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PFAFFOSIDES, NORTRITERPENOID SAPONINS, FROM *PFAFFIA PANICULATA**

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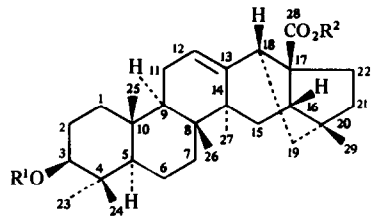
Key Word Index—*Pfaffia paniculata*; Amaranthaceae; Brazil ginseng; nortriterpenoid saponin; pfaffosides.

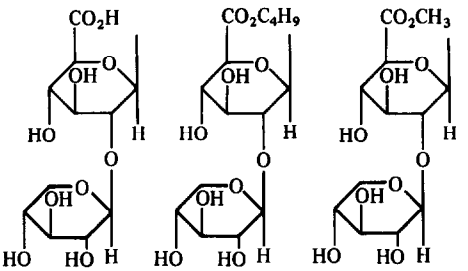
Abstract—Three new nortriterpene saponins having inhibitory effects on the growth of cultured tumor cells, named pfaffosides D, E and F, have been isolated from *Pfaffia paniculata*. Their structures have been established as 3β-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-(6-*O*-*n*-butyl) glucuronopyranosyl]-pfaffic acid-(28 → 1)-β-D-glucopyranosyl ester, 3β-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-(6-*O*-methyl) glucuronopyranosyl]-pfaffic acid-(28 → 1)-β-D-glucopyranosyl ester and 3β-*O*-[β-D-glucuronopyranosyl]-pfaffic acid, respectively, based on their chemical and spectroscopic properties.

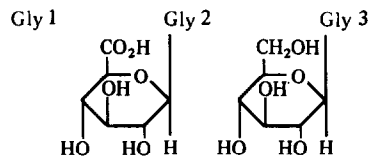
The root of *Pfaffia paniculata* Kuntze, known in Brazil as 'Brazil ginseng', have been used as a tonic, an aphrodisiac and as a folk medicine for antidiabetic purposes [2]. In our previous studies on the constituents of this plant, we reported the isolation and the structural elucidation of a new nortriterpene named pfaffic acid and three new saponins named pfaffosides A(**1**), B(**2**) and C(**3**), together with other known compounds [1, 3]. In the present paper, the structural elucidation of three more saponins named pfaffosides D(**4**), E(**5**) and F(**6**) is described.

The roots of *Pfaffia paniculata*, collected in the Goias area of Brazil, were treated with hot methanol and partitioned in an *n*-butanol–water mixture. The water soluble portion of the *n*-butanol layer was passed through a column of charcoal and purified by chromatography on silica gel to yield the pfaffosides (**1–6**).

Pfaffoside D (**4**), $C_{50}H_{70}O_{18}\cdot 4H_2O$, mp 185°, $[\alpha]_D^{22}$ −1.3° (*c* 0.42, MeOH), and pfaffoside E (**5**), $C_{47}H_{72}O_{18}\cdot 3\frac{1}{2}H_2O$, mp 197–199° $[\alpha]_D^{22}$ −1.5° (*c* 0.48, MeOH), contained hydroxyl groups (3400 cm^{-1}) and ester groups (1730 and 1740 cm^{-1}, respectively), as judged from the IR spectra. Acid hydrolysis of both **4** and **5** yielded pfaffic acid [3] as the aglycone and xylose, glucuronic acid and glucose as the sugar moieties, respectively. The ^{13}C NMR spectrum of **4** showed 50 carbon signals (Table 1). The spectra of both **2** and **4** suggested that **4** was the *n*-butyl ester at C-6 of the glucuronopyranosyl unit of **2**, as the chemical shift of the C-6 signal of the glucuronopyranosyl unit of **4** was displaced up-field by 2.7 ppm from that of **2** and an additional four signals at δ65.1 (*t*), 30.9 (*t*), 19.2 (*t*) and 13.8 (*q*) were attributed to an *n*-butyl group [4]. The above conclusion was further confirmed by the GC detection of *n*-butanol on alkaline hydrolysis of **4**. Based on the above results, the structure of **4** has been established as 3β-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-(6-*O*-*n*-butyl)glucuronopyranosyl]-pfaffic acid-(28 → 1)-β-D-glucopyranosyl ester. In the comparison with **4**, the ^{13}C NMR spectrum of **5** which showed 47 carbon signals (Table 1) revealed that **5** was the methyl







Gly 4 Gly 5

1	R^1 = Gly 1	R^2 = H
2	R^1 = Gly 1	R^2 = Gly 5
3	R^1 = Gly 4	R^2 = Gly 5
4	R^1 = Gly 2	R^2 = Gly 5
5	R^1 = Gly 3	R^2 = Gly 5
6	R^1 = Gly 4	R^2 = H

*Part 2 in the series "Pfaffosides". For Part 1 see ref. [1].

Table 1. ^{13}C NMR chemical shifts of pfaffosides A (1), B (2), C (3), D (4), E (5) and F (6)

Carbon	1	2	3	4	5	6
1	38.7 *t*	38.7 *t*	38.7 *t*	38.7 *t*	38.7 *t*	38.6 *t*
2	26.6 *t*	26.6 *t*	26.6 *t*	26.7 *t*	26.6 *t*	26.6 *t*
3	89.4 *d*	89.4 *d*	89.2 *d*	89.5 *d*	89.6 *d*	89.2 *d*
4	39.6 *s*	39.6 *s*	39.6 *s*	39.6 *s*	39.6 *s*	39.6 *s*
5	56.0 *d*	56.0 *d*	55.9 *d*	56.0 *d*	56.0 *d*	55.9 *d*
6	18.5 *t*	18.7 *t*	18.5 *t*	18.5 *t*	18.5 *t*	18.5 *t*
7	33.8 *t*	33.3 *t*	33.3 *t*	33.3 *t*	33.3 *t*	33.8 *t*
8	39.6 *s*	39.6 *s*	40.1 *s*	40.0 *s*	40.0 *s*	40.1 *s*
9	47.7 *d*	47.7 *d*	47.8 *d*	47.8 *d*	47.8 *d*	47.7 *d*
10	36.9 *s*	36.9 *s*	37.0 *s*	37.0 *s*	36.9 *s*	36.9 *s*
11	23.3 *t*	23.4 *t*	23.4 *t*	23.4 *t*	23.4 *t*	23.4 *t*
12	120.3 *d*	121.1 *d*	121.1 *d*	121.0 *d*	121.0 *d*	120.2 *d*
13	145.6 *s*	144.7 *s*	144.7 *s*	144.7 *s*	144.7 *s*	145.6 *s*
14	40.7 *s*	40.9 *s*	40.9 *s*	40.9 *s*	40.9 *s*	40.7 *s*
15	29.1 *t*	29.0 *t*	29.0 *t*	29.0 *t*	29.0 *t*	29.0 *t*
16	52.1 *d*	51.8 *d**	51.9 *d**	51.8 *d**	51.8 *d**	52.1 *d*
17	56.4 *s*	56.2 *s*	56.2 *s*	56.2 *s*	56.2 *s*	56.4 *s*
18	52.1 *d*	52.2 *d**	52.0 *d**	52.1 *d**	52.1 *d**	52.1 *d*
19	41.6 *t*	41.4 *t*	41.4 *t*	41.4 *t*	41.5 *t*	41.5 *t*
20	44.4 *s*	44.4 *s*	44.5 *s*	44.4 *s*	44.4 *s*	44.4 *s*
21	39.5 *t*	39.0 *t*	39.1 *t*	39.0 *t*	39.0 *t*	39.6 *t*
22	32.2 *t*	32.2 *t*	32.2 *t*	32.1 *t*	32.2 *t*	32.1 *t*
23	30.2 *q*	30.1 *q*	30.1 *q*	30.1 *q*	30.1 *q*	30.2 *q*
24	16.2 *q*	16.2 *q*	16.8 *q*	16.2 *q*	16.2 *q*	16.2 *q*
25	15.3 *q*	15.4 *q*	15.4 *q*	15.4 *q*	15.4 *q*	15.3 *q*
26	16.7 *q*	17.3 *q*	17.3 *q*	17.3 *q*	17.2 *q*	18.6 *q*
27	27.8 *q*	27.8 *q*	28.2 *q*	27.8 *q*	27.8 *q*	28.1 *q*
28	177.8 *s*	174.2 *s*	174.2 *s*	174.1 *s*	174.1 *s*	177.8 *s*
29	18.7 *q*	18.5 *q*	18.5 *q*	18.5 *q*	18.5 *q*	18.7 *q*
Glucuronic acid						
1	105.4 *d*	105.4 *d*	107.4 *d*	105.5 *d*	105.4 *d*	107.3 *d*
2	83.6 *d*	83.7 *d*	75.6 *d*	83.5 *d*	83.4 *d*	75.5 *d*
3	77.4 *d**	77.5 *d**	78.3 *d*	77.7 *d*	77.6 *d*	78.0 *d*
4	73.2 *d*	73.2 *d*	73.6 *d*	72.8 *d*	72.9 *d*	73.5 *d*
5	77.8 *d**	77.8 *d**	77.9 *d*	77.0 *d*	76.9 *d*	77.2 *d*
6	172.9 *s*	172.9 *s*	173.3 *s*	170.2 *s*	170.6 *s*	173.0 *s*
6–O$\underline{C}$H$_2$–				65.1 *t*	52.1 *q*	
–OCH$_2\underline{C}$H$_2$–				30.9 *t*	(6–O–$\underline{C}$H$_3$)	
–$\underline{C}$H$_2$CH$_3$				19.2 *t*		
–CH$_2\underline{C}$H$_3$				13.8 *q*		
Xylose						
1	107.0 *d*	107.1 *d*		107.1 *d*	107.0 *d*	
2	76.6 *d*	76.6 *d*		76.6 *d*	76.6 *d*	
3	78.2 *d*	78.2 *d*		78.2 *d*	78.2 *d*	
4	71.1 *d*	71.2 *d*		71.1 *d*	71.1 *d*	
5	67.5 *t*	67.6 *t*		67.6 *t*	67.6 *t*	
Glucose						
1		95.7 *d*	95.8 *d*	95.7 *d*	95.7 *d*	
2		74.2 *d*	74.2 *d*	74.2 *d*	74.2 *d*	
3		79.0 *d*	79.1 *d*	79.0 *d*	79.0 *d*	
4		71.4 *d*	71.4 *d*	71.4 *d*	71.4 *d*	
5		78.9 *d*	79.0 *d*	78.9 *d*	78.9 *d*	
6		62.4 *t*	62.5 *t*	62.4 *t*	62.4 *t*	

^{13}C NMR were recorded on a JEOL FX-100 FT-NMR spectrometer (25.15 Hz). The chemical shifts were expressed in δ-values in ppm relative to TMS used as internal standard.

*These values are interchangeable within their respective columns.

ester at C-6 of the glucuronopyranosyl unit of **2**, as the signal due to a methyl group instead of those due to an *n*-butyl group in the spectrum of **4** was observed at 52.1 (*q*). The above conclusion was also confirmed by the GC detection of the methanol on alkaline hydrolysis of **5**. Based on the above results, the structure of **5** has been established as 3*β*-*O*-[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-(6-*O*-methyl)glucuronopyranosyl]-pfaffic acid-(28 → 1)-*β*-D-glucopyranosyl ester.

As we had reservations regarding **4** and **5** as artifacts, the roots of this plant were extracted with hot water, purified without using *n*-butanol and methanol, and subjected to TLC to identify **4** and **5**, which were no longer observed on TLC. The above result suggested the possibility that **4** and **5** were artifacts formed from **2** during the procedures of extraction and separation using *n*-butanol and methanol.

Pfaffoside F (**6**), $C_{35}H_{52}O_9 \cdot 3\frac{1}{2}\ H_2O$, mp 243–244°, $[\alpha]_D^{22}$ + 32.4° (*c* 0.32, MeOH), contained hydroxyl groups (3400 cm^{-1}) and carboxyl groups (1730 and 1700 cm^{-1}), as judged from the IR spectrum. Acid hydrolysis of **6** yielded pfaffic acid as the aglycone and glucuronic acid as the sugar moiety. The comparison of the ^{13}C NMR spectrum of **6** with those of **1** and **3** revealed that the signals due to the aglycone moiety and the glucuronic acid moiety of **6** were superimposable with those due to the aglycone moiety of **1** and to the glucuronic acid moiety of **3** (Table 1). Further, alkaline hydrolysis of **3** yielded **6**. Accordingly, the structure of **6** was established as 3*β*-*O*-[*β*-D-glucuronopyranosyl]-pfaffic acid.

Pfaffosides D (**4**), E (**5**) and F (**6**) show inhibitory effects on the growth of cultured tumor cell melanomas (B-16) at concentrations of *ca* 70, *ca* 120 and *ca* 30 μg/ml, respectively, using the method reported previously [1]. It is interesting that the inhibitory effect of **6** is the highest among the pfaffosides A–F (**1**–**6**) [1].

EXPERIMENTAL

General remarks. Mps are uncorr. ^{13}C NMR spectra taken in C_5D_5N using TMS as internal standard. TLC was conducted on Kieselgel 60 F_{254} (Merck) using the lower phase of $CHCl_3$–MeOH–H_2O (65:35:10) as solvent and spots were detected by spraying with 10% H_2SO_4, followed by heating.

Plant material. The plant material was same as described in the preceding paper [1].

Isolation of pfaffosides (**4**, **5** *and* **6**). In the previous paper [1], we have described the isolation of pfaffosides by column chromatography, that is, crude saponin (5 g) has been obtained from the air-dried roots (*ca* 2 kg) and chromatographed on a silica gel column (150 g). The fraction, eluted with $CHCl_3$–MeOH–H_2O (8:2:0.5, lower phase), afforded crude **4** and **5** which were repeatedly subjected to CC on silica gel and eluted with the same solvent to afford chromatographically pure pfaffosides. Pure samples of **4** and **5** were obtained by recrystallization from MeOH–EtOAc–Et_2O: amorphous (140 mg) and amorphous (180 mg), respectively. The fraction eluted with $CHCl_3$–MeOH–H_2O (7:3:1, lower phase) afforded crude **6** besides **1**, **2** and **3**. Crude **6** was purified by the same method used in the purification of **1**, **2** and **3**. A pure sample of **6** was obtained, by recrystallization from MeOH–EtOAc, as colorless fine crystals (20 mg).

Pfaffoside D (**4**). Mp 185°, $[\alpha]_D^{22}$ − 1.3° (*c* 0.42, MeOH). (Found: C, 57.9; H, 8.2. $C_{50}H_{78}O_{18} \cdot 4H_2O$ requires: C, 57.8; H, 8.3%). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (–CO_2–). ^{13}C NMR: Table 1.

Pfaffoside E (**5**). Mp 197–199°, $[\alpha]_D^{22}$ − 1.5° (*c* 0.48, MeOH). (Found: C, 57.0; H, 7.9. $C_{47}H_{72}O_{18} \cdot 3\frac{1}{2}H_2O$ requires: C, 57.1; H, 8.1%). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (–CO_2–). ^{13}C NMR: Table 1.

Pfaffoside F (**6**). Mp 243–244°, $[\alpha]_D^{22}$ + 32.4° (*c* 0.32, MeOH). (Found: C, 61.9; H, 8.5. $C_{35}H_{52}O_9 \cdot 3\frac{1}{2}H_2O$ requires: C, 61.8; H, 8.8%). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 and 1700 (CO_2H). ^{13}C NMR: Table 1.

Acid hydrolysis of pfaffosides D (**4**), *E* (**5**) *and F*(**6**). Complete acid hydrolysis of pfaffosides was carried out by the method described in the previous paper [1].

Alkaline hydrolysis of pfaffosides D (**4**) *and E* (**5**). Compound **4** (5 mg) and 1 N KOH (2 ml) were mixed in a glass tube, which was flushed with nitrogen, sealed and then heated at 95°. After 2 hr, the contents of the tube were subjecte/ to GC. Compound **5** was analysed in the same manner. (GC: detector, FID; carrier gas, N_2 at 50 ml/min; inj. temp., 150°; Column temp. 75°; Packed column, 2 m × 3 mm, 20% PEG 6000; methanol, R_t = 3.4 min; *n*-butanol, R_t = 15.7 min).

Alkaline hydrolysis of pfaffoside C (**3**). A soln of **3** (16 mg) in 1 N KOH (3 ml) was treated by the method reported previously [1] to yield colorless fine crystals (10 mg) identical with **6** as determined by mmp, TLC, IR and elemental analysis.

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