies the transformation of iridoid glycosides (aucubin contained in the leaves of *Eucommia ulmoides* Oliv., gardenoside and geniposide contained in the leaves and fruits of *Gardenia jasminoides* Ellis) and a secoiridoid (swertiamarin contained in the *Swertia japonica* (Maxim.) Makino) to the corresponding nitrogen-containing compounds using human intestinal bacteria. Recently, we found that harpagoside can be transformated to two new alkaloids named harpagometabolin I (**1**), II (**2**), and one known alkaloid acubinine B (**3**) by human intestinal bacteria including *Bifidobacterium angulatum*, *B. bifidum* a E319, *Clostridium butyricum*, *Escherichia coli* O-127, *Eubacterium aerofaciens*, *Fusobacterium nucleatum*, *Lactobacillus acidophilus* ATCC 4356, *L. Brevis* II-46, *L. Fermentum* ATCC 9338, *L. xylosus*, *Peptostreptococcus tetradius* G-0608, *Proteus mirabilis* S2, *Ruminococcus sp.* PO 1-3. In the present paper, we describe the structural elucidation of these metabolites.

Metabolite **1** was isolated as a yellowish oil. The IR spectrum displayed absorp-tion bands at 3425, 1715 and 1605 cm^{-1} for the –OH, C=N and C=C. The positive FAB-MS showed peaks at *m/z* 206 $[M+Na]^+$, 184 $[M + 1]^+$. Its molecular

formula $C_9H_{14}NO_3$ was determined by the positive ion HR-FAB-MS m/z 184.0989 $[M + 1]^+$ (calcd for $C_9H_{14}NO_3$ 184.0973).

The ^1H NMR spectrum of metabolite **1** showed the presence of a methylene (δ 1.54 and 2.79, each dd, J=12.5, 18.9Hz and 7.3, 18.9Hz, respectively), two methine (δ 5.30, dd, J=4.5, 7.3Hz and 2.84, s) and a methyl (δ 1.25, s). Compared with the ^1H and ^{13}C NMR spectra of harpagoside and 6′-O-acetylharpagoside[2], metabolite **1** have also the structure part of five-member ring of 5β, 6β, 8β-trihydroxyl-8α-methyl. In addition, the ^1H NMR spectrum of metabolite **1** showed also the presence of three aromatic protons at δ 8.74, 8.53, and 8.45 which were ascribable to two alpha and a beta protons (H-1, H-3 and H-4) in the dihydropyridine nucleus, on the basis of the chemical shifts and the spin-spin coupling constants and HMBC. These findings led us to conclude the structure of metabolite **1** as shown in **Scheme 1**, named harpagometabolin I. The ^{13}C NMR spectrum also supported the proposed structure.

Scheme 1 The structures of compounds **1**, **2** and **3**



1 **2** **3**

Table 1 ^1H and ^{13}C NMR* Spectral Data of Metabolites **1**, **2** and **3** (δ Relative to TMS in $CDCl_3$)

No.	^1H			^{13}C		
	1	**2**	**3**	**1**	**2**	**3**
1	8.74 (br s)	9.00 (br s)	8.97 (br s)	144.8	150.8	149.0
3	8.53 (d, 4.5)	8.81 (d, 4.5)	8.70 (d, 4.5)	147.3	148.4	148.3
4	8.45 (d, 4.5)	7.58 (d, 4.5)	7.56 (d, 4.5)	120.6	116.1	116.1
5				68.3	133.9	142.3
6	5.30 (dd, 4.5, 7.3)			74.3	150.8	205.9
7	1.54 (dd, 12.5, 18.9) 2.79 (dd, 7.3, 18.9)	5.58 (br s)	3.01 (dd, 7.0, 19.5) 2.34 (dd, 2.0, 19.5)	42.3	127.5	45.2
8		3.67 (m)	3.59 (m)	72.7	44.1	29.6
9	2.84 (s)			51.3	149.1	152.9
10	1.25 (s)	1.51 (d, 7.0)	1.49 (d, 7.0)	19.6	21.1	22.5

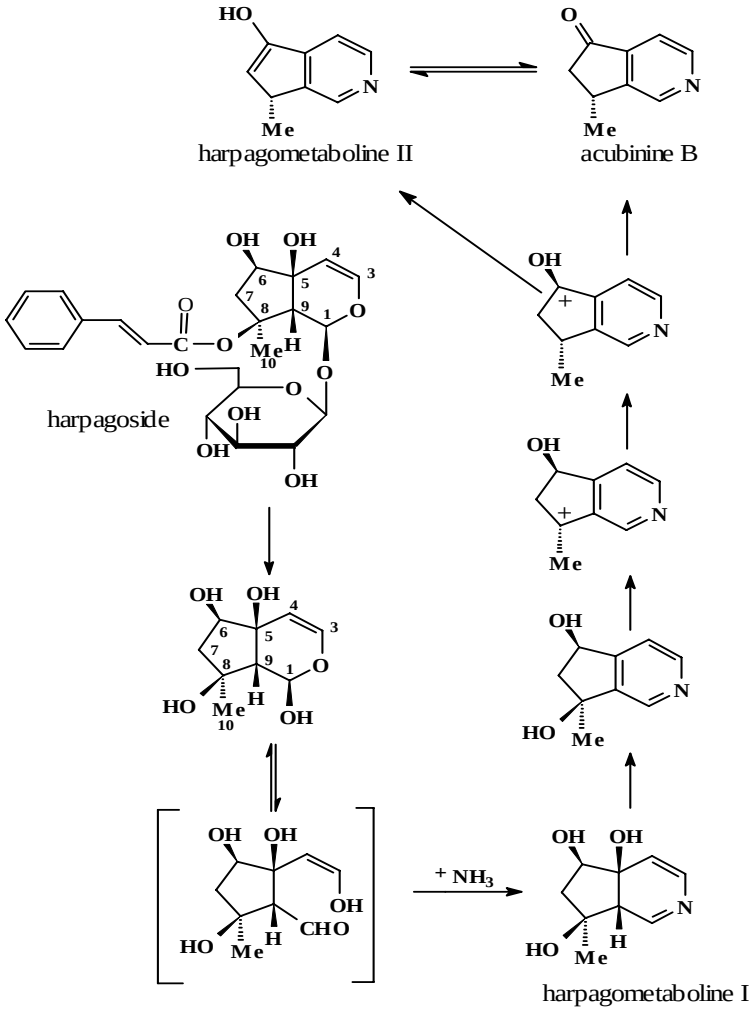
* ^1H: 500MHz; ^{13}C: 125MHz

Metabolite **2** was also obtained as a yellowish oil. The ^1H and ^{13}C NMR spectra of metabolite **2** lacked the signals corresponding to the 5β, 8β-dihydroxyl group found in **1**. The EI-MS showed peaks at *m/z* 147 $[M]^+$, 132 $[M\text{-}Me]^+$, 118 $[M\text{-}C_2H_5]^+$, 104 $[M\text{-}C_3H_7]^+$, and 91 $[M\text{-}C_3H_7\text{-}N]^+$. The IR spectrum showed absorption bands at 3435, 1725 and 1660 cm^{-1} for the –OH, C=N and C=C. In addition, three aromatic protons at δ 9.00 (br s), 8.81 (d, J=4.5Hz), and 7.58 (d, J=4.5Hz), which were ascribable to two

alpha and a beta protons (H-1, H-3 and H-4) in the pyridine nucleus, were observed. These were consistent with pyridine nucleus part of aucubinine B[3].

Compared with the ^{1}H and ^{13}C NMR spectra of metabolite **1**, signals of two conjugated double band, a doublet methyl and a hydroxyl group in five-member ring were observed in metabolite **2**. Further confirmation was provided by HR-EI-MS m/z 147.0696 $[M]^{+}$ (calcd for C_9H_9NO 147.0684) which established the molecular formula as

Scheme 2 Possible metabolic processes of harpagoside by human intestinal bacteria



C_9H_9NO. ^{1}H-^{1}H COSY analysis established the presence of a olefinic proton at C_7. On the basis of these findings, the structure of metabolite **2** was determined as shown in **Scheme 1**, named harpagometabolin II.

Metabolite **3** was also obtained as a yellowish oil. The IR spectrum showed absorption bands at 3420, 1725 and 1600 cm^{-1} for the –OH, C=N and C=C. The EI-MS

showed the fragment ion peaks at *m/z* 147 $[M]^+$, 132 $[M\text{-}Me]^+$, 118 $[M\text{-}C_2H_5]^+$, 104 $[M\text{-}C_3H_7]^+$, and 91 $[M\text{-}C_3H_7\text{-}N]^+$. Its molecular formula C_9H_9NO was determined by HR-EI-MS m/z 147.0699 $[M]^+$ (calcd for C_9H_9NO 147.0684). It was identified as acubinine B (**Scheme 1**) by comparison of the ^1H and ^{13}C NMR data with those reported in literature[3].

The iridoid glucoside, harpagoside, was transformated to the monoterpene alkaloids by anaerobic incubation with fecal flora of human and with individual strains of human intestinal bacteria. **Scheme 2** shows the possible metabolic processes of harpagoside.

Acknowledgment

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