Notes

Phenolic Constituents of *Phenax angustifolius*

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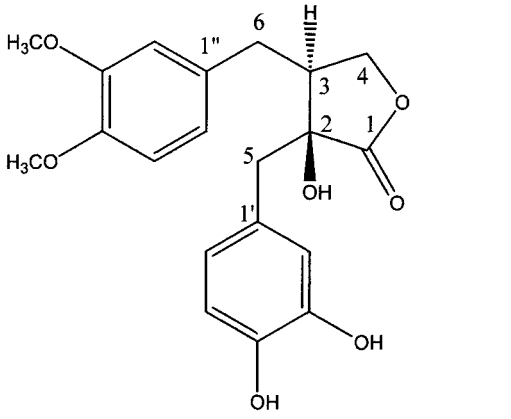
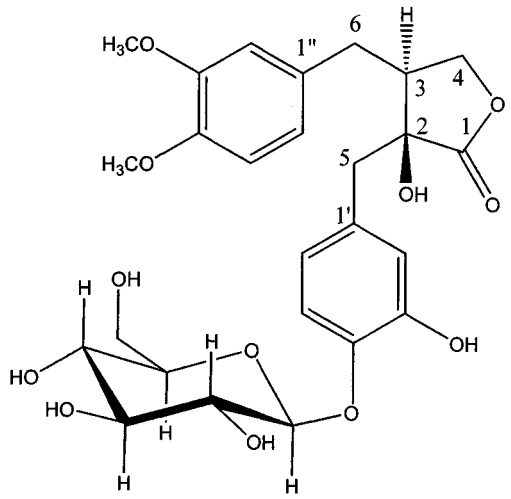
Fractionation of an ethanolic extract of the leaves of *Phenax angustifolius* has resulted in the isolation of two new lignans, 2-hydroxy-2-(3′,4′-dihydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)methyl-γ-butyrolactone (**1**) and 2-hydroxy-2-(4′-*O*-β-D-glucopyranosyl-3′-hydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)methyl-γ-butyrolactone (**2**), and three known compounds, vitexin, isovitexin, and quercetin 3-*O*-α-L-rhamnopyranoside. The structures of **1** and **2** were determined using spectroscopic methods.

As a part of our program to discover and develop potential therapeutic agents from Central America flora, *Phenax angustifolius* (H.B.K.) Weddell was collected near Limon, Costa Rica, and phytochemically investigated. *Phenax* is a herbaceous genus of the family Urticaceae found throughout Central America. These species grow in the Costa Rican tropical forests and have traditional uses as insecticidal agents.[1] So far, no chemical studies have been reported on any *Phenax* species. In this paper we present the isolation and structural elucidation of two new lignans (**1** and **2**), together with three known flavonoids, from *Phenax angustifolius* leaves.

The dried leaves of *P. angustifolius* were extracted with EtOH−H_2O (7:3). Fractionation of the dried residue on a Sephadex LH-20 column and final purification by HPLC gave five compounds. Three of them were identified as vitexin, isovitexin, and quercetin 3-*O*-α-L-rhamnopyranoside on the basis of interpretation of their spectroscopic data and specifically by comparison of their NMR values with those in the literature.[2,3] The last two compounds were identified as new lignans 2-hydroxy-2-(3′,4′-dihydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)methyl-γ-butyrolactone (**1**) and 2-hydroxy-2-(4′-*O*-β-D-glucopyranosyl-3′-hydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)methyl-γ-butyrolactone (**2**), on the basis of the evidence outlined below.

The EIMS of compound **1** showed an M^+ ion at *m*/*z* 374, consistent with the molecular formula $C_{20}H_{22}O_7$ also deduced using ^{13}C NMR and DEPT analysis. The 600 MHz ^1H NMR spectrum (Table 1) showed signals for two methylenes, an oxymethylene, and two methoxyl groups in the aliphatic region. Also it revealed signals (δ 6.59, 6.71, 6.73, 6.89) integrating for six protons in the aromatic region and attributable to two phenyl groups. Its ^{13}C NMR spectrum (Table 1) indicated the presence of a lactone carbonyl (δ_C 180.6) and 12 aromatic carbon resonances, six of which were fully substituted. The remaining carbons (2 CH_2, 1 CH_2O, 1 CH, 1 C−OH) suggested the structure of a diarylbutyrolactone-type of lignan.[4,5] Analysis of the 1D and 2D NMR spectra of **1** with homo- and heteronuclear direct





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and long-range correlations allowed the assignments of all ^1H and ^{13}C NMR signals as listed in Table 1. Each proton was assigned by means of the $^1H-^1H$ DQF−COSY technique, which showed correlations (H_2-4/H-3/H_2-6) within the three spin system containing the H_2-4 resonance (δ 4.00, br t, J = 10.0 Hz), typical of methylene protons of a 2,3-*trans*-γ-butyrolactone.[6] The COSY spectrum also established the presence of two 1,3,4-trisubstituted phenyl

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Table 1. ^{13}C NMR and ^{1}H NMR Data of Compounds **1** and **2** in CD_3OD[a]

	1		**2**	
position[c]	δ_C	δ_H (J_{H-H} in Hz)[b]	δ_C	δ_H (J_{H-H} in Hz)[b]
1	180.6		180.4	
2	77.4		77.3	
3	44.6	2.47 m	44.6	2.50 m
4	71.8	4.00, 2H, br t (10.0)	71.8	4.01, 2H, br t (10.0)
5	41.9	3.14 d (13.5), 2.98 d (13.5)	41.8	3.15 d (13.5) 2.95 d (13.5)
6	32.2	2.88 dd (13.0, 3.5) 2.53 dd (13.0, 11.0)	32.2	2.86 dd (13.0, 4.5) 2.52 dd (13.0, 11.5)
1′	128.2		132.7	
2′	114.9	6.73 br	115.9	6.82 br d (1.5)
3′	146.7		147.2	
4′	148.7		147.2	
5′	116.1	6.73 d (8.0)	117.8	7.10 d (8.0)
6′	124.1	6.59 dd (8.0, 1.5)	124.1	6.72 dd (8.0, 1.5)
1″	133.4		133.4	
2″	113.2	6.71 br d (1.5)	113.2	6.74 br d (1.5)
3″	149.1		149.1	
4″	150.1		150.6	
5″	113.8	6.89 d (8.0)	114.0	6.88 d (8.0)
6″	122.2	6.71 dd (8.0, 1.5)	122.2	6.72 dd (8.0, 1.5)
Glc-1‴			101.9	5.47 d (7.5)
Glc-2‴			74.0	3.30 dd (9.5, 7.5)
Glc-3‴			78.1	3.43 t (9.5)
Glc-4‴			71.0	3.57 t (9.5)
Glc-5‴			78.3	3.37 m
Glc-6‴			62.1	3.70 dd (12.2, 4.5) 3.85 dd (12.2, 3.5)
−OMe	56.4	3.77 3 H, s	56.4	3.78 3 H, s
	56.5	3.80 3 H, s	56.5	3.80 3 H, s

[a] Assignments confirmed by 1D TOCSY and 2D DQF−COSY, HSQC, HMBC experiments; the main HMBC correlations are reported in the text. [b] $^{1}H-^{1}H$ coupling constants in the sugar unit were measured from TOCSY and COSY spectra in Hz. [c] Glc = β-D-glucopyranosyl.

groups showing connectivities between the hydrogens resonating at δ 6.59 (H-6′) and 6.73 (H-2′ and H-5′) on ring A and δ 6.89 (H-5″) and 6.71 (H-2″ and H-6″) on ring B. The HSQC spectrum of **1** indicated all of the protonated carbon correlations, thereby leading to the elucidation of the structural skeleton. Readily identifiable constitutive units of compound **1** included a lactone, a CH_2 linked to a quaternary carbon, the $CH_2-CH-CH_2OR$ chain (as also evidenced by COSY experiment), and a tertiary alcoholic group (C-2). The ^{13}C NMR chemical shift of C-2 (δ_C 77.4) was almost superimposable to that of *trans*-fused butyrolactones such as benchequiol[8] and different from that reported for *cis*-fused butyrolactones such as guayadequiol[7] (δ_C 75.9). Also, the resonances of C-5 and C-6, diagnostic for the distinction between *cis*- and *trans*-fused butyrolactone rings,[4] confirmed a 2,3-*trans*-γ-butyrolactone structure for compound **1**. The chemical shifts of the aryl carbons and hydrogens suggested a 3,4-dihydroxy and a 3′,4′-dimethoxy substitution on the phenyl groups.

Multiple-bond heteronuclear correlation (HMBC) data established the structure of **1** unambiguously. Correlations were observed between H_2-4 (δ 4.00) and CH_2-6 (δ 32.2), CH-3 (δ 44.6), the tertiary alcoholic carbon (δ 77.4), and the lactone (δ 180.6) groups, confirming a diaryl-γ-butyrolactone structure. Cross-peaks were also observed between H_2-5 (δ 3.14 and 2.98) and CH-3 (δ 44.6), C-2 (δ 77.4), C-1 (δ 180.6), and C-1′, C-2′, and C-6′, establishing that CH_2-5 was attached to ring A. Correlations seen between H_2-6 (δ 2.88 and 2.53) and C-3, C-4, C-2 and C-1″, C-2″, and C-6″ established that the benzyl group B was at the C-6 position. Other diagnostic correlations were observed between signals at δ 3.77 and 3.80 (−OMe) and C-3″ and C-4″, between the overlapped signals assigned to H-2″ and H-6″ (δ_H 6.71) and C-6, C-1″, C-3″, C-4″, C-5″, and between the H-5″ signal (δ_H 6.89) and C-1″, C-3″, C-4″, and C-6″. This connectivity information confirmed the location of two −OMe groups at C-3″ and C-4″ of ring B and that the 3,4-dimethoxyphenyl group was linked to C-6. The cross-peaks observed between H-2′/H-5′ (δ_H 6.73) and C-1′, C-3′, C-5′, and C-6′ signals and between H-6′ (δ_H 6.59) and C-1′, C-2′, C-4′, and C-5′ signals supported the 3,4-dihydroxy substitution on ring A. All the connectivity information inferred by the HMBC spectrum was compatible only with the structure **1**. Accordingly compound **1** was determined as 2-hydroxy-2-(3′,4′-dihydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)methyl-γ-butyrolactone.

Compound **2** was assigned a molecular formula of $C_{26}H_{32}O_{12}$ as deduced by MS and ^{13}C NMR and DEPT analysis. The ^{1}H NMR spectrum of **2** was similar in chemical shifts and multiplicities of the signals to that of **1** except for the downfield shift of H-5′ (δ 6.73 in **1**, 7.10 in **2**) and small downfield shifts observed for H-6′ and H-2′ in the aromatic region as well as for the presence of signals attributable to a sugar moiety in the aliphatic region (Table 1). This evidence clearly suggested that **2** is a glycosyl derivative of **1**. The glycosidic linkage of **2** was determined to be at the C-4′ position based on the cross-peaks due to ^{3}J long-range coupling between the anomeric proton (δ 5.47, H-1‴) and C-4′ (δ 147.2) in the HMBC spectrum. Also, the observed upfield shift of C-4′ (−1.5 ppm) and downfield shifts of the *ortho*-correlated C-5′ (+1.7 ppm) and the *para*-correlated C-1′ (+4.5 ppm) and C-3′ (+0.5 ppm) carbons, in comparison with those observed in **1**, were indicative of glycosidation at C-4′. These shifts were almost superimposable on those observed in arctiin and traxilloside in comparison with arctigenin and traxillagenin.[9] The sugar substituent at C-4′ was identified as β-D-glucopyranosyl from the following evidence: the ^{1}H and ^{13}C NMR data of the key hydrogens and carbons (C-2, C-3, and C-5) indicated a β-configuration at the anomeric position ($J_{H-1-H-2}$ = 7.5 Hz); a 1D TOCSY[10] subspectrum obtained by irradiating at the well-resolved anomeric proton at δ 5.47 showed a set of coupled protons at δ 3.30, 3.43, 3.57, 3.37 (all CH) and 3.70 and 3.85 (CH_2); and the DQF-COSY spectrum established the proton sequence within this monosaccharide as H-1‴ to H_2-6‴; analysis of the correlated ^{13}C NMR signals in the HSQC spectrum led to the identification of glucopyranose.[11] Therefore the structure of **2** was determined as 2-hydroxy-2-(4′-*O*-β-D-glucopyranosyl-3′-hydroxyphenyl)methyl-3-(3″,4″-dimethoxyphenyl)-methyl-γ-butyrolactone.

The CD spectra of **1** and **2** showed no Cotton effects; therefore their absolute configurations have remained undetermined.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell in 0.5% w/v solutions in MeOH at 25 °C. UV spectra were obtained with a Perkin-Elmer 550 SE spectrophotometer. CD spectra were measured on a JASCO J720 spectropolarimeter. For NMR experiments in CD_3OD a Bruker DRX-600 spectrometer was used, operating at 599.2 MHz for ^{1}H and 150.9 for ^{13}C and using the UXNMR software package; DEPT, $^{1}H-^{1}H$ DFQ-COSY (double quantum filtered COSY), $^{1}H-^{13}C$ HSQC, and HMBC experiments were obtained using conventional pulse sequence. 1D TOCSY[10] (selective excitation spectra) were acquired using waveform generator-based GAUSS shaped pulses, with mixing times ranging from 100 to 120 ms, and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5-ms trim

pulse. Chemical shifts are expressed in δ (ppm) referring to the following solvent peaks: δ_H 3.34 and δ_C 40.0 for CD_3OD. The FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy 2−6 KV); the EIMS, on a VG-Prospec mass spectrometer (70 eV). HPLC separations were carried out on a Waters apparatus equipped with a refractive index detector.

Plant Material. The leaves of *P. angustifolius* (H.B.K.) Weddell were collected at Limon, Costa Rica, in February 1996 and identified by Prof. Luis Poveda, CIPRONA. A specimen of the plant (P.A. 3, 1996) used in this study has been deposited at the Herbarium of CIPRONA, Universidad de Costa Rica.

Extraction and Isolation. The powdered, dried leaves (650 g) were extracted at room temperature with EtOH−H_2O (7:3) to give 12.14 g of a lyophilized extract. Part of the extract (8 g) was partitioned between *n*-BuOH and H_2O to afford a *n*-BuOH-soluble portion (5 g), which was chromatographed over a Sephadex LH-20 column (100 × 5 cm) using MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si gel, *n*-BuOH−HOAc−H_2O (60:15:25)]. Fractions 9−12 (75 mg), containing the crude lignan mixture, were submitted to RP-HPLC on a C_{18} μ-Bondapack column (30 cm × 7.8 mm, flow rate 2.5 mL min^{-1}) using MeOH−H_2O (4:6) as the eluent to yield compounds **1** (25 mg; t_R, 20.6 min) and **2** (7 mg; t_R, 17.1 min). Fractions 60−65 (300 mg) were separated under the same conditions giving vitexin (8 mg; t_R, 15 min), isovitexin (4 mg; t_R, 16 min), and quercetin 3-*O*-α-L-rhamnopyranoside (4 mg; t_R, 12 min).

Compound 1: $[\alpha]^{25}_D$ −39.1° (*c* 0.5, MeOH); UV (MeOH) λ_{max} 233 (4.15), 282 (3.75) nm; ^1H and ^{13}C NMR (see Table 1); EIMS *m*/*z* 374 $[M]^+$; *anal.* C 64.27%, H 5.88%, calcd for $C_{20}H_{22}O_7$, C 64.16%, H 5.92%.

Compound 2: $[\alpha]^{25}_D$ −57.5° (*c* 0.5, MeOH); UV (MeOH) λ_{max} 230 (4.08), 280 (3.80) nm; ^1H and ^{13}C NMR (see Table 1); EIMS *m*/*z* 536 $[M]^+$; FABMS *m*/*z* 535 $[M - H]^-$, 373 $[(M - H) - 162)]^-$; *anal.* C 58.21%, H 5.97%, calcd for $C_{26}H_{32}O_{12}$, C 58.20%, H 6.01%.

Known Compounds. The known compounds were identified as vitexin, isovitexin, and quercetin 3-*O*-α-L-rhamnopyranoside, respectively, on the basis of their spectroscopic data and specifically by ^1H and ^{13}C NMR in comparison with literature values.[2,3]

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