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PHENYLETHANOID GLYCOSIDES FROM *PLANTAGO DEPRESSA*

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Key Word Index—*Plantago depressa*; Plantaginaceae; phenylethanoid glycosides; β-oxoacteoside.

Abstract—The five known phenylethanoid glycosides, cistanoside F, β-hydroxyacteoside, campenoside I, acteoside and orobanchoside, and a new phenylethanoid glycoside, β-oxoacteoside, were isolated from the aerial parts of *Plantago depressa*. The structure of β-oxoacteoside was deduced from chemical and spectral evidence to be β-oxo-β-(3,4-dihyroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-(4-*O*-caffeoyl)-glucopyranoside.

INTRODUCTION

The aerial parts of *Plantago depressa* have been used since ancient times as a diuretic, an anti-asthmatic and an antiinflammatory drug (oriental medicinal herb) in China. It is listed as 'Plantaginis Herba' in the Chinese Pharmacopeia [1]. In a previous paper [2], we reported the isolation of four phenylethanoid glycosides, 3,4-dihydroxyphenethyl alcohol-6-*O*-caffeoyl-β-D-glucoside, acteoside, plantamajoside and hellicoside, and a flavonoid, plantaginin, from *P. asiatica* and the enzyme inhibitory activities of phenylethanoid glycosides on cyclic AMP phosphodiesterase, 5-lipoxygenase and the lens aldose reductase, respectively. We have now examined the phenolic compounds of the aerial parts of *P. depressa*, and isolated a new phenylethanoid glycoside together with five known phenylethanoid glycosides.

RESULTS AND DISCUSSION

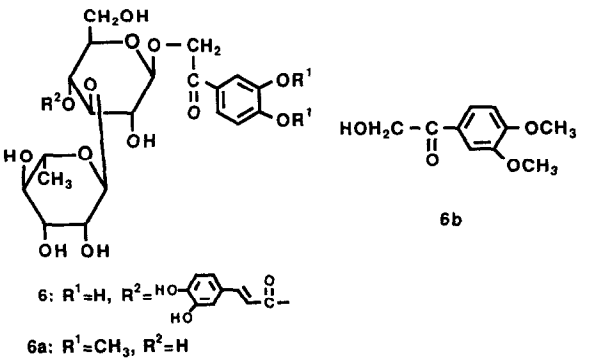
The HPLC chromatogram of a methanol extract of the aerial parts of *P. depressa* showed the presence of six phenolic compounds. This extract was fractionated by successive extractions with ether, ethyl acetate and *n*-butanol–ethyl acetate (5:1). The ethyl acetate extract yielded **1**–**6** on column chromatography over Sephadex LH-20. The *n*-butanol–ethyl acetate extract after column chromatography on Sephadex LH-20 furnished **1**, **2** and **4**.

Compounds **1**–**5** were identified as cistanoside F [3], β-hydroxyacteoside [4], campenoside I [5], acteoside [4] and orobanchoside [6] by comparing their various physical and spectral data with those in the literature or by direct comparison with authentic samples.

Compound **6**, designated as β-oxoacteoside, was obtained as an amorphous powder whose M_r was confirmed by the observation of *m/z* 639 $[M(C_{29}H_{34}O_{16})+H]^+$ and *m/z* 661 $[M(C_{29}H_{34}O_{16})+Na]^+$ by positive ion FAB mass spectrometry. The UV spectrum of **6** showed absorption maxima at 222sh, 232, 287 and 326 nm. Its IR spectrum suggested the presence of a conjugated ester (1705 cm^{-1}), a carbonyl group (1695 cm^{-1}) and aromatic rings (1605 cm^{-1} and 1521 cm^{-1}), while its ^1H NMR spectrum showed signals due to methyl protons (δ1.0), anomeric protons of a sugar moiety (δ4.38 and 5.13), aromatic protons of a phenethyl moiety bearing a carbonyl group at the β-position (δ6.74 and 7.30–7.35) and protons of a caffeoyl moiety (δ6.18, 6.68, 6.85, 6.96 and 7.50).

The reaction of **6** in methanol with excess diazomethane gave deacyl-β-oxoacteoside dimethyl ether (**6a**) as an amorphous powder ($C_{22}H_{32}O_{13}$) and 4-(3,4-dimethoxyphenyl)-2-pyrazoline-3-carboxylic acid methyl ester (**6c**) [7].

Acid hydrolysis of **6a** gave **6b**, D-glucose and L-rhamnose. Compound **6b** was identical with β-oxo-β-(3,4-dimethoxyphenyl)-ethanol which was synthesized by the reaction of 3,4-dimethoxyacetophenone with iodobenzene [8]. These results clearly suggested that **6** consists of a β-oxo-β-(3,4-dihydroxyphenyl)-ethyl moiety and a rhamno-glucosyl moiety containing a caffeoyl group. The



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^{13}C NMR spectrum of **6** supported the attachment of the caffeoyl moiety at C-4 of the inner glucose, the rhamnosyl moiety at C-3 of the inner glucose and linkage of the inner glucosyl moiety to the α-position of β-oxo-β-(3,4-dihydroxyphenyl)-ethanol. Consequently, the structure of **6** has been established as β-oxo-β-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-(4-*O*-caffeoyl)-glucopyranoside.

A novel phenylethanoid glycoside bearing a carbonyl group at the β-position of a phenethyl moiety is reported for the first time. Also it is biogenetically noteworthy that a series of compounds which may indicate metabolic pathways of phenylethanoid glycosides was isolated. In addition, it should be noted from a medicinal point of view that the phenylethanoid glycosides of *P. depressa* are different from those of *P. asiatica* as the origin of "Plantaginis Herba" in the Japanese Pharmacopeia XII [9].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively; chemical shifts are given in δ relative to TMS as int. std. Precoated TLC plates, silica gel 60_{F254} (Merck), were used for prep. TLC.

Plant material. Plantago depressa Willd. was collected in August 1990 at Liaoning, China. A voucher specimen has been deposited in the Department of Traditional Chinese Materia Medica, Liaoning College of Traditional Chinese Medicine.

Isolation. Dried aerial parts (5 kg) were extracted ×3 with MeOH for 1 hr at 80°. The extract was concd *in vacuo* at 40° to one-fifth; H_2O was added and the suspension filtered. The filtrate was extracted successively with Et_2O, EtOAc and *n*-BuOH–EtOAc (5:1). The extracts were evapd to dryness *in vacuo* at 40°. The EtOAc extract (3.9 g) was subjected to CC on Sephadex LH-20, eluting with H_2O to give **1** (18 mg), **2** (126 mg), **3** (24 mg), **4** (362 mg), **5** (83 mg) and **6** (43 mg). The *n*-BuOH–EtOAc extract (5 g) was subjected to CC on Sephadex LH-20, eluting with H_2O to give **1** (35 mg), **2** (48 mg) and **4** (31 mg).

Cistanoside F (**1**). Amorphous powder. FAB-MS *m/z* 511 $[M+Na]^+$. IR, UV, ^{1}H and ^{13}C NMR spectral data were in agreement with reported data [3].

β-Hydroxyacteoside (**2**). Amorphous powder. FAB-MS *m/z* 663 $[M+Na]^+$. IR, UV, ^{1}H and ^{13}C NMR spectral data were consistent with those of an authentic sample [4].

Campenoside I (**3**). Amorphous powder. FAB-MS *m/z* 677 $[M+Na]^+$. IR, UV, ^{1}H and ^{13}C NMR spectral data were in agreement with reported data [5].

Acteoside (**4**). Amorphous powder. FAB-MS *m/z* 647 $[M+Na]^+$. IR, UV, ^{1}H and ^{13}C NMR data were consistent with those of an authentic sample [4].

Orobanchoside (**5**). Amorphous powder. FAB-MS *m/z* 645 $[M-H_2O+Na]^+$. IR, UV, ^{1}H and ^{13}C NMR, HPLC spectral data were consistent with those of an authentic sample [6].

β-Oxoacteoside (**6**). Amorphous powder, mp 153–158° (uncorr.). $[\alpha]_D^{20}$ −72.2° (MeOH; *c* 0.3). FAB-MS *m/z* 639 $[M+1]^+$, 661 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 222 (4.31) sh, 232 (4.30), 287 (4.20), 326 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 1705, 1695 (C=O), 1605, 1521 (arom. C=C). ^{1}H NMR (CD_3OD): δ1.00 (3H, *d*, *J*=6 Hz, Rha-Me), 4.38 (1H, *d*, *J*=8 Hz, Glc-H-1), 5.13 (1H, *d*, *br s*, Rha-H-1), 6.74 (1H, *d*, *J*=8 Hz, H-5), 7.30–7.35 (2H, *m*, H-2, H-6), 6.18 (1H, *d*, *J*=16 Hz, H-8′), 6.68 (1H, *d*, *J*=8 Hz, H-5′), 6.85 (1H, *dd*, *J*=8, 2 Hz, H-6′), 6.96 (1H, *d*, *J*=2 Hz, H-2′), 7.50 (1H, *d*, *J*=16 Hz, H-7′). ^{13}C NMR see Table 1.

Reaction of compound **6** *with excess diazomethane.* A soln of **6** (30 mg) in MeOH was treated with excess CH_2N_2 and the mixt. left to stand overnight at 5°. The reaction mixt. was then evapd to dryness. The residue was extd with $CHCl_3$. The insol. portion was purified by prep. TLC using $CHCl_3$–MeOH–H_2O (13:7:2) to give **6a** (10 mg), amorphous powder, $C_{22}H_{32}O_{13}$. FAB-MS *m/z* 527 $[M+Na]^+$. The sol. portion was purified by prep. TLC using $CHCl_3$–EtOAc (1:1) to give **6c** (5 mg), needles (MeOH), mp 119–120°, which was identical with 4-(3,4-dimethyoxyphenyl)-2-pyrazoline-3-carboxylic acid Me ester obtained by the reaction of 3,4-dimethoxycinnamic acid in MeOH with excess CH_2N_2 [7].

Table 1. ^{13}C NMR spectral data for compounds **6**, **6a** and **6b** (in CD_3OD, 100 MHz)

	β-Oxo-dihydroxy-phenethyl moiety				Rhamno-glucosyl moiety			Caffeoyl moiety
C	**6**	**6a**	**6b**	C	**6**	**6a**	C	**6**
1	127.5	128.9	128.5	Glc-1	103.9	104.1	1′	127.7
2	115.5	111.2	111.1	Glc-2	76.0	75.5	2′	115.1
3	152.7	155.8	155.4	Glc-3	81.0	84.3	3′	146.6
4	146.4	150.4	150.6	Glc-4	70.2	70.1	4′	149.5
5	115.8	111.6	111.8	Glc-5	76.1	77.8	5′	116.3
6	122.6	123 4	123.5	Glc-6	62.2	62.5	6′	123.0
α	72.1	72.1	66.0	Rha-1	102.7	102.6	7′	147.8
β	196.5	196.7	198.6	Rha-2	71.9	71.9	8′	114.4
OMe	—	56.4	56.4	Rha-3	71.8	72.1	9′	168.0
		56.5	56.5	Rha-4	73.6	73.9		
				Rha-5	70.2	70.0		
				Rha-6	18.2	17.7		

Acid hydrolysis of compound **6a**. Compound **6a** in 1% H_2SO_4 soln was heated at 100° for 1 hr, then cooled. The mixt. was extracted with Et_2O. The Et_2O layer was washed and evapd to dryness. The residue was purified by prep. TLC using $CHCl_3$–EtOAc (1:1) to give **6b**, $C_{10}H_{12}O_4$, EIMS *m/z* 196 $[M]^+$. Compound **6b** was identical with β-oxo-β-(3,4-dimethoxyphenyl)-ethanol which was synthesized by the reaction of 3,4-dimethoxyacetophenone with iodobenzene [8].

The aq. layer was neutralized with $BaCO_3$ and the ppt. filtered off. The filtrate was evapd to dryness and the residue analysed by GC to identify L-rhamnose and D-glucose as TMSi ethers in a ratio of 1:1.

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