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IRIDOIDS FROM *SCROPHULARIA NINGPOENSIS*

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Key Word Index—*Scrophularia ningpoensis*, Scrophulariaceae; roots; iridoid; phenylpropanoid glycoside, harpagide, harpagoside

Abstract—Three new compounds were isolated from Scrophulariae Radix, the dried root of *Scrophularia ningpoensis*, together with the known compounds harpagide and harpagoside. The structure of one was shown to be similar to that of acteoside and angoroside A, the other two were characterized as iridial derivatives by spectroscopic means.

INTRODUCTION

The root of *Scrophularia ningpoensis* has been used for treatment of inflammation in Chinese traditional medicine [1]. We were interested that the aqueous extract of this plant shows a hypotensive action and embarked upon the investigation of constituents of this plant as a part of our chemical studies on antihypercholesterolemic crude drugs. The methanol extractive prepared from commercial Scrophulariae Radix was partitioned between *n*-butanol and water. The organic layer was subjected to CC on silica gel, Sephadex LH-20 and Bondapak C-18 with various solvent systems to yield compounds **1**–**5**. Among these compounds, compounds **1** and **2** were elucidated as harpagide and harpagoside [2], respectively, which have already been isolated [3] from the same origin.

RESULTS AND DISCUSSION

Compound **3**, paste, $[\alpha]_D$ −62.9° (MeOH) showed complicated signals in the sp^2-carbon and oxygenated carbon region in the ^{13}C NMR spectrum, suggesting that it is a glycoside of an aromatic compound. It was also revealed that the glycosidic moiety consisted of three moles of sugar based on the existence of three anomeric carbon signals at δ 103.0, 104.0 and 105.4 in the ^{13}C NMR spectrum. Compound **3** was then hydrolysed with hydrochloric acid in methanol to yield 2-(3-hydroxy-4-methoxyphenyl) ethanol as an aglycone, and methyl ferulate as an ester part, and methyl glucopyranoside, methyl rhamnoside and methyl arabinopyranosides as sugar parts together with 2-(3-hydroxy-4-methoxyphenyl)ethyl 1-*O*-β-D-glucopyranoside (**6**). The structure of the aglycone was determined by making a comparison between the ^1H NMR spectrum of the compound with that of authentic 2-(3-methoxy-4-hydroxyphenyl) ethanol (=homovanillyl alcohol). Moreover, in order to determine the structure of the glycosidic moiety, the saponified compound (**7**) of **3** was partially hydrolysed with hydrochloric acid to yield **6** and two other compounds **8** and **9**. Comparison of the ^{13}C NMR spectrum with that of **6** showed the presence of a set of signals assignable to a rhamnosyl residue in the spectrum of **8**. Moreover, it was shown that the rhamnosyl moiety was linked to C-3 of **6** based on the observation of glycosidation shifts [4–6] of signals due to C-3 (downfield shift by 5.2 ppm) and C-4 (upfield shift by 2.3 ppm). In the same way, it was revealed that compound **9** has a terminal arabinose in **6**, and that the arabinose is joined to C-6 of **6**, based on the observation of glycosidation shifts of the signals due to C-6 (downfield shift by 6.8 ppm) and C-5 (upfield shift by 1.2 ppm). According to these results, **7** is suggested to be 2-(3-hydroxy-4-methoxyphenyl) ethyl 1-*O*-[α-L-arabinopyranosyl (1→6)]-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside. A comparative study of the ^{13}C NMR spectra of **3** and **7** showed that the feruloyl group is linked to the C-4 position of glucose Therefore, the structure of **3** was determined to be 2-(3-hydroxy-4-methoxyphenyl) ethyl 1-*O*-[α-L-arabinopyranosyl (1→6)]-[feruloyl (1→4)]-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside; the compound has two additional methyl groups on phenolic oxygens at C-3 and C-4′ of angoroside A recently reported by Sticher *et al.* [7].

Compound **4**, an amorphous powder, $[\alpha]_D$ 181.0° (MeOH), showed a total of nine carbon signals in the ^{13}C NMR, which included two olefinic carbons and four oxygenated ones. The ^1H NMR spectrum of the acetate (**10**) of **4** shows two acetyl groups at δ 2.08 and 2.07, which means that only two among the four oxygenated carbons are acylable carbinols. Decoupling experiments were also performed revealing the existence of four fragmental structures of **10**. Assigned signals and the corresponding partial structures are shown in Fig 1. Moreover, measurement of the ^1H–^1H COSY spectrum (Fig. 2) indicates the correlation that was not clarified by the

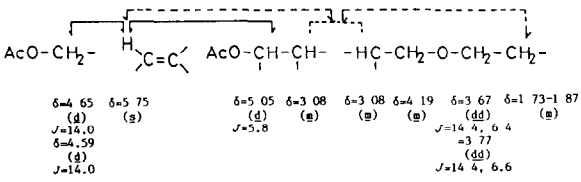

Fig. 1. ^1H NMR (in $CDCl_3$) of compound **10** (*J* in Hz).

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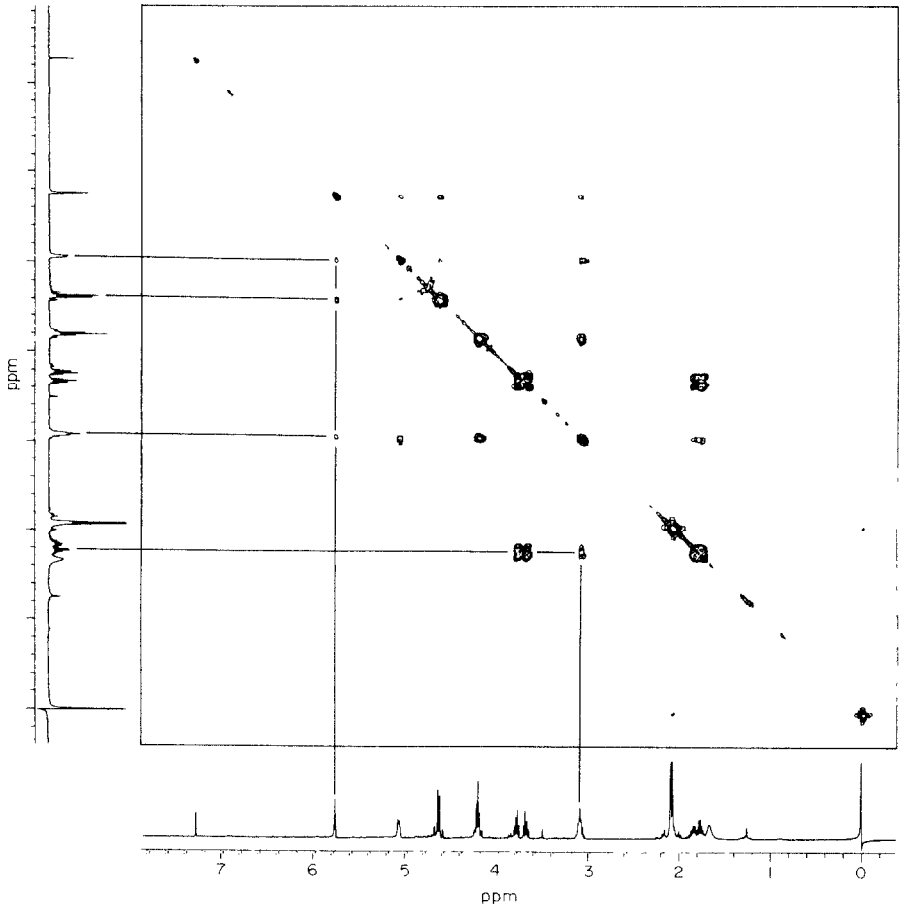
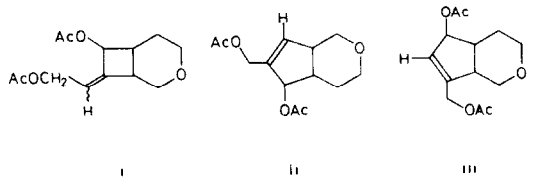


Fig 2 ^1H–^1H COSY spectrum of compound **10**



i ii iii

Fig 3 Plausible structures of compound **10**

decoupling experiments Plausible bondings are shown by arrows and dotted arrows in Fig 1 However, two methine protons at δ3 08 had the same chemical shift, thus, it could not be clarified which proton is coupled with a vinylic proton at δ5 75 and a methylene proton at δ1 73–1 87. Accordingly, the structure of **10** was suggested as one of three shown in Fig. 3. Among these structures, **iii** was regarded as the most plausible one from the viewpoint of biogenesis

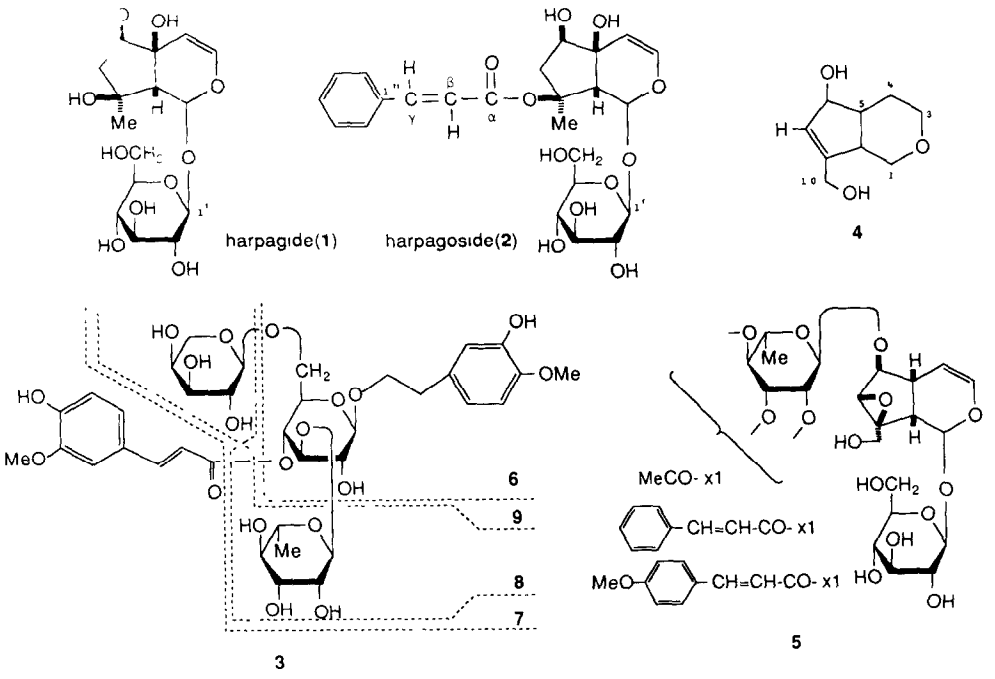
Compound **5**, an amorphous powder, showed many complex signals, containing several peaks in the ester carbonyl region in its ^{13}C NMR Alkaline hydrolysis yielded 6-rhamnosyl catalpol (**11**) [8], methyl *p*-methoxycinnamate and methyl cinnamate Comparison of the ^{13}C NMR spectrum of **5** with that of a saponified compound (**11**) showed that the acyl groups were joined to the rhamnosyl moiety This was confirmed by the EI-mass spectral fragment peaks of the acetate (**12**) of **5** at *m/z* 331 (Glc 4 × Ac) and 447 (Rha · 1 × Ac 1 × C_6H_5-CH=CH-CO 1 × MeO-C_6H_4-CH=CHCO) The location of each of the acyl groups, however, could not be clarified

EXPERIMENTAL

Isolation The MeOH extract (460 g) of Scrophulariae Radix, the dried root of *S ningpoensis* L, purchased in a market in Japan was partitioned between *n*-BuOH and H_2O The organic layer was sepd by CC on silica gel (hexane–Me_2CO, 2 3/EtOAc–MeOH, 40 1/$CHCl_3$–MeOH, 100 0–20 1/$CHCl_3$–MeOH–H_2O, 90 10 1–40 10 1–70 30 3), Sephadex LH-20 (MeOH) and Bondapak C-18 (60% MeOH) to give five compounds.

Compound **1** Amorphous powder, $[\alpha]_D$ −135 7° (MeOH; *c* 0 5) ^{13}C NMR (C_5D_5N) δ 93 6 (C-1), 141 2 (C-3), 109 2 (C-4), 72 8 (C-5), 77 9 (C-6), 47 2 (C-7), 77 5 (C-8), 59 9 (C-9), 25 3 (C-10), 99 3 (C-1′), 74 7 (C-2′), 78 7 (C-3′), 71 3 (C-4′), 78 7 (C-5′), 62 3 (C-6′)

Compound **2** Amorphous powder, $[\alpha]_D$ −44 9° (MeOH, *c* 0 5) ^{13}C NMR (C_5D_5N) δ 94 7 (C-1), 142 3 (C-3), 107 9 (C-4), 73 2 (C-5), 76 9 (C-6), 46 0 (C-7), 87 6 (C-8), 55 4 (C-9), 22 7 (C-10), 99 4 (C-1′), 74 8 (C-2′), 78 6 (C-3′), 71 7 (C-4′), 78 6 (C-5′), 62 9 (C-6′), 166 9 (C-α), 135 6 (C-β), 144 5 (C-γ), 130 5 (C-1″), 129 2 (C-2″,6″), 128 5 (C-3″,5″), 120 2 (C-4″)



Compound **3** Syrup, $[\alpha]_D$ −62.9° (MeOH, *c* 0.5). ^{13}C NMR (C_5D_5N) see Table 1.

Alkaline hydrolysis of **3**. Compound **3** was saponified with 5% KOH in MeOH under reflux for 20 min The reaction mixt. was neutralized with HCl–MeOH and the solvent evapd The resultant residue was purified by CC on silica gel ($CHCl_3$–MeOH–H_2O, 14·6 1) to afford **7** as an amorphous powder. ^{13}C NMR (C_5D_5N) see Table 1.

Acid hydrolysis of **3**. Compound **3** was treated with 1 M HCl in MeOH and refluxed for 1 hr, followed by neutralization with 3% KOH–MeOH The generated salt and sugar were removed by Sephadex LH-20 (MeOH) CC. The sugar-containing fr. was collected and analysed by TLC ($CHCl_3$–MeOH–H_2O, 14:6:1) to reveal the existence of Me glucopyranoside, Me rhamnoside and arabinopyranoside. The other fr collected was purified by CC on silica gel (hexane–Me_2CO, 3 1–1 1/$CHCl_3$–MeOH–H_2O, 90.20.1) to yield 2-(3-hydroxy-4-methoxyphenyl) ethanol and Me ferulate, which were identified by means of ^{1}H NMR, and **6** which was converted to its acetate derivative whose structure was then determined by ^{13}C NMR (Table 1).

Methyl ferulate ^{1}H NMR ($CDCl_3$) δ 7 17 (1H, *d*, *J* = 2.0 Hz, Ar-H), 7.06 (1H, *dd*, *J* = 2.0 and 8 3 Hz, Ar-H), 6.80 (1H, *d*, *J* = 8.3 Hz, Ar-H), 7.61, 6.35 (each 1H, *d*, *J* = 15 6, –CH=CH–), 3.89 (3H, *s*, –COOMe), 3 76 (3H, *s*, –OMe).

2-(3-hydroxy-4-methoxyphenyl) ethanol. ^{1}H NMR δ 6.81 (1H, *d*, *J* = 7 8 Hz, Ar-H), 6.68 (1H, *d*, *J* = 2.0 Hz, Ar-H), 6 64 (1H, *dd*, *J* = 7.8 and 2.0 Hz, Ar-H), 3.81 (3H, *s*, –OMe), 3.68 (2H, *t*, *J* = 8.0 Hz, $-CH_2OH$), 2.69 (2H, *t*, *J* = 8 Hz, Ar-CH_2).

Compound **4**. Amorphous powder, $[\alpha]_D$ + 181.0° (MeOH; *c* 1.02). ^{13}C NMR (C_5D_5N) δ· 150 5 (*s*), 126.2 (*d*), 87 3 (*d*), 67 4 (*t*), 61 8 (*t*), 60 5 (*t*), 49 6 (*d*), 43.6 (*d*), 28.5 (*t*).

Diacetate (**10**) *of* **4**. Compound **4** (68.5 mg) was acetylated with Ac_2O–pyridine for 1 hr at room temp. After the reaction was stopped by adding a small amount of MeOH, the solvent was evapd and the residue purified by CC on silica gel (hexane–Me_2CO, 3:1) to yield a diacetate (69.2 mg) as an oily product. ^{1}H NMR ($CDCl_3$) δ· 5 75 (1H, *br s*), 5.05 (1H, *d*, *J* = 5.8 Hz), 4.65 (1H, A part of AB, *d*, *J* = 14 0 Hz), 4.59 (1H, B part of AB, *d*, *J* = 14 0 Hz), 4.19 (2H, *m*), 3.75 (1H, A part ABX, *dd*, *J* = 14.4 and 6 6 Hz), 3.67 (1H, B part of ABX, *dd*, *J* = 14.4 and 6.4 Hz), 3.08 (2H, *m*), 2.08, 2 07 (each 3H, *s*, –OAc × 2), 1.73–1.87 (2H, *m*).

Compound **5**. Amorphous powder ^{13}C NMR (C_5D_5N) δ: 94 7 (C-1), 141.7 (C-3), 102 6 (C-4), 36 6 (C-5), 84.7 (C-6), 58.6 (C-7), 66 4 (C-8), 43.2 (C-9), 60.1 (C-10), 100.2 (C-1'), 74.9 (C-2'), 79.0 (C-3'), 71.5 (C-4'), 78.3 (C-5'), 62.7 (C-6'), 97.2 (C-1''), 70.9 (C-2''), 70.1 (C-3''), 71.3 (C-4''), 67.5 (C-5''), 17.8 (C-6''), 166.8 (C-α), 117.9 (C-β), 146.2 (C-γ), 134.5 (Ar-1), 129.1 (Ar-2,6), 128.7 (Ar-3,5), 130.8 (Ar-4), 166 2 (C-α'), 114.0 (C-β'), 133.1 (C-γ'), 127.3 (Ar-1'), 130 5 (Ar-2',6'), 114.8 (Ar-3',5'), 160 9 (Ar-4'), 55.4 (–OMe), 170.2 (Me–CO–), 20.6 (Me–CO–)

Alkaline hydrolysis of **5**. Compound **5** (50 9 mg) was hydrolysed with 5% KOH in MeOH for 1 hr at room temp. After reaction, the reaction mixt was worked-up as described for the alkaline hydrolysis of **3** and purified by silica gel CC ($CHCl_3$–MeOH, 50 1) to give Me *p*-methoxycinnamate and Me cinnamate (both identified by MS) and compound **11** (30.6 mg).

Compound **11** Amorphous powder. ^{13}C NMR (C_5D_5N) δ. 94.8 (C-1), 141.3 (C-3), 103.2 (C-4), 36.8 (C-5), 82.9 (C-6), 58.6 (C-7), 66 3 (C-8), 43.2 (C-9), 60.4 (C-10), 100.4 (C-1'), 74 9 (C-2'), 79 0 (C-3'), 71.5 (C-4'), 78.2 (C-5'), 62 6 (C-6'), 100 1 (C-1''), 72.5 (C-2''), 72.3 (C-3''), 73.8 (C-4''), 70.1 (C-5''), 18 5 (C-6'').

Methyl cinnamate. EIMS *m/z* 162 $[M]^+$, 131 $[M-OMe]^+$.

Methyl p-methoxycinnamate EIMS *m/z* 192 $[M]^+$, 161 $[M-OMe]^+$, 133 $[M-CO_2Me]^+$.

Acetylation of **5** Compound **5** (22.6 mg) was acetylated, worked up by the usual method and the residue subjected to silica gel CC (hexane–EtOAc, 5.2) to give compound **12** (8.3 mg) as an oil EIMS *m/z* 331 [Glc·4 × Ac]$^+$, 443 [Rha·1 × Ac·1 × C_6H_5–CH=CH–CO·1 × MeO–C_6H_4–CH=CH–CO]$^+$.

Table 1 ^{13}C NMR (C_5D_5N) spectra of compounds **3**, **7**–**9** and **6**-Ac

		3	**7**	**8**	**9**	**6**-Ac
	1	103 0	102 6	102 4	104 5	100 3
	2	74 2	75 2	75 1	74 9	72 5
Glc	3	80 3	83 3	83 4	78 4	71 5
	4	72 5	69 2	69 4	71 7	70 9
	5	75 6	76 7	78 0	77 0	68 2
	6	70 2	69 7	62 2	69 6	61 7
	1	104 0	104 1	103 9	—	—
	2	72 4	72 2	72 2	—	—
Rha	3	72 4	72 3	72 4	—	—
	4	73 9	73 9	73 8	—	—
	5	70 1	69 4	69 4	—	—
	6	18 8	18 5	18 6	—	—
	1	105 4	105 4	—	105 4	—
	2	72 5	72 5	—	72 3	—
Ara	3	74 6	74 2	—	74 3	—
	4	69 2	69.2	—	69 2	—
	5	66 8	66 7	—	66 6	—
	1	132 3	132 2	132 7	132 5	130 5
3-Hydroxy	2	112 6	112 6	112.1	112 7	111 8
-4-methoxy	3	146 8	147 1	147 4	148 0	148 9
phenyl	4	148 0	147 8	144 5	147 1	138 9
ethanol	5	117 5	117 4	117 0	117 5	126 7
	6	120 0	120 0	119 4	120 1	122 7
		71 0	70 9	70 6	71 0	—
		35 9	35 9	35 8	36 1	—
	–OMe	56 0	56 1	—	—	—
	1	126 5	—	—	—	—
	2	123 9	—	—	—	—
Feruloyl	3	151 2	—	—	—	—
	4	147 1	—	—	—	—
	5	116 7	—	—	—	—
	6	123 1	—	—	—	—
		148 9	—	—	—	—
		114 8	—	—	—	—
		167 0	—	—	—	—
	–OMe	55 8	—	—	—	—

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