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TWO CHALCONE-PRENYLCOUMARIN DIELS–ALDER ADDUCTS FROM *BROSIMUM RUBESCENS*

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Key Word Index—*Brosimum rubescens*; Moraceae; bark; chalcone; prenylcoumarin; Diels–Alder type adduct; spectroscopic analysis; Peruvian folk medicine.

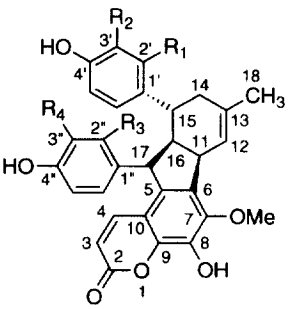
Abstract—Two new Diels–Alder type adducts, named palodesagretins I and II, were isolated from a Peruvian folk medicine "palo de sangre" (*Brosimum rubescens*). The structure of these new isolates indicated them to be adducts consisting of chalcone derivatives and a prenylcoumarin produced a Diels–Alder-type addition reaction, with a five-membered ring-closure. Structural elucidation is based mainly on 2D-NMR analyses. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Brosimum rubescens, a tree growing up 30 to 50 m high, is fairly common in Amazonia [1]. It is known as "palo de sangre", "palisangre" and "paro negro" in Peru, and "muirapiranga" in Brazil [2]. This tree is commonly used in carpentry and an alcoholic extract is used in medicine as a tonic. Its reddish heartwood contains large amounts of xanthyletin and several other coumarins [2–4]. Based on previous research, five novel Diels–Alder-type adducts, named palodesangrens A–E, having antiandrogenic activity, together with three known coumarins have been isolated [5]. We have investigated the minor chemical constituents of this material and have obtained further related compounds. The isolation and structural elucidation of two new compounds, palodesagretins I (**1**) and II (**2**) are reported in the present paper. Their structures, adducts consisting of chalcone derivatives and a prenylcoumarin with an additional five-membered ring, were elucidated by spectroscopic analyses, including 2D-NMR.

RESULTS AND DISCUSSION

The CH_2Cl_2-soluble portion obtained from the MeOH extract of the bark during column chromatography over silica gel followed by Sephadex LH-20 column chromatography and medium-pressure liquid chromatography on silica gel and/or ODS resulted in the isolation of two new compounds, palodesangretins I (**1**; 0.0002%) and II (**2**; 0.0003%). They were characterized using spectroscopic techniques, including high-field one- and two-dimensional NMR.



1: R_1 = H, R_2 = OH, R_3 = OMe, R_4 = H
2: R_1 = OMe, R_2 = H, R_3 = H, R_4 = OH

Compound **1** was obtained as a pale brownish amorphous solid with an M_r of 528, as determined from the positive FAB mass spectrum, after the addition of 1 M NaI, *vis.* $[M+Na]^+$ at *m/z* 551 and $[M+H]^+$ at *m/z* 529. HRFAB mass spectrometry indicated the molecular formula to be $C_{31}H_{28}O_8$. The ^1H NMR spectrum suggested that **1** contained two sets of trisubstituted benzene rings {δ_H 6.85 (1H, *dd*, J = 2.0, 7.9 Hz), 7.28 (1H, *d*, J = 7.9 Hz), and 7.29 (1H, *d*, J = 2.0 Hz); 6.88 (1H, *d*, J = 1.9 Hz), 6.70

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(1H, *br s*), and 6.72 (1H, *dd*, J = 1.9, 8.4 Hz)}. Also, ^{1}H NMR signals assignable to five methines {δ_H 2.81 (1H, *ddd*, J = 4.2, 6.8, 8.4 Hz), 3.02 (1H, *br dd*, J = 8.0, 13.8 Hz), 4.26 (1H, *br s*), 5.06 (1H, *d*, J = 4.0 Hz) and 6.22 (1H *br s*)}, one set of methylenes {2.36 (1H, *br dd*, J = 5.4, 17.8 Hz), 2.45 (1H, *br dd*, J = 5.4, 17.8 Hz) and one methyl group {δ_H 1.77 (3H, *s*, long-range coupled)} were observed. Coupling correlations as revealed by H-H COSY are listed in Table 1. These connectivities were thought to form a cyclohexene unit; this was confirmed by ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectroscopy (HMBC) (Table 1). Furthermore, the presence of signals assignable to one α,β-unsaturated ketone group {δ_H 6.09 (1H, *d*, J = 9.7 Hz) and 7.46 (1H, *d*, J = 9.7 Hz); δ_C 161.0 (*s*)} and six quaternary carbons (δ_C 112.4, 133.8, 136.5, 137.9, 144.9 and 148.3), suggested formation of a tetrasubstituted coumarin unit; H-C long-range correlations in the HMBC spectrum tentatively supported this assumption. The remaining signals that appeared in the NMR were two methoxyl groups {δ_H 3.74 (3H, *s*) and 4.21 (3H, *s*)} linked with the aromatic rings. These data and the unsaturation ratio, suggested the presence of six ring systems in the molecule. The molecular formula of **1** shows four additional hydrogens, suggesting four exchangeable hydroxyl groups that exhibited IR absorption around 3399 cm^{-1}. The connection between these units was based on a combination of HMBC and ROESY. HMBC correlations between the methine proton at δ_H 3.02 and aromatic carbons at δ_C 116.5, 119.8 and 137.9, and between the methine proton at δ_H 5.06 and other aromatic carbons at δ_C 122.8, 129.3 and 158.9, revealed the connecting positions of the two aromatic rings to the cyclohexene unit. The methoxyl group (δ_H 3.74) linked with one of the aromatic rings was also confirmed. Other methoxyl group protons (δ_H 4.21) had an HMBC correlation with the carbon at δ_C 1.48 on the coumarin unit. In the coumarin unit, the carbons at δ_C 133.8 and 136.5 exhibited HMBC correlations with the methine protons at δ_H 2.81 and 5.06. However, these correlations failed to confirm the connecting position of the coumarin unit to the cyclohexene unit. Supporting information was obtained from ROE correlations between the methine proton (δ_H 5.06) of the cyclohexene unit and the olefinic proton (δ_H 7.46) of the coumarin unit, and between the olefinic proton (δ_H 6.22) of the cyclohexene unit and the methoxyl group protons (δ_H 4.21) on the coumarin unit. These data corroborated the structure of **1**, which formed an additional five-membered ring between the cyclohexene and coumarin units. The relative configuration of the cyclohexene part was based on ROESY. In this spectrum, strong ROE correlations were observed between the geminal methine protons, H-11 and H-16, and between the benzyl methine protons H-15 and H-16, which confirmed the *cis*-configuration between H-11 and H-16, and the equatorial orientation of H-15. ROE was also observed between the benzyl methine proton, H-17, and one of the methylene protons, H-14b, and between H-17 and H-15. These configurations satisfied all of the ROE correlations for a *cis*-β fusion between C-11 and C-16, and an anticlinal one between H-16 and H-17. Consequently, the structure of compound **1**, palodesangretin I, was determined.

Compound **2**, a pale brownish amorphous solid, had the same molecular formula, $C_{31}H_{28}O_8$, as **1**, as established by HRFAB mass spectrometry. ^{1}H and ^{13}C NMR spectra suggested that **2** contained two substituted benzene ring unit, one coumarin and one cyclohexene units, identical to those of **1**. A possible difference between **1** and **2** appeared to be the replacement of the respective aromatic rings linking at C-15 and C-17, or in geometrical isomerism of the conjugation positions of the coumarin unit. Careful inspection of the ^{1}H NMR signals revealed chemical shift changes in the signals assignable to H-15 (δ_H 3.02 for **1** and 3.59 for **2**) and H-17 (δ_H 5.06 for **1** and 4.54 for **2**), resulting from replacement of the respective aromatic rings. This phenomenon appeared to be the result of the influence of the 2-substituted methoxyl groups on the aromatic ring units, that is, the deshielding effect of the lone pair of the methoxyl group on the respective benzyl methines [5]. This presumption was confirmed by HMBC which showed H-C long-range correlations between the respective methine and aromatic carbons (δ_H 3.59/δ_C 124.6, 129.1 and 159.3 for H-15/C-1′, 6′ and 2′; δ_H 4.54/δ_C 115.7, 119.0 and 136.7 for H-17/C-2″, 6″ and 1″). Also, this spectrum confirmed the positions of conjugation of the coumarin unit and the methoxyl group linking-positions. Thus, the structure of compound **2**, palodesangretin II, was determined.

A possible route for the biosynthesis of palodesangretins was considered to be from chalcone derivatives and a 6-prenyl-7-methoxy-8-hydroxycoumarin by a Diels–Alder-type addition reaction, followed by a five-membered ring closure. This route is similar to that for parodesangrens, previously reported compounds possessing a 6-prenyl-7-hydroxycoumarin unit and a pyrane ring [5].

EXPERIMENTAL

General

Mp are uncorr. Optical rotations are given in 10^{-1} cm^2 g^{-1}. Medium-pressure liquid chromatography (MPLC) was performed with a CIG column system (22 mm i.d. × 300 mm or 22 mm i.d. × 100 mm, Kusano Scientific) packed with 10 μm or 5 μm silica gel and octadecyl silica gel (ODS). HPLC was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, GL Science) packed with 10 μm ODS. TLC was done on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and spots detected by heating after spraying with 10% H_2SO_4. 1D, 2D and ^{1}H and ^{13}C NMR were recorded on a Unity plus 400 spectrometer (Varian)

Table 1. ^{13}C and ^{1}H NMR data and H-H COSY, HMBC and ROESY correlations for compound **1***

Assignment	δ_C	δ_H	Multiplicity	J (Hz)	H-H COSY	HMBC	ROESY
2	160.97						
3	113.69	6.09	*d*	9.7	H-4	C-2, C-10	H-4
4	141.86	7.46	*d*	9.7	H-3	C-2, C-5, C-9, C-10	H-3, H-17
5	133.75						
6	136.48†						
7	148.29						
8	137.88†						
9	144.92						
10	112.71						
11	43.27	4.26	*br s*		H-12, H-16, H-18		H-12, H-16, H-6″
12	121.92	6.22	*br s*		H-11, H-18		H-11, H-18, 7-OMe
13	134.25						
14a	36.88	2.36	*br dd*	7.8, 17.8	H-14b, H-15, H-18	C-13	H-18, H-2′, H-6′
14b		2.45	*br dd*	5.4, 17.8	H-14a, H-15, H-18	C-12, C-13	H-15, H-17, H-18
15	41.27	3.02	*br dd*	8.0, 13.8	H-14a, H-14b, H-16	C-1′, C-2′, C-6′	H-14b, H-16, H-17, H-2′, H-6′
16	54.33	2.81	*ddd*	4.2, 6.8, 8.4	H-11, H-15, H-17	C-6	H-11, H-15, H-17, H-2′, H-6′, H-6″
17	45.10	5.06	*d*	4.0	H-16	C-5, C-6, C-15, C-16, C-1″, C-2″, C-6″	H-4, H-14b, H-15, H-16, H-6″
18	23.84	1.77	*s*		H-11, H-12, H-14a, H-14b	C-12, C-13, C-14	H-12, H-14a, H-14b
7-OMe	60.65	4.21	*s*			C-7	H-12
1′	137.88						
2′	116.52‡	7.29	*d*	2.0	H-6′	C-4′, C-6′	H-14a, H-15, H-16, H-6′
3′	147.04						
4′	145.47						
5′	116.37‡	7.28	*d*	7.9	H-6′	C-1′, C-3′	H-6′
6′	119.84	6.85	*dd*	2.0, 7.9	H-2′, H-5′	C-2′, C-4′	H-14a, H-15, H-16, H-2′
1″	122.78†						
2″	158.48						
3″	100.20	6.88	*d*	1.9	H-5″	C-1″, C-4″, C-5″	2″-OMe
4″	158.92						
5″	108.23	6.72†	*dd*	1.9, 8.4	H-3″, H-6″	C-1″, C-3″	H-6″
6″	129.31	6.70†	*br s*		H-5″	C-17, C-2″, C-4″	H-16, H-17, H-5″
2″-OMe	55.34	3.74	*s*			C-2″	H-3″

* Measurements in C_5D_5N at 100 MHz for ^{13}C and at 400 MHz for ^{1}H, 300 K.
† Signals bearing this superscript overlapped each other or on solvent signals.
‡ Assignments for values bearing this superscript may be reversed.

Table 2. ^{13}C and ^{1}H NMR data and H-H COSY, HMBC and ROESY correlations for compound **2***

Assignment	δ_C	δ_H	Multiplicity	J (Hz)	H-H COSY	HMBC	ROESY
2	160.93						
3	113.77	6.11	*d*	9.7	H-4	C-2, C-10	H-4
4	141.96	7.50	*d*	9.7	H-3	C-2, C-5, C-9, C-10	H-3, H-17, H-2″
5	133.27						
6	134.97						
7	149.33						
8	138.29						
9	144.79						
10	112.93						
11	43.79	4.32	*br s*		H-12, H-16, H-18		H-12, H-16, 7-OMe, H-2″, H-6″
12	121.90	6.33	*br s*		H-11, H-14, H-18	C-16, C-18	H-11, H-18, 7-OMe
13	134.63						
14	36.45	2.32	*br m*	7.8, 17.8	H-12, H-15, H-18	C-13, C-12, C-16	H-15, H-16, H-17, H-18, H-6′
15	34.45	3.59	*br m*	8.0, 13.8	H-14, H-16	C-14, C-16, C-1′, C-2′, C-6′	H-14, H-16, H-17, H-6′
16	53.62	2.98	*ddd*	2.8, 7.0, 10.2	H-11, H-15, H-17	C-6, C-1″	H-11, H-14, H-17, H-6′, H-2″, H-6″
17	52.21	4.54	*d*	2.8	H-16	C-5, C-6, C-11, C-15, C-16, C-1″, C-2″, C-6″	H-4, H-14, H-15, H-16, H-2″, H-6″
18	23.85	1.78	*s*		H-11, H-12, H-14	C-12, C-13, C-14	H-12, H-14
7-OMe	60.51	4.16	*s*			C-7	H-11, H-12
1′	124.55						
2′	159.32						
3′	100.34	6.91	*d*	2.0	H-5′	C-1′, C-2′, C-4′, C-5″	2′-OMe
4′	158.58						
5′	108.37	6.97	*dd*	2.0, 8.1	H-3′, H-6′	C-1′, C-3′, C-4′	H-6′
6′	129.06	7.40	*d*	8.1	H-5′	C-15, C-2′, C-3′, C-4′	H-14, H-15, H-16, H-17, H-5′
2′-OMe	55.20	3.59	*s*			C-2′	H-3′
1″	136.69						
2″	115.74	6.88	*d*	1.9	H-6″	C-17, C-3″, C-4″, C-6″	H-11, H-15, H-16, H-17, H-6″
3″	147.24						
4″	145.75						
5″	116.74	7.20	*d*	7.9	H-6″	C-1″, C-3″, C-4″	H-6″
6″	119.01	6.59	*dd*	1.9, 7.9	H-2″, H-5″	C-17, C-2″, C-4″	H-11, H-15, H-16, H-17, H-5″, H-2″

* Measurements in C_5D_5N at 100 MHz for ^{13}C and at 400 MHz for ^{1}H, 300 K.

at 300 K using standard pulse-sequences. ROESY expts used a mixing time of 300 ms. Delay to value to optimize one-bond correlations in HSQC and to suppress them in HMBC was 150 ms and the evolution delay for long-range couplings in HMBC was 63 ms.

Plant material

Bark of *B. rubescens* Tauber, commonly known as "palo de sangre" in Peru, was purchased at Lima, Peru, in 1992. Identification was made by Dr Franklin Ayala Flores (Herbarium Amazonese, Universidade Nacional Amazonian Peru, Iquitos, Peru). A voucher specimen is deposited at the National Institute of Health Sciences, Japan.

Extraction and isolation

Bark (2.4 kg) was milled and extracted with hot MeOH (5 l × 3) to give an extract (152 g), which was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-sol. fr. (25.2 g) was subjected to a silica gel CC using a *n*-hexane–EtOAc gradient (9:1–0:1), followed by an EtOAc–MeOH gradient (9:1–0:1), to give 12 frs (I–XII). Fr. V was subjected to Sephadex LH-20 CC using *n*-hexane–CH_2Cl_2–MeOH (4:5:1) and the frs obtained subjected to silica gel MPLC with *n*-hexane–EtOAc (7:3), then to ODS MPLC with MeOH–H_2O (3:1) to obtain a mixt. of **1** and **2**. Compounds **1** (5 mg) and **2** (7 mg) were finally sepd by ODS HPLC using MeCN–H_2O (2:3).

Palodesangretin I (**1**). Pale brownish amorphous solid (5 mg). $[\alpha]_D$ +7.1° (*c* 0.056, MeOH). UV λ_{max} (MeOH) nm (log ε): 261 (3.96), 287 (3.91), 306 (3.89). IR ν_{max} (KBr) cm^{-1}: 3399, 2930, 1707, 1603, 1508, 1435, 1292, 1198, 1154, 1032, 957, 833. ^1H NMR (C_5D_5N, 400 MHz): Table 1. ^{13}C NMR (C_5D_5N, 100 MHz): Table 2. Positive FABMS (with 1 M NaI) *m/z* (rel. int): $[M+Na]^+$ 551 (58), $[M+H]^+$ 529 (28); HRFABMS *m/z*: $[M+H]^+$ 529.1865 ($C_{31}H_{29}O_8$ requires 529.1862).

Palodesangretin II (**2**). Pale brownish amorphous solid (7 mg). $[\alpha]_D$ +12.5° (*c* 0.064, MeOH). UV λ_{max} (MeOH) nm (log ε): 260 (3.99), 287 (3.98), 304 (3.95). IR ν_{max} (KBr) cm^{-1}: 3360, 2932, 1707, 1601, 1508, 1435, 1281, 1198, 1154, 1034, 957, 831. ^1H NMR (C_5D_5N, 400 MHz): Table 1. ^{13}C NMR (C_5D_5N, 100 MHz): Table 2. Positive FABMS *m/z* (rel. int): $[M+H]^+$ 529 (35); positive FABMS (with 1 M NaI) *m/z* (rel. int): $[M+Na]^+$ 551 (28), $[M+H]^+$ 529 (22); HRFABMS *m/z*: $[M+H]^+$ 529.1855 ($C_{31}H_{29}O_8$ requires 529.1862).

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