Pergamon

0031-9422(94)00299-1

Phytochemistry, Vol. 38, No. 3, pp. 741–743, 1995

0031-9422/95 $9.50 + 0.00

A PHENYLETHANOID GLYCOSIDE FROM *PLANTAGO ASIATICA*

SANSEI NISHIBE,* YASUHIKO TAMAYAMA, MICHIKO SASAHARA and CLAUDE ANDARY†

Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen-University, Ishikari-Tobetsu, Hokkaido 061-02, Japan; †Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Université de Montpellier, 34060 Montpellier Cedex 01, France

(*Received* 21 *February* 1994)

Key Word Index—*Plantago asiatica*; Plantaginaceae; phenylethanoid glycoside; plantasioside; orobanchoside.

Abstract—A new phenylethanoid glycoside, plantasioside, was isolated from the aerial parts of *Plantago asiatica*. The structure of plantasioside was deduced from chemical and spectral evidence to be 1′,2′-[β(3,4-dihydroxyphenyl)-α,β-dioxoethanol]-6′-*O*-caffeoyl-*O*-β-D-glucopyranoside. In addition, the structure of orobanchoside from *P. depressa* and *P. camtschatica* was revised to be 1′,2′-[β(3,4-dihydroxyphenyl)-α,β-dioxoethanol]-4′-*O*-caffeoyl-*O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-β-D-glucopyranoside from β-hydroxy-[β(3,4-dihydroxyphenyl)-ethyl]-4′-*O*-caffeoyl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside.

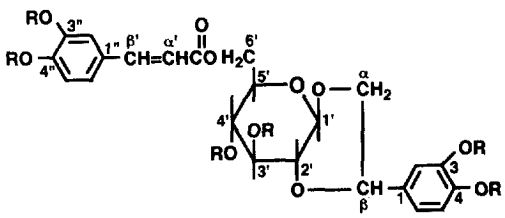
INTRODUCTION

The aerial parts of *Plantago asiatica*, 'Plantago Herba', have been used since ancient times as a diuretic, an anti-inflammatory and an anti-asthmatic drug, in China and Japan [1]. The herb is listed in the Japanese Pharmacopoeia XII as an important crude drug and an aqueous extract is also used as a medicine [2].

In a previous paper [3], we reported the isolation of five phenylethanoid glycosides, hellicoside, plantamajoside, isoplantamajoside, acteoside and 3,4-dihydroxyphenethyl alcohol-6-*O*-caffeoyl-β-D-glucoside (3,4-DPCG) and a flavone glucoside, plantaginin, from *P. asiatica*. We have now further examined the phenylethanoid glycosides of the aerial parts of *P. asiatica* and isolated a new phenylethanoid glycoside. In addition, according to the results of structural elucidation of a new phenylethanoid glycoside and previous work on orobanchoside [unpublished data and 7], we have compared the structure of different molecules named 'orobanchoside' which were respectively isolated from *Orobanche rapum-genistae* [4], *Plantago depressa* [5] and *P. camtschatica* [6].

RESULTS AND DISCUSSION

The methanol extract of the aerial parts of *P. asiatica* was fractionated by successive extractions with diethyl ether and ethyl acetate. The ethyl acetate extract after column chromatography on Sephadex LH-20, followed by successive column chromatography on silica gel and Sephadex LH-20, furnished **1**.



1 : R=H

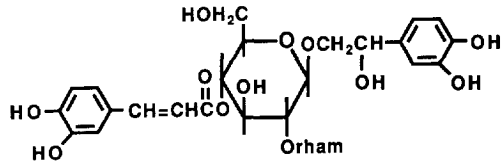
1a : R=Ac

Compound **1**, designated as plantasioside, was obtained as an amorphous powder, mp 159–163°, $[\alpha]_D^{26}$ +33.2° (MeOH), whose molecular formula ($C_{23}H_{24}O_{11}$) was confirmed by the observation of ions at *m/z* 499 $[M+Na]^+$ and *m/z* 477 $[M+H]^+$ by positive ion FAB-mass spectrometry. The UV spectrum of **1** showed absorption maxima at 290 and 329 nm, which were very similar to those of 3,4-DPCG. Its IR spectrum suggested the presence of a conjugated ester (1696 cm^{-1}), a conjugated double bond (1632 cm^{-1}) and aromatic rings (1604 and 1522 cm^{-1}), while its ^1H NMR spectrum resembled that of 3,4-DPCG except for displaying signals at δ4.53 (1H, *dd*, *J* = 3, 10 Hz) assignable to a C-β proton and δ3.93 (1H, *dd*, *J* = 3, 13 Hz), δ3.65 (1H, *m*) assignable to C-α protons instead of δ2.77 (2H, *t*, *J* = 7 Hz), δ3.56 (1H, *m*) and δ3.73 (1H, *m*) in the phenethyl moiety. The ^1H NMR spectrum of the acetate (**1a**) showed the presence of two alcoholic acetoxy (δ1.97 and 1.99) and four phenolic acetoxy (δ2.21, 2.22, 2.23 and 2.24) groups but no presence of a proton at the benzyl position bearing an acetoxy group as that of β-hydroxyacteoside acetate [3]. Hydrolysis of **1** with acid afforded only glucose on TLC examination.

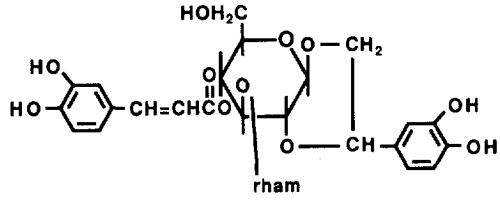
*Author to whom correspondence should be addressed.

These data suggested that **1** bore a marked structural resemblance in the linkage between a glucose and a phenethyl moiety to that of oraposide which contains, besides the glycosidic linkage, an ether linkage between a glucose and a phenethyl moiety [7]. The ^{13}C NMR spectrum of **1** was correlated with those of 3,4-DPCG isolated from *P. asiatica* [3] and oraposide isolated from the genus *Orobanche* [7]. The spectrum of **1** supported the attachment of the caffeoyl moiety at the C-6′ position of the glucose moiety and the presence of an ether linkage between a glucose moiety and phenethyl moiety (−5.1, +5.8 and −3.0 ppm shifts at C-1′, C-2′ and C-3′ positions of glucose moiety as compared to those of 3,4-DPCG), besides the glucosidic linkage. Consequently, the structure of **1** has been established as 1′,2′-[*β*(3,4-dihydroxyphenyl)-α,*β*-dioxoethanol]-6′-*O*-caffeoyl-*O*-*β*-D-glucopyranoside.

In the process of structural elucidation of **1**, we found that the chemical shifts at the C-α and *β* positions of the phenethyl moiety in the ^{13}C NMR spectrum of oroban-



Former structure of orobanchoside



Revised structure of orobanchoside

Table 1. ^{13}C NMR spectral data

C-atom	Plantasioside (1)*	3,4-DPCG*	Oraposide*	Orobanchoside*	Orobanchoside†	Crenatoside‡
Phenethyl moiety						
1	129.9	131.4	129.8	129.7	128.1	129.9
2	114.8	116.4	114.5	114.3	113.7	114.5
3	146.5	146.1	146.4	146.2	145.3	146.9
4	146.3	144.6	146.4	146.2	145.3	146.9
5	116.5	117.1	116.2	116.1	115.5	116.3
6	119.5	121.3	118.9	118.7	117.3	119.0
β	78.7	36.7	78.4	78.3	76.3	79.0
α	72.8	72.3	73.0	72.8	71.1	73.0
D-Glucose						
1′	99.4	104.5	99.1	98.9	97.1	99.1
2′	80.8	75.0	82.0	81.8	80.6	82.0
3′	74.9	77.9	77.4	77.2	74.5	77.5
4′	72.0	71.7	70.2	70.0	68.9	70.2
5′	77.2	75.4	77.8	77.7	76.3	77.9
6′	64.5	64.6	62.1	61.9	60.7	62.1
L-Rhamnose						
1	—	—	102.2	102.0	100.4	102.2
2	—	—	72.1	71.9	70.5	72.1
3	—	—	72.0	71.8	70.5	72.0
4	—	—	73.6	73.4	71.6	73.6
5	—	—	70.4	70.2	68.9	70.5
6	—	—	18.3	18.1	18.0	18.4
Caffeoyl moiety						
1″	127.7	127.7	127.7	127.5	125.6	127.7
2″	115.2	114.9	115.3	115.1	114.8	115.3
3″	146.8	146.7	146.8	146.6	145.7	146.4
4″	149.6	149.6	149.8	149.6	148.7	149.4
5″	116.2	116.5	116.5	116.3	113.4	116.5
6″	123.0	123.1	123.3	123.0	121.6	123.3
β′	147.2	147.2	148.3	148.0	146.0	148.3
α′	115.0	115.1	114.5	114.3	115.9	114.5
C=O	169.0	169.1	168.0	167.8	165.6	168.0

*Solvent : CD_3OD. Spectra were measured at 100.6 MHz.
†Solvent : DMSO-d_6 + CF_3CO_2H (2 drops). Spectrum was measured at 62.8 MHz [4].
‡Solvent : CD_3OD. Spectrum was measured at 100.6 MHz [8].

choside isolated from the genus *Plantago* were consistent with those of **1** and oraposide, but different from that of β-hydroxyacteoside [6]. In addition, the positive ion FAB-mass spectrum of orobanchoside gave only the ion at *m/z* 645 as $[M+Na]^+$, which was consistent with that of oraposide (molecular formula $C_{29}H_{32}O_{14}$). Thus, the structure of orobanchoside reported as β-hydroxy-[β(3,4-dihydroxyphenyl)-ethyl]-4′-*O*-caffeoyl-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside [4–6] should be replaced by that of oraposide [unpublished and 7]. Furthermore, the ^{13}C NMR spectra of oraposide, and crenatoside which was recently isolated from *Orobanche crenata* by Afifi *et al.* [8], were compared. As a result, the ^{13}C NMR spectra of orobanchoside, oraposide and crenatoside in methanol-d_4 were completely superimposed. The spectral data of orobanchoside [FAB-mass, UV, IR and ^{1}H NMR spectra] were also in agreement with those of oraposide and crenatoside.

We conclude that orobanchoside, oraposide and crenatoside are the same compound, that is, 1′,2′-[β(3,4-dihydroxyphenyl)-α,β-dioxoethanol]-4′-*O*-caffeoyl-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-glucopyranoside.

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. standard.

Plant material. 'Plantaginis Herba' from a Japanese market (dried aerial parts of *P. asiatica* L.) was used. A voucher specimen is deposited in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen-University.

Isolation. Crushed 'Plantaginis Herba' (1.2 kg) was extracted × 3 with 30% EtOH–H_2O (2:1) for 1 hr at 80°. The extract was concd *in vacuo* at 40° to one-fifth and the suspension filtered. The filtrate was extracted successively with Et_2O (1 l × 2) and EtOAc (1 l × 5). The extracts were evapd to dryness *in vacuo* at 40°. The EtOAc extract (117.1 g) was subjected to CC on Sephadex LH-20 eluting with H_2O, followed by a 10–70% MeOH–H_2O gradient. Frs (50 ml) were monitored by HPLC [Develosil ODS-5 (4.6 × 250 mm), eluant: H_2O–HOAc–MeOH, 29:2:9, det. UV: 330 nm; flow rate: 1.2 ml min^{-1}, temp: 40°]. The extract from frs containing **1** was chromatographed on a silica gel column with a 2.5–20% EtOH–$CHCl_3$ gradient to give frs 1–5. The extract from fr. 1 was subjected to CC on Sephadex LH-20 eluting with a 20–30% MeOH–H_2O gradient to give **1** (27 mg).

Plantasioside (**1**). Amorphous powder. Mp 159–163° (uncorr.). $[\alpha]_D^{26}$ +33.2° (MeOH; *c* 0.6). FAB-MS *m/z* 499 $[M+Na]^+$, 477 $[M+H]^+$. UV λ_{max}^{MeOH} nm (log ε): 219 (4.28), 233 (4.15) sh, 248 (3.98) sh, 290 (4.11), 329 (4.21). IR ν_{max}^{KBr} cm^{-1}: 3404 (OH), 1696 (C=O), 1632 (C=C), 1604, 1522 (arom. C=C). ^{1}H NMR (CD_3OD): δ3.19 (1H, *dd*, *J* = 7, 10 Hz, H-2′), 3.46 (1H, *t*, *J* = 10 Hz, H-4′), 3.62 (1H, *t*, *J* = 10 Hz, H-3′), 3.65 (1H, *m*, H-α_{ax}), 3.72 (1H, *m*, H-5′), 3.93 (1H, *dd*, *J* = 3, 13 Hz, H-α_{eq}), 4.33 (1H, *dd*, *J* = 6, 12 Hz, H-6′), 4.44 (1H, *d*, *J* = 7 Hz, H-1′), 4.53 (1H, *dd*, *J* = 3, 10 Hz, H-β_{ax}), 4.55 (1H, *dd*, *J* = 2, 12 Hz, H-6′), 6.29 (1H, *d*, *J* = 16 Hz, H-α′), 6.70 (1H, *dd*, *J* = 2, 8 Hz, H-6), 6.73 (1H, *d*, *J* = 8 Hz, H-5), 6.77 (1H, *d*, *J* = 8 Hz, H-5″), 6.84 (1H, *d*, *J* = 2 Hz, H-2), 6.94 (1H, *dd*, *J* = 2, 8 Hz, H-6″), 7.05 (1H, *d*, *J* = 2 Hz, H-2″), 7.57 (1H, *d*, *J* = 16 Hz, H-β′). ^{13}C NMR: see Table 1.

Acetate (**1a**). Prepd via Ac_2O–pyridine. Amorphous powder. ^{1}H NMR ($CDCl_3$): δ1.97 (3H, *s*, alcoholic Ac), 1.99 (3H, *s*, alcoholic Ac), 2.21 (3H, *s*, phenolic Ac), 2.22 (3H, *s*, phenolic Ac), 2.23 (3H, *s*, phenolic Ac), 2.24 (3H, *s*, phenolic Ac), 4.45 (1H, *d*, *J* = 7 Hz, H-1′), 6.35 (1H, *d*, *J* = 16 Hz, H-α′), 7.09–7.35 (6H, *m*, arom. H), 7.58 (1H, *d*, *J* = 16 Hz, H-β′).

Acid hydrolysis of compound **1**. Compound **1** was treated with 1% H_2SO_4 soln. The presence of D-glucose in the hydrolysate was shown by TLC examination.

Acknowledgements—We thank Mr Yoshitaka Ohta of Alps Pharmaceutical Co. Ltd, Japan for supplying 'Plantaginis Herba' from a Japanese market.

REFERENCES

1. Mitsuhashi, H. (ed.) (1988) *Illustrated Medicinal Plants of the World in Colour*, p. 493. Hokuryukan, Tokyo.
2. Nippon Kouteisho Kyokai (1991) in *Japanese Pharmacopoeia XII*, p. D-436. Hirokawa, Tokyo.
3. Ravn, H., Nishibe, S., Sasahara, M. and Xuebo, L. (1990) *Phytochemistry* **29**, 3627.
4. Andary, C., Wylde, R., Laffite, C., Privat, G. and Winternitz, F. (1982) *Phytochemistry* **21**, 1123.
5. Nishibe, S., Sasahara, M., Jiao, Y., Yuan, C. L. and Tanaka, T. (1993) *Phytochemistry* **32**, 975.
6. Jiao, Y., Sasahara, M., Nishibe, S., Yuan, C. L. and Tanaka, T. (1993) *Shoyakugaku Zasshi* **47**, 330.
7. Andary, C. (1989) *Brevet d'invention pour la France*, n°89/12369 (20 September).
8. Afifi, M. S., Lahloub, M. F., El-Khayaat, S. A., Anklin, C. G., Rüegger, H. and Sticher, O. (1993) *Planta Med.* **59**, 359.