Isolation of Two New Antiinflammatory Biflavanoids from *Sarcophyte piriei*

Abiodun Ogundaini,† Mohamed Farah, Premila Perera, Gunnar Samuelsson, and Lars Bohlin*

Division of Pharmacognosy, Biomedical Centre, Uppsala University, Box 579, S-751 23 Uppsala, Sweden

Received November 2, 1995[⊗]

Two new flavano flavanone glycosides, diinsininol (**1**) and diinsinin (**2**), have been isolated from the rhizome of *Sarcophyte piriei*, together with one known flavanone glycoside, naringenin 5-glucoside. Their structures were elucidated on spectroscopic evidence as 5,7,3′,4′-tetrahydroxyflavanyl-7-*O*-*β*-glucosyl-(4*β*-8;2*β*-*O*-7)-eriodictyol (**1**) and 5,7,3′,4′-tetrahydroxyflavanyl-7-*O*-*β*-glucosyl-(4*β*-8;2*β*-*O*-7)-naringenin (**2**), respectively. The compounds were tested for their ability to inhibit prostaglandin synthesis, with **1** and **2** giving IC_{50} values of 9.20 μM and 13.14 μM, respectively, and in the inhibition of platelet-activating-factor-induced exocytosis, IC_{50} values of 49 and 39 μM.

Sarcophyte piriei Hutch. (Balanophoraceae; vernacular name, *diinsi*) is a parasitic plant that grows on the root of *Acacia* species. A decoction of its underground tuber is in Somalia, a popular folk remedy against bruises, toothache, sore throat, and abdominal pain.[1] In continuation of the search for antiinflammatory compounds from medicinal plant sources,[2−4] the tubers of *S. piriei* were procured for antiinflammatory evaluation and phytochemical investigation. Preliminary evaluation showed that the aqueous extract has antiinflammatory activity both in vivo in carragenan-induced edema of the rat paw, with maximal activity obtained after 10 h (50% inhibition at a dose of 1000 mg/kg) compared to 3 h for sodium salicylate (73% at a dose of 200 mg/kg) and, in vitro in the inhibition of prostaglandin synthesis (IC_{50} 0.2 mg/mL, comparable to 0.2 mg/mL for aspirin).[5]

The results from these tests have encouraged us to work on the isolation and identification of *S. piriei* constituents with the guidance of *in vitro* testing, using prostaglandin synthesis and platelet-activating-factor (PAF)-induced exocytosis assays. This paper deals with the isolation and structure elucidation of two new biflavanoid glycosides (**1** and **2**) from the tubers of *S. piriei* and the determination of their antiinflammatory activity in these test systems. The present study, to our knowledge, reports for the first time on the occurrence of flavano-flavanones of this type within the Balanophoraceae family.

Results and Discussion

The EtOAc fraction from the 50% aqueous MeOH extract of *S. piriei* was fractionated by Si gel chromatography to yield several fractions that were further purified by LC on polyamide and RP-8 silica to give the new biflavanoids diinsininol (**1**) and diinsinin (**2**), along with naringenin 5-glucoside.

The positive ion FABMS of **1** indicated $[M + H]^+$ peak at *m/z* 721 corresponding to $C_{36}H_{32}O_{16}$ and consistent with a glycosylated biflavanoid moiety.[6] The complete structural elucidation of **1** was derived from the MS, chemical shifts, and *J* values of the ^{1}H-NMR spectrum, HMQC (heteronuclear multiple-quantum correlations), and HMBC (heteronuclear multiple-bond correlations) experiments and by comparison with similar structures in literature.[7−9] The ^{1}H-NMR spectrum confirmed the biflavanoid nature of **1**, because it showed the characteristic ABX system signals of a flavan moiety at δ 4.88 (H-4u) and 2.00 (H-3u) with $J_{3,4}$ = 3Hz and, a flavanone nucleus at δ 5.45 (H-2u) and 2.74 (H-3l) with $J_{2ax,3ax}$ = 17Hz).

Table 1. ^{1}H -NMR Spectral Data of Compounds **1** and **2** in $(CD_3)_2SO$[a]

	compound	
proton	**1**	**2**
H-2u[b]		
L[c]	5.45 dd (3, 12) 1H	5.53 dd (3, 12) 1H
H-3u	2.00 dd (3, 13) 2H	2.10 dd (2.5, 13.5) 2H
L	2.74 dd (3, 17) 1H	2.74 dd (3, 17) 1H
	3.13 dd (12, 17) 1H	3.16 dd (12, 17) 1H
H-4u	4.88 br s 1H	4.92 br s 1H
L		
H-6u	6.26d (2) 1H	6.27 d (2.1) 1H
L	5.95 s 1H	6.07 s 1H
H-8u	6.05 d (2.2) 1H	6.07 d (2.1) 1H
L		
H-2′u	6.98 d (2) 1H	6.98 d (2) 1H
L	6.84 d (2.1) 1H	6.80 d (8) 2H
H-3′u		
L		7.42 d (8) 1H
H-5′u	6.77 d (8) 1H	6.77 d (8) 1H
L	6.77 d (8) 1H	7.42 d (8) 1H
H-6′u	6.89 dd (8, 2.1) 1H	6.86 dd (2, 8) 1H
L	7.02 d (8.2, 2) 1H	6.80 d (8) 2H
anomeric proton	4.55 d (8) 1H	4.53 d (8) 1H

[a] Values in parentheses are *J*; s = singlet, br s = broad singlet, d = doublet, t = triplet, dd = double doublet. [b] u = Upper flavan unit. [c] L = Terminal flavananone unit.

The aromatic region for the A rings displayed two doublets (δ 6.05 and 6.26, each 1H, J = 2 Hz) and a singlet (δ 5.95, 1H), showing that **1** has a tetrasubstituted and a pentasubstituted benzene. The B rings for the upper and lower units were the same with characteristic ABC system signals for C-5′, C-6′ and C-2′ (Table 1). The ^{13}C-NMR data (Table 2) also complement these assignments. From the data above, **1** was thought to be a flavan upper moiety joined to the flavanone, eriodictyol. The positions of the interflavan linkages were deduced from the UV shift measurements with NaOAc, and the ^{1}H and ^{13}C-NMR data shift indicated that C-7L OH is not free. The absence of the H-2 signal at about 5.01 ppm (compared to xanthorrone), and the appearance of H-6L as a singlet (δ 5.95) indicated that the C-2u and C-8L are without hydrogen atoms. A ketal

† Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1996.

S0163-3864(96)00386-2 CCC: $12.00

Table 2. ^{13}C-NMR Spectral Data of Compounds **1** and **2** in $(CD_3)_2SO$

	compound		
carbon	**1**	**2**	HMBC
C-2u[a]	98.6	98.6	H-4u, H-2′u, H-6′u
L[b]	79.3	79.2	H-2′L, H-6′L
C-3u	33.2	33.6	
L	42.9	43.0	
C-4u	19.5	19.9	
L	196.3	196.5	
C-5u	157.2	157.0	C-5u O*H*
L	160.4	160.1	C-5L O*H*
C-6u	97.2	98.4	C-5u O*H*, H-8u
L	95.5	97.1	C-5L O*H*
C-7u	156.1	156.1	H-1″, H-6u
L	160.6	160.8	
C-8u	96.2	96.7	H-6u
L	106.3	106.5	H-4u
C-9u	152.7	152.6	H-4u, H-8u
L	160.6	159.1	H-4u
C-10u	107.3	107.5	H-6u, H-8u
L	103.2	103.1	C-5L O*H*
C-1′u	131.5	131.4	H-5′u
L	129.5	128.5	H-5′L
C-2′u	113.1	115.0	C-3′u O*H*, H-6′u
L	114.4	128.0	C-3′L O*H*, H-6′L
C-3′u	144.9	144.7	H-6′u
L	145.3[c]	115.1	
C-4′u	145.2[c]	145.7	
L	145.8	157.2	H-6′L
C-5′u	115.5	116.2	
L	115.1	115.1	
C-6′u	116.4	116.3	H-2′u
L	117.5	128.0	
C-1″	103.5	103.9	
C-2″	72.3	72.2	
C-3″	77.0	77.5	
C-4″	69.7	69.6	
C-5″	76.8	76.9	
C-6″	60.5	61.1	

[a] u = Upper flavan unit. [b] L = Lower flavananone unit. [c] Values could be interchanged.

carbon resonance at 98.4 (C-2u), the appearance of C-4u (δ19.9) as a −CH, and a downfield shift of C-8L to 106.5 ppm in ^{13}C data (compared to 95 ppm in eriodictyol) also showed the involvement of C-2u, C-8L, and C-4u in the linkages in **1**. Confirmation of the linkages as 2u-*O*-7L and 4u-8L is from the HMBC experiments that showed cross peaks between H-2′u, H-6′u, H-4u, and C-2u, on one hand, and H-4u and C-8L, on the other (Figure 1). The small coupling constant (δ 4.88, br s) established the stereochemistry at C-4u, with the lower flavanone unit adopting the β orientation.[10]

The ion at *m*/*z* 559 $[(M + H) - 162]^+$ indicated the loss of a hexose from **1**, presumably attached to the upper unit as indicated by the peak at *m*/*z* 433 corresponding to the loss of the eriodictyol lower unit. Retro Diels−Alder fission of ring C (upper) to give the ion at *m*/*z* 423 from *m*/*z* 559 showed that the sugar is not attached to ring B of the flavan moiety. The hexose attached to the upper portion was identified as glucose by hydrolysis/GC analysis with authentic sugar samples as references. The location of the glucose at C-7u OH was confirmed by a cross peak between the anomeric proton (δ 4.55, 1H) and the C-7u in the HMBC experiment. The large coupling constant, J = 8 Hz, of the anomeric proton indicated a β-linkage. Thus, the structure of **1** was established as 5,7,3′,4′-tetrahydroxyflavan-7-*O*-β-glucosyl- (4β-8:2β-*O*-7)-eriodictyol.

The FABMS of **2** revealed the molecular ion peak *m*/*z* 705 corresponding to $C_{36}H_{32}O_{15}$ showing one oxygen unit

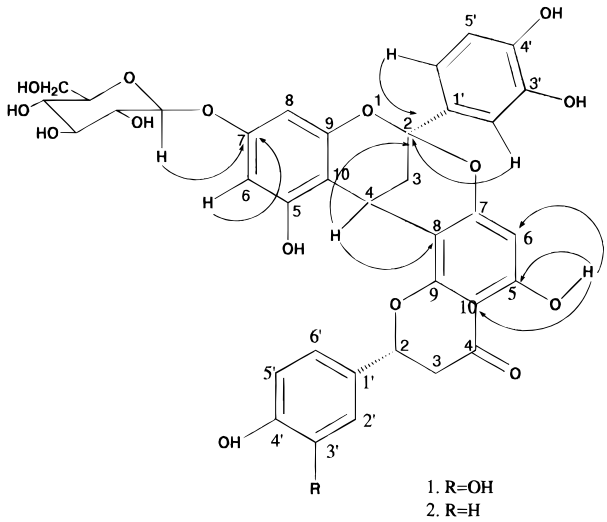

Figure 1. Important ^{1}H-^{13}C couplings observed in the HMBC spectrum of **1**.

less than in **1**. The ^{1}H-NMR of **2** was quite similar to that of **1** except for the aromatic resonances arising from the B ring of the lower unit, which displayed a pair of signals of the A_2B_2 type (6.80 and 7.42, each 2H, J = 8 Hz) suggesting a *p*-substituted benzene ring. This observation was substantiated by a pair of signals in the ^{13}C-NMR spectrum (115.1, 128.0, each 2C) suggesting the lower unit to be naringenin. This was confirmed by the fragment ion at *m*/*z* 543 due to loss of glucose and at 423 due to the Retro Diels-Alder fission of ring C. Thus, **2** was assigned as 5,7,3′,4′-tetrahydroxyflavan-7-*O*-β-glucosyl-(4β-8:2β-*O*-7)-naringenin.

Diinsininol (**1**) and diinsinin (**2**) exhibited IC_{50} values of 9.20 μM and 13.14 μM, respectively, in the inhibition of prostaglandin synthesis assay compared with indomethacin which had an IC_{50} value of 0.56 μM. The IC_{50} for **1** in PAF-induced exocytosis was calculated by regression analysis to be 49 μM; and for **2**, 39 μM, which makes them more potent inhibitors of PAF-induced exocytosis than the known PAF antagonist ginkgolide BN 52021, isolated from the tree *Ginkgo biloba* (IC_{50} of 80 μM).

Experimental Section

General Experimental Procedures. Positive ion FABMS were recorded with a JEOL SX 102 instrument with glycerol as matrix. NMR spectra were recorded using a Varian VXR-400 and Varian Unity 500 instrument at 25 °C with $(CD_3)_2SO$ as solvent and TMS as internal standard, 400 MHz (for ^{1}H) and 101 MHz (for ^{13}C); inverse heteronuclear correlations HMBC and HMQC were obtained at 500 MHz. UV spectra were recorded on a Shimadzu UV−160A spectrophotometer. Melting points are uncorrected and were determined using a Digital Melting Point Apparatus (8103, Electrothermal Engineering Ltd., England).

TLC was carried out using precoated Si gel plates (E. Merck, DC Alufolien, Kieselgel 60$F_{254,}$ 0.25 mm) with toluene−Me_2CO−H_2O (10:20:1) (A) and on RP-8 $F_{254}S$ (0.25 mm, Merck) using MeOH−H_2O (1:1) (B) as the solvent systems. Chromatograms were examined under UV light (254 and 366 nm) and sprayed with vanillin−H_2SO_4 spray with heating at 90 °C for 5 min.

Adsorption column chromatography was performed with Si gel 60 (70−230) mesh, (E. Merck, Darmstadt,

Germany) and Polyamide 6S (Riede-de Haen AG). Reversed-phase low pressure liquid chromatography was accomplished with RP-8 Lobar columns (size B, 40−63 μM, E. Merck). The crude and semipurified fractions were tested as a 0.2-mg/mL solution in the inhibition of prostaglandin synthesis assay.

Plant Materials. Underground rhizomes of *Sarcophyte piriei* (Hutch) were bought in the market in Luuq Somalia, and stored in a cold room. The commercial rhizomes were identical to the rhizome of a fresh plant identified by Mats Thulin (Department of Systematic Botany, Uppsala University), and a voucher specimen coded SMP 152 has been deposited at the herbarium of Uppsala University, Sweden.

Extraction and Isolation. Powdered rhizomes (1.0 kg) of *S. piriei* was extracted twice with 50% aqueous MeOH (2 × 10 L) at room temparature for 24 h on each occasion. The extract was filtered and concentrated *in vacuo* to about 1.5 L. After removal of precipitate by centrifugation, it was extracted successively with $CHCl_3$ (4 × 500 mL) and EtOAc (10 × 1 L). The $CHCl_3$ extract after drying and evaporation *in vacuo* gave an oily semisolid residue (9.2 g) and the EtOAc extract gave a light brown solid substance (11.0 g).

The EtOAc fraction (9.0 g), after adsorption onto silica, was chromatographed on a gravity flow column of Si gel (250 g, 4.5 mm diameter) with a stepwise gradient of toluene−Me_2CO 4:1 (4 L) toluene−Me_2CO 2:1 (4.2 L); toluene−Me_2CO 1:1 (4.0 L); toluene−Me_2CO 1:2 (6.6 L); toluene−Me_2CO 1:4 (3.0 L); Me_2CO 100% (4.0 L); and finally 20% MeOH in Me_2CO (2.4 L). In each case, the preceeding solvent/solvent mixture was gradually enriched with the solvent immediately following it in the gradient.

Fractions of 25 mL were collected, and those exhibiting similar TLC profiles (solvent system A) were combined. Altogether, 20 fractions (F001−F020) were obtained, and they were evaluated for activity in the inhibition of prostaglandin synthesis asssay. Activity was concentrated in fractions F014 (2.15 g, 50% inhibition) and F015 (0.91 g, 55% inhibition). These fractions were each subjected to chromatography on polyamide (50 g) with isocratic elution using EtOH (95%) and final purification on RP-8 column with H_2O-MeOH (1:1) as the eluent. F014 afforded naringenin 5-*O*-β-glucoside (54 mg) and diinsinin (**2**, 42 mg). F015 afforded diinsininol (**1**, 70 mg).

Sugar Analysis. About 1 mg of the sample was dissolved in a few drops of 2 M HCl and heated in a stoppered vial for 30 min. Into this reaction mixture were added two drops of DMF and bis(trimethylsilyl)-trifluoroacetamide (BSTFA) were added and the vial kept at 70 °C for 15 min. The derivatized sugars were then analyzed by GC, using authentic samples as reference.

Naringenin 5-*O*-β-glucoside (54 mg) was identified by comparison of ^{1}H- and ^{13}C-NMR and UV data with those in literature:[11,12] mp 166−168 °C (R_f = 0.48, solvent system B); UV λ max (MeOH) 298, 310sh (+ $AlCl_3$) 298, 310sh (+ NaOAc) 324 nm.

5,7,3′,4′-Tetrahydroxyflavanyl-7-*O*-β-glucosyl-(4β-8:2β-*O*-7)-eriodictyol (diinsininol) (1): white amorphous solid, mp 221−223 °C dec (R_f = 0.35, solvent system B); UV λ max (MeOH) 292 nm (+ $AlCl_3$) 314 nm, (+ NaOAc) 290 nm; FABMS *m/z* $(M + H)^+$ 721, $[(M + H) - 162]^+$ 559, $[(M + H) - 288]^+$ 433; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

5,7,3′,4′-Tetrahydroxyflavan-7-*O*-β-glucosyl-(4β-8:2β-*O*-7)-naringenin (diinsinin) (2): white amorphous solid, mp 223−224 °C dec (R_f = 0.28, solvent system B); UV λ max (MeOH), 298 nm (+ $AlCl_3$), 313 nm (+ NaOAc), 296 nm; FABMS *m/z* $(M + H)^+$ 705, $[(M + H) - 162]^+$ 543; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2, respectively.

Prostaglandin Synthesis Assay. The experiments were performed according to Pongprayoon *et al.*[2] Bovine seminal vesicle microsomes (10 μL, 20−30 μg protein) were preincubated with 50 μL of cofactor solution (reduced glutathione and *l*-adrenalin, 0.3 mg/mL each in Tris buffer, pH 8.2) in an ice−water bath for 15 min. Vehicle or solution of test substance (20 μL) and 20 μL of [^{14}C]-arachidonic acid (16 Ci/μmol, 30 μM) were added and the mixture incubated at 37 °C for 10 min. A blank was kept in the ice-water bath. After incubation, 5 μL of a carrier solution of unlabeled prostaglandins (0.2 mg/mL) was added, and the reaction was terminated by adding 5 μL of 2 N HCl. The unreacted arachidonic acid was separated from the prostaglandin products by chromatography on a Biosil column and elution with hexane-dioxane-glacial HOAc (70:30:1). The prostaglandins formed were then eluted with EtOAc-MeOH (85:15), and the radioactivity of the samples was counted in a Packard scintillation spectrometer. Regression analysis was used to calculate IC_{50} (concentration that gives 50% inhibition). Indomethacin was used as the reference compound.

PAF-Induced Exocytosis Assay. The PAF-induced exocytosis assay was performed according to the procedure described by Tunon and Bohlin.[4] Test solutions or distilled H_2O (10 μL) was added to 750 μL of the incubation medium. Neutrophil suspension (100 μL) was added, and the test tubes were incubated for 5 min at 37 °C. The reaction was started with the addition of 100 μL of PAF at a final concentration of 0.1 μM. Blank tubes without the addition of PAF were run in parallel. After incubation at 37 °C for 10 min the reaction was stopped by addition of 250 μL 2% citric acid, and the tubes were centrifuged at 600 × *g* for 10 min. The absorbance of each sample was measured at 405 nm, using a Hitachi U-1100 spectrophotometer. All samples were run in duplicate. The absorbance of the blank tubes was subtracted from the absorbance of the corresponding sample. The inhibition of PAF-induced exocytosis was calculated as the relative decrease in absorbance of the samples containing test substance as compared to distilled H_2O. The ginkgolide BN 52021 was used as a standard inhibitor, and IC_{50} values were calculated using regression analysis.

Acknowledgment. The authors are grateful to SAREC (Swedish Agency for Research Cooperation with Developing Countries) for the sponsorship of the joint Somali-Swedish research project on medicinal plants and to Rolf Andersson of the Agricultural University, Uppsala, for the skillful running of the NMR spectra. A.O. is grateful to IPICS (International Program in Chemical Sciences, Uppsala University) for a one-year fellowship in Sweden to enable his participation in the project.

References and Notes

(1) Samuelsson, G.; Farah, M. H.; Claeson, P.; Hagos, M.; Thulin, M.; Hedberg, O.; Warfa, A. M.; Hassan, A. O.; Elmi, A. H.; Abdulrahman, A. D.; Elmi, A. S.; Abdi, Y.A.; Alin, M. H. *J. Ethnopharmacol.* **1991**, *35*, 25.
(2) Pongprayoon, U.; Baeckström, P.; Jacobsson, U.; Lindström, M.; Bohlin, L. *Planta Med*. **1991**, *57*, 515.
(3) Farah, M. H.; Samuelsson, G. *Planta Med*. **1992**, *58*, 14.
(4) Tunon, H.; Bohlin, L. *Phytomedicine* **1995**, *2*(2), 31.
(5) Samuelsson, G.; Farah, M. H.; Claeson, P. Unpublished results.
(6) Karchesy, J. J.; Hemingway, R. W.; Foo, Y. L.; Barofsky, E.; Barofsky, D. *Anal. Chem*. **1986**, *58*, 2563.
(7) Gujer, R.; Magnolato, D.; Self, R. *Phytochemistry* **1986**, *25*(6), 1431.
(8) Birch, A. J.; Dahl, C. J.; Pelter, A. *Tetrahdron Lett*. **1967**, *6*, 481.
(9) Kasahara, Y.; Hikino, H. *Heterocycles* **1983**, *20*(10), 1953.
(10) Cai, Y.; Evans, F. J.; Roberts, M. F.; Phillipson, J. D.; Zenk, M. H.; Gleba, Y. Y.; *Phytochemistry* **1991**, *30*(6), 2033.
(11) Shen, C.-C.; Chang, Y.-S.; Ho, L.-K. *Phytochemistry* **1993**, *34*(3), 843.
(12) Cubucku, B.; Yuksel, V. *J. Nat. Prod*. **1982**, *45*(2), 137.

NP960386K