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IRESINOSIDE, A YELLOW PIGMENT FROM *PFAFFIA IRESINOIDES*

YOSHINORI SHIOBARA, SHUN-SUKE INOUE, YUKARI NISHIGUCHI, KEIKO KATO, TSUNEMATSU TAKEMOTO, NOBUSHIGE NISHIMOTO,* FERNANDO DE OLIVEIRA,† GOKITHI AKISUE,† MARIA KUBOTA AKISUE† and GORO HASHIMOTO‡

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan; †Department de Farmácia da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, São Paulo 30786, Brazil; ‡Centro de Pesquisas História Natural, Vila Leopoldina, São Paulo 05318, Brazil

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Key Word Index—*Pfaffia iresinoides*; Amaranthaceae; Brazil ginseng; roots; yellow pigment; styrylpyrone glucoside.

Abstract—Iresinoside, a new yellow pigment, has been isolated from the crude drug 'Brazil ginseng', the roots of *Pfaffia iresinoides*. Its structure was elucidated by chemical and extensive spectral analyses.

INTRODUCTION

In the course of our continuing search for chemical constituents of 'Brazil ginseng'. dried roots of *Pfaffia iresinoides* Spreng. we have isolated a large amount of ecdysterone [1] together with ecdysteroid glucosides [2]. Further survey of the glycoside fractions led to the isolation of a novel yellow pigment, named iresinoside, which has an extended styryl-2-pyrone structure.

RESULTS AND DISCUSSION

Iresinoside (**1**) was obtained as a yellow amorphous powder. Its NMR spectrum (Table 1) indicated the presence of a *ca* 2:1 ratio of two components, each one giving a major and a minor set of signals. In particular, the observation of two sets of AB quartet signals ($J = 16.5$ and 12.5 Hz) suggested that **1** was a mixture of *trans* and *cis* double bond isomers. On examination of the yellow pigment by HPLC, two peaks were detectable. Attempts to isolate the individual compounds using HPLC were unsuccessful, because on working-up the fractions each compound changed to give the same mixture described above. These results suggested that the two components could isomerize to each other.

Fortunately, methylation of **1** with Me_2SO_4–K_2CO_3 afforded two trimethylates in a ratio of *ca* 5:1 and the major product (**2**) could be separated by fractional recrystallization. Compound **2**, $C_{31}H_{34}O_{12}$ was obtained as yellow needles, which gave glucose and aglycone (**3**) on enzymatic hydrolysis. Its ^{1}H NMR spectrum (Table 1) indicated the presence of a carbomethoxyl group, two methoxyl groups on an aromatic ring, two sets of A_2B_2 quartets which was characteristic of a *p*-substituted phenyl group, methine and methydne groups coupled to each other, an AB quartet due to the *trans* olefinic protons and an isolated sp^2 methine, besides signals assigned to a glucose moiety. The partial structures, *p*-methoxystyryl- and *p*-methoxyphenethyl- were deduced by ^{1}H–^{1}H COSY analysis. The aromatic protons [H-2(6)′ and H-2(6)″] were long-range coupled with the *trans* olefinic proton (H-8) and the sp^3 methine proton (H-9), respectively. In addition, the ^{13}C NMR spectrum (Table 2) indicated the presence of three sp^2 quaternary carbons, two of which were oxygen-bearing, and a conjugated carbonyl carbon. These data along with the IR absorptions (1710, 1670, 1540 cm^{-1}) suggested the presence of a trisubstituted pyrone ring. Furthermore, the UV spectrum of **3** was similar to that of yangonin [3], that is, 6-(*p*-methoxystyryl)-4-methoxy-2-pyrone. Long-range ^{13}C–^{1}H COSY experiments were carried out under various conditions in order to clarify the connectivities of the partial structures and the substituent groups (Table 3.) The sp^3 methine proton (H-9) was correlated with four quaternary carbons (C-3, C-4, C-2 and C-1″), two of which of the former were correlated with H-5. Furthermore, H-5 was correlated with the sp^2 oxygen-bearing carbon (C-6) and the vinylic carbon of styryl group (C-7). The anomeric proton and the sp^3 methylene protons (H-10) were correlated with C-4 and the methoxycarbonyl carbon (C-11), respectively. Thus, the connectivities centred around the pyrone ring were revealed to give the extended styrylpyrone structure of **2**.

The aglycone (**3**) obtained from enzymatic hydrolysis of **2** was considered to exist in a 2-pyrone form because no marked difference in the UV spectra was observed between **2** and **3** [4]. The β-D-glucopyranosyl linkage was assigned by means of the coupling constant of the anomeric proton (*d*, $J = 7.4$ Hz) and comparison of the molecular rotation difference, $[M]_D$ (**2**) − $[M]_D$ (**3**) = −159° with that of methyl glucoside [5]. The assignments of ^{1}H and ^{13}C NMR of **3** were performed through a comparison with that of compound **2** (Tables 1 and 2). Thus, the structure of **2** was established as shown in the formula except for the absolute configuration of the C-9 position.

From the above data it was suggested that iresinoside (**1**) was a mixture of *trans* and *cis* Δ^7 isomers. As expected,

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds **1a**, **b**, **2**–**4** (400 MHz, in DMSO-d_6, δ values in ppm from TMS)

H	1a	1b	2	3	4
5	6.51 *s*	6.54 *s*	6.60 *s*	6.20 *s*	6.37 *s*
7	6.72 *d* (16.5)	5.97 *d* (12.5)	6.79 *d* (16.1)	6.86 *d* (16.5)	2.68 *m*
8	7.18 *d* (16.5)	6.67 *d* (12.5)	7.26 *d* (16.1)	7.20 *d* (16.5)	2.75 *m*
9	4.65 *t* (8.0)	4.60 *t* (8.0)	4.73 *t* (8.0)	4 62 *t* (8.0)	4.62 *t* (7.9)
10	3.00, 3.14 *dd* (16.0, 8.0)	2.96, 3.12 *dd* (16.0, 8.0)	3.15, 3.27 *dd* (16.0, 8.0)	3.17 *d* (8.0)	2.95, 3.08 *dd* (15.7, 7 9)
11-OMe	—	—	3.52 *s*	3 53 *s*	—
2(6)′	7.46 *d* (8.6)	7.35 *d* (8.6)	7.59 *d* (8.8)	7.61 *d* (8 6)	7.02 *d* (8.6)
3(5)′	6.80 *d* (8.6)	6.70 *d* (8.6)	6.98 *d* (8.8)	6.96 *d* (8.6)	6.68 *d* (8.6)
4′-OMe	—	—	3.79 *s*	3.79 *s*	—
2(6)″	7.23 *d* (8.6)	7.20 *d* (8.6)	7.34 *d* (8.8)	7.25 *d* (8.6)	7.20 *d* (8.6)
3(5)″	6.60 *d* (8.6)	6.58 *d* (8.6)	6.78 *d* (8.8)	6.82 *d* (8.6)	6.61 *d* (8.6)
4″-OMe	—	—	3.70 *s*	3.70 *s*	—
Glc-1	5.04 *d* (7.4)	4.92 *d* (7.4)	5.06 *d* (7.4)	—	4.96 *d* (7.4)
2–6	3.20–3.70	3.20–3.70	3.30–3.75	—	3.20–3.70

Values in parentheses are coupling constants in Hz.

when the yellow pigment was catalytically hydrogenated, a single product (**4**) was obtained in 80% yield. Compound **4** gave a $[M+H]^+$ ion at *m/z* 559 in its FAB mass spectrum consistent with the molecular formula $C_{28}H_{30}O_{12}$. The ^{1}H NMR spectrum of **4** indicated the presence of A_2B_2-type methylene groups. In the UV spectrum no absorption was observed at wavelengths longer than 287 nm. Extensive analyses using ^{1}H–^{1}H, ^{13}C–^{1}H and long-range ^{13}C–^{1}H COSY experiments allowed the assignments of the ^{1}H and ^{13}C spectra. Furthermore, it was possible to assign the ^{1}H and ^{13}C NMR signals of iresinoside (**1**) due to the major (**1a**) and the minor component (**1b**) by comparison with those of compounds **2** and **4** (Tables 1 and 2).

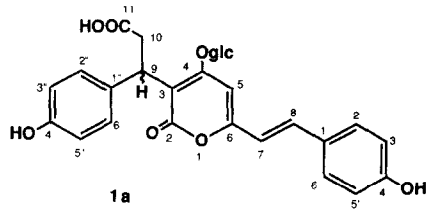
It has been reported that yangonin undergoes a *trans–cis* photoisomerization to give an equilibrium mixture in aqueous MeOH, but in organic solvents no change occurs [6]. Considering a similar reaction to occur in the present study, the behaviour of **1** on HPLC could be explained. In the case of compounds **2** and **3**, the *trans* isomers obtained by fractional recrystallization, no isomerization was observed through experimental measurements in DMSO-d_6 for structural elucidation. Iresinoside (**1**) is probably biosynthesized from bis-noryangonin [7] by the addition of a phenylpropanoid unit (e.g. *p*-coumaric acid) and subsequent glycosylation.

EXPERIMENTAL

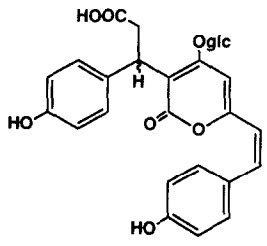
Mps: uncorr. TLC was performed on Kiesel gel 60KF$_{254}$ precoated silica gel plates (Merck) using solvent A: $CHCl_3$–MeOH–H_2O (13:7:2, lower phase); solvent B: *n*-BuOH–HOAc–H_2O (4:1:1); solvent C: $CHCl_3$–MeOH (4:1). Spots were visualized in UV light or by spraying with 10% H_2SO_4 and heating at 150°. HPLC was carried out on a 15 cm × 4.6 mm i.d. column of Cosmosil 5 Ph. (Nacalai tesque) using 40% MeOH containing 0.1% HOAc; flow rate: 1 ml min^{-1}; UV detector: 300 nm. LC was carried out on a 50 cm × 22 mm i.d. column of silica gel (TLC-Kieselgel 60H, 15 μm, Merck).

Plant material. The material was the same as that described in the preceding paper [2].

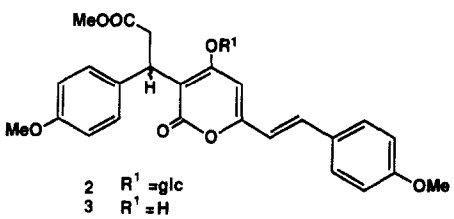
Isolation of iresinoside (**1**). In the previous paper [2], we have described the isolation of ecdysteroid glycosides by CC in which the residue (40 g) containing glycosides was obtained from dried



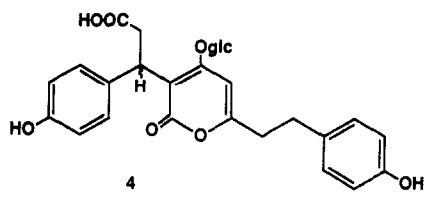
1a



1b



2 R¹ =glc
3 R¹ =H



4

Table 2. ^{13}C NMR spectral data of compounds **1a**, **b**, **2**–**4** (100 MHz, in DMSO-d_6, δ values in ppm from TMS)

C	1a	1b	2	3	4
2	163.8[a]	162.2[a]	162.3	162.8[a]	163.3
3	109.0	109.8	108.7	104.8	108.1
4	164.1[a]	162.5[a]	164.2	165.2[a]	164.0
5	97.9	97.9	98.2	100.8	97.1
6	159.0[b]	157.8[b]	157.9	157.6[b]	163.7
7	116.6	117.8	117.3	117.3	34.9
8	134.2	136.5	133.9	133.3	31.2
9	35.1	35.1	35.0	35.1	35.2
10	36.2	36.2	35.4	36.3	36.7
11	173.8	173.8	172.3	172.3	174.0
11-OMe	—	—	51.3	51.2	—
1′	126.5	126.5	127.8	127.9	130.3
2(6)′	129.3	131.4	129.1	129.1	129.1
3(5)′	115.2	116.0	114.5	114.3	115.3
4′	158.0[b]	158.0[b]	160.4	160.2	155.8
4′-OMe	—	—	55.3	55.3	—
1″	132.5	132.4	133.9	134.7	132.8
2(6)″	128.8	128.8	128.8	128.3	128.8
3(5)″	114.8	114.8	113.3	113.5	114.6
4″	155.7	155.7	157.7	157.1[b]	155.6
4″-OMe	—	—	55.0	54.9	—
Glc-1	99.7	99.7	99.7	—	99.6
2	73.2	73.2	73.2	—	73.2
3	76.9	76.9	76.9	—	76.9
4	69.5	69.5	69.5	—	69.6
5	77.4	77.4	77.4	—	77.5
6	60.5	60.4	60.5	—	60.5

Values in parentheses are coupling constants in Hz.
[a,b]Values with the same superscript in the same column are interchangeable.

Table 3. Long-range couplings observed in long-range ^{13}C–^{1}H COSY experiments* of compounds **2** and **4**

H	2 Correlated carbon $^{2}J_{CH}$ coupling	2 Correlated carbon $^{3}J_{CH}$ coupling	4 Correlated carbon $^{2}J_{CH}$ coupling	4 Correlated carbon $^{3}J_{CH}$ coupling
5	C-4,† 6	C-3, 7	C-4	C-3
7	C-6	C-1′	C-6	—
8	—	C-6, 2(6)′	—	C-2(6)′
9	C-3, 1″	C-2, 4, 2(6)″	C-3, 1″	C-2, 4, 2(6)″
10	C-11	C-3	—	—
2(6)′	—	C-8, 4′	—	C-4′
3(5)′	—	C-1′	—	C-1′
4′-OMe	—	C-4′	—	—
2(6)″	—	C-4″	—	C-4″
3(5)″	—	C-1″	—	C-1″
4″-OMe	—	C-4″	—	—
11-OMe	—	C-11	—	—
Glc-1	—	C-4‡	—	—

Measured under the conditions of $^{*}\Delta_2 = 25$ msec ($J = 10$ Hz) at 25°, $^{\dagger}\Delta_2 = 40$ msec ($J = 6.25$ Hz) at 25° and $^{\ddagger}\Delta_2 = 50$ msec ($J = 5$ Hz) at 42°.

roots (60 kg) by chromatography on silica gel (400 g) using $CHCl_3$–MeOH–H_2O (8:2:1, lower phase, 24 l) and $CHCl_3$–MeOH–H_2O (15:5:2, lower phase, 21 l) and the collection of 300 ml frs. Frs 120–150 were concd and the residue (6.8 g) passed through a column of Toyopearl HW-40F (Toyo Soda) using $CHCl_3$–MeOH (1:1) to give a residue (3.1 g), which was subjected to LC using EtOAc–MeOH–H_2O (18:2:1); 20 ml frs collected. Frs 85–105 were concd to afford iresinoside (**1**) as a

yellow amorphous powder (1.6 g), which showed a single spot on TLC (solvent A, R_f 0.15). $[\alpha]_D$ −43.5° (MeOH; *c* 1). FAB-MS: *m/z* 557 $[M+H]^+$. UV λ_{max}^{EtOH} nm (log ε): 223 (4.30), 284 (4.05), 378 (4.40). IR ν_{max}^{KBr} cm^{-1}: 1720, 1700, 1660, 1600, 1580, 1550, 960, 830.

Behaviour of iresinoside (**1**) *on HPLC.* Iresinoside was subjected to prep. HPLC and the component corresponding to R_t 5.9 and 9.1 min collected. On re-examination of each eluate after removal of the solvent by HPLC, two peaks were again detectable.

Methylation of iresinoside (**1**). Iresinoside (300 mg) was methylated in the usual manner with Me_2SO_4–K_2CO_3. The reaction product was chromatographed on silica gel using $CHCl_3$–MeOH (19:1). The eluate was concd and recrystallized from EtOAc–MeOH to afford **3** (210 mg) as yellow needles, mp 174–176°. $[\alpha]_D$ −71.4° (MeOH; *c* 1). Found: C, 60.62; H, 5.53. Calc for $C_{31}H_{34}O_{12} \cdot H_2O$:C, 60.38; H, 5.78. UV λ_{max}^{EtOH} nm (log ε): 223 (4.43), 268sh (4.07), 276 (4.12), 374 (4.50). IR ν_{max}^{KBr} cm^{-1}: 3400, 1735, 1710, 1670, 1640, 1600, 1540, 960, 825.

Enzymatic hydrolysis of compound **2**. Compound **2** (50 mg) was incubated at 37° in H_2O (10 ml) containing a few drops of EtOH with cellulase (50 mg). After 24 hr, the reaction mixt. was evapd and the residue chromatographed on silica gel using $CHCl_3$ and $CHCl_3$–MeOH (7:3). The residue obtained from the first eluate was recrystallized from EtOAc–MeOH to afford **3** (20 mg) as yellow needles. The second eluate showed a spot identical to glucose on TLC (solvent A, R_f 0.08; solvent **B**, R_f 0.26).

Compound **3**. Mp 192–194°. $[\alpha]_D$ −61.5° (MeOH; *c* 1). HREI-MS *m/z*: 436.1487 (calc for $C_{25}H_{24}O_7$: 436.1522). UV λ_{max}^{EtOH} nm (log ε): 224 (4.38), 265 (4.01), 273sh (4.05), 369 (4.34). IR ν_{max}^{KBr} cm^{-1}: 3450, 1735, 1710, 1660, 1600, 1555, 960, 828.

Catalytic reduction of iresinoside (**1**). Iresinoside (250 mg) was hydrogenated over PtO_2 (25 mg) in MeOH (10 ml) for 20 hr. The residue obtained by usual work-up was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (15:5:2, lower phase) to afford compound **4** (198 mg) as a powder. $[\alpha]_D$ +43.7° (MeOH; *c* 0.75). FAB-MS: *m/z* 559 $[M+H]^+$. UV λ_{max}^{EtOH} nm (log ε): 223 (4.18), 287 (3.97). IR ν_{max}^{KBr} cm^{-1}: 3300, 1700, 1680, 1600, 1555, 825.

REFERENCES

1. Nishimoto, N., Shiobara, Y., Fujino, M., Inoue, S., Takemoto, T., Oliveira, F., Akisue, G., Akisue, M., Hashimoto, G., Tanaka, O., Kasai, R. and Matsuura, H. (1987) *Phytochemistry* **26**, 2505.
2. Nishimoto, N., Shiobara, Y., Inoue, S., Fujino, M., Takemoto, T., Yeoh, C. L., Oliveira, F., Akisue, G., Akisue, M. and Hashimoto, G. (1988) *Phytochemistry* **27**, 1665.
3. Mors, W. B., Magalhães, M. T. and Gottlieb, O. R. (1962) *Fortschritte Chem. Org. Naturst.* **20**, 131.
4. Scott, A. I. (1964) *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 141. Pergamon Press, London.
5. Klyne, W. (1955) *Determination of Organic Structures by Physical Methods* (Braude, E. A. and Nachod, F. C., eds), p. 73. Academic Press, New York.
6. Smith, R. M., Thakrar, H., Arowolo, T. A. and Shafi, A. A. (1984) *J. Chromatogr.* **283**, 303.
7. Hatfield, G. M. and Brady, L. R. (1968) *Lloydia* **31**, 225.