Antiandrogenic Natural Diels−Alder-Type Adducts from *Brosimum rubescens*

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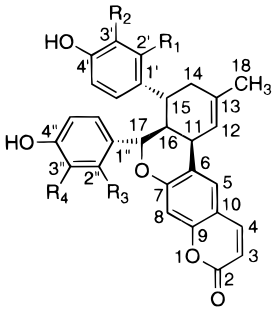
The isolation and structure elucidation of five novel natural Diels−Alder-type adducts, named palodesangrens A−E (**1**−**5**), from the Peruvian folk medicine known as "palo de sangre" (*Brosimum rubescens*) is described. The structures of the Diels−Alder adducts, consisting of chalcone derivatives and a prenylcoumarin, were elucidated by analysis of spectroscopic data including 2D NMR. Some of these compounds showed potent inhibitory activity towards 5α-dihydrotestosterone (DHT) binding with an androgen receptor to form a DHT-receptor complex that causes androgen-dependent diseases.

Brosimum rubescens Taubert, an arboreous Moraceae tree growing 30 to 50 m high, is fairly common in Amazonia.[1] It is known as "palo de sangre", "palisangre", and "palo negro" in Peru, and "muirapiranga" in Brazil.[2] This tree is commonly used in carpentry, and its alcoholic extract is used medicinally as a tonic. There are three chemical reports indicating that its reddish heartwood contains a large amount of xanthyletin and several other coumarins.[2−4]

Chemical investigation of the bark of Peruvian "palo de sangre" led us to isolate five novel natural Diels−Alder-type adducts, which we named palodesangrens A−E (**1**−**5**), together with three known coumarins: xanthyletin, (*R*)-aegelinol, and 7-demethylsuberosin. The structures of palodesangrens A−E were elucidated by a combination of several spectroscopic data including 2D NMR. Palodesangrens C (**3**), D (**4**), and E (**5**) showed potent inhibition of binding of 5α-dihydrotestosterone (DHT) with the androgen receptor.



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
3: R_1 = H, R_2 = H, R_3 = OMe, R_4 = H
4: R_1 = OMe, R_2 = H, R_3 = H, R_4 = H
5: R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = H

Results and Discussion

The CH_2Cl_2-soluble portion (25.2 g) of the MeOH extract (152 g) of the bark of *Brosimum rubescens* (2.4 kg) was subjected to Si gel column chromatography. The fractions were further separated by Sephadex LH-20 column chromatography and by Si gel and ODS medium-pressure liquid chromatography (MPLC) and/or HPLC to give palodesangrens A (**1**; 0.00104%), B (**2**; 0.00187%), C (**3**; 0.00083%), D (**4**; 0.00075%), and E (**5**; 0.00054%), and three known coumarins: xanthyletin (0.0458%), (*R*)-aegelinol (0.00287%), and 7-demethylsuberosin (0.00033%).

Compound **1** was obtained as a colorless amorphous solid with elemental composition $C_{30}H_{26}O_7$, as established by HRFABMS. The ^{1}H-NMR spectrum suggested that **1** contained two 1,2,4-trisubstituted benzene rings; a methoxyl group on an aromatic ring; a 1,2,4,5-tetrasubstituted benzene ring; and an α,β-unsaturated ester group. The presence of the latter moiety suggested that the tetrasubstituted benzene ring forms part of a coumarin unit. The H−H COSY spectrum also revealed the presence of a cyclohexene unit, as shown in Figure 1, to which the aromatic units were joined. All these observations were confirmed by analysis of HMBC data. One aryl proton of the coumarin unit (δ_H 7.30; H-5) showed a long-range correlation to a methine carbon of the cyclohexene unit (δ_C 32.1; C-11), and the oxygenated methine proton of the cyclohexene unit (δ_H 5.74; H-17) gave a cross peak to the oxygenated aromatic carbon of the coumarin unit (δ_C 158.6; C-7). Therefore, the coumarin unit was linked to the cyclohexene unit by connection of C-6 with C-11 and C-7 with C-17 through an oxygen. The oxygenated methine proton of the cyclohexene unit (δ_H 5.74; H-17) also showed HMBC correlations to the aromatic carbons at δ_C 119.4, 158.7, and 128.73 of one trisubstituted benzene ring unit, to which the methoxyl group (δ_H 3.73) was linked, and the methine proton at δ_H 3.00 (H-15) correlated to the aromatic carbons at δ_C 136.5, 116.2, and 119.4 of the second trisubstituted benzene ring unit. These correlations confirmed the locations of the two benzene sub-

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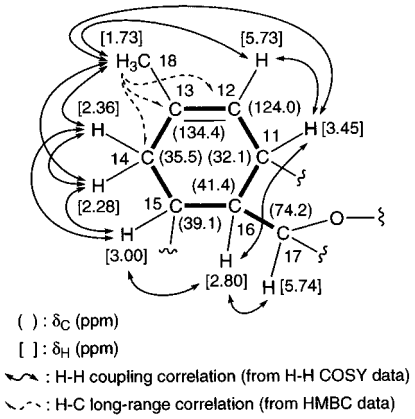

Figure 1. Cyclohexene unit of palodesangren A (**1**).

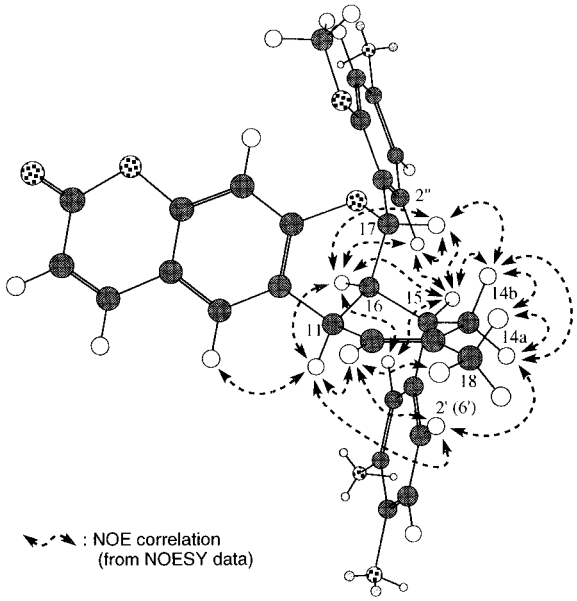

Figure 2. Relative stereochemistry of palodesangren A (**1**).

stituents at C-17 and C-15. Thus, the gross structure of **1** was established as shown.

The relative stereochemistry of the cyclohexene unit of **1** was elucidated by detailed analysis of phase-sensitive NOESY data (Figure 2). In this spectrum, a strong NOE correlation between the H-11 and the H-16 methine protons corroborated their *cis* relationship. This *cis* relationship was also supported by the small coupling value (J < 1; observed as broadened signals) between the H-11 and the H-16 methine protons. The H-16 methine proton showed a NOE correlation with the H-6″ aromatic proton on the 2″-methoxy-4″-hydroxybenzene substituent at C-17. Also, one of the H-14 methylene protons (δ_H 2.36; H-14b) had strong NOE correlations with the H-15 methine proton and the H-17 oxygenated methine proton. Another H-14 methylene proton (δ_H 2.28; H-14a) correlated with the H-2′ and the H-6′ aromatic protons on the 3′,4′-dihydroxybenzene substituent at C-15. And these H-2′ and H-6′ aromatic protons also had NOE correlations with the H-11, the H-15, and the H-16 methine protons. All of these correlations confirmed the relative stereochemistry of the cyclohexene unit of **1**, including the *cis*-β fusion between C-11 and C-16, and the configuration at C-17, as shown in Figure 2. Consequently, the structure of palodesangren A was assigned as **1**.

Compound **2** had the same elemental composition $C_{30}H_{26}O_7$ as **1**, as established by HRFABMS, as in compound **1**. The NMR spectra suggested that **2** had two trisubstituted benzene ring units, a coumarin unit, and one cyclohexene unit. The differences between **1** and **2** were evidenced by minor chemical shifts of the cyclohexene unit. In this unit, the signals corresponding to the methine units at positions 15 and 17 were shifted as follow; δ_H 3.00/δ_C 39.1 to δ_H 3.44/δ_C 32.3 at position 15, and δ_H 5.74/δ_C 74.2 to δ_H 5.19/δ_C 79.3 at position 17. These changes suggested that the positions of the two aromatic ring units were interchanged. The differences of chemical shifts observed in **1** and **2** were consistent with the expected deshielding influence of the 2-substituted methoxyl groups on the aromatic ring units. The HMBC spectrum of **2** confirmed the locations of these two aromatic ring units. Although the methoxyl-group-containing aryl unit (2-methoxy-4-hydroxybenzene unit) solved no long-range correlations with the broadened H-15 methine proton signal (δ_H 3.44, br d, J = 4.0), carbon signals (δ_C 131.9, 115.7 and 119.2) of the other aromatic ring unit (3,4-dihydroxybenzene unit) did show H−C long-range correlations with the H-17 oxygenated methine proton (δ_H 5.19, d, J = 7.0). The relative stereochemistry of **2** was assigned to be the same as that of **1** based on analysis of phase-sensitive NOESY data. Considering this spectroscopic evidence, the structure of palodesangren B was assigned as **2**.

Compound **3** was assigned the elemental composition $C_{30}H_{26}O_6$ based on HRFABMS data. The NMR spectra indicated that **3** again has two aromatic ring substituents, but with one of them differing from those present in **1** and **2**. This aromatic ring unit was easily assigned to a 1,4-disubstituted benzene. Other NMR signals, including these for the remaining 1,2,4-trisubstituted benzene ring and the chemical shift values of H-15/C-15 and H-17/C-17, were virtually the same as those of **1**. Therefore, it was suggested that the 3,4-dihydroxybenzene unit of **1** was replaced with a 4-hydroxybenzene unit in **3**. This difference was verified by analysis of HMBC data. The H-15 methine proton showed correlations to aromatic carbons of the 4-hydroxybenzene unit (δ_C 135.3, 129.4 × 2) and oxygenated methine proton H-17 correlated with the aromatic carbons of the 2-methoxy-4-hydroxybenzene unit (δ_C 119.4, 158.6, and 128.7). Thus, the structure of palodesangren C was assigned as **3**.

Compound **4** has the same elemental composition as **3**. The NMR spectra suggested that the 4-hydroxybenzene unit and the 2-methoxy-4-hydroxybenzene unit of **3** were interchanged in **4**, so the methine protons and carbons at positions 15 and 17 were shifted as observed in **1** and **2**. This presumption was verified by analysis of the HMBC spectrum, which showed H−C correlations between the respective methine and aromatic carbons. Consequently, the structure of palodesangren D was assigned as **4**.

Compound **5**, $C_{31}H_{28}O_7$, contains two aromatic ring units, each bearing a methoxyl group. Characteristic NMR shifts for positions 15 ($^1H/^{13}C$: 3.54/31.9) and 17 ($^1H/^{13}C$: 5.81/73.6) suggested that two 2-methoxy-4-hydroxybenzene units were present in these positions. This conjecture was confirmed by HMBC data in the same manner as with **1** to **4**, allowing confirmation of the structure of palodesangren E as **5**.

Table 1. ^{1}H-NMR Chemical Shifts (ppm, number of protons, multiplicity, and *J* in Hz) for Compounds **1**−**5**[a]

assignment	compound **1**	compound **2**	compound **3**	compound **4**	compound **5**
H-3	6.28 1H d 9.4	6.28 1H d 9.5	6.29 1H d 9.5	6.28 1H d 9.4	6.26 1H d 9.5
H-4	7.68 1H d 9.4	7.65 1H d 9.5	7.69 1H d 9.5	7.67 1H d 9.4	7.66 1H d 9.5
H-5	7.30 1H s	7.26 1H s	7.35 1H s	7.26 1H s	7.27 1H s
H-8	6.92 1H s	6.90 1H s	6.95 1H s	6.94 1H s	6.92 1H s
H-11	3.45 1H br s	3.39 1H br s	3.42 1H br s	3.37 1H br s	3.41 1H br s
H-12	5.73 1H br s	5.70 1H br s	5.78 1H br s	5.66 1H br s	5.69 1H br s
H-14a	2.28 1H br dd 5.4, 18.1	2.23 1H br d 16.5	2.25 1H br dd 5.7, 18.3	2.20 1H brd 17.2	2.18 1H br d 18.1
H-14b	2.36 1H br dd 5.5, 18.1	2.33 1H br d 16.5	2.33 1H br dd 5.6, 18.3	2.36 1H br d 17.2	2.47 1H br d 18.1
H-15	3.00 1H br dd 5.9, 12.1	3.44 1H br d 4.0	3.01 1H br dd 5.9, 12.5	3.37 1H br s	3.54 1H br d 4.9
H-16	2.80 1H br dd 5.9, 12.1	2.91 1H br d 4.8	2.79 1H br dd 5.1, 12.5	2.89 1H br s	2.90 1H br dd 5.3, 12.2
H-17	5.74 1H br d 5.7	5.19 1H d 7.0	5.68 1H d 5.8	5.16 1H d 7.9	5.81 1H d 7.7
H-18	1.73 3H s	1.73 3H s	1.78 3H s	1.82 3H s	1.80 3H s
H-2′	7.26 1H br s		7.33 1H d 8.5		
H-3′		6.83 1H br s	7.23 1H d 8.5	6.84 1H s	6.85 1H d 2.0
H-4′					
H-5′	7.24 1H br d 8.0	6.85 1H br d 8.8	7.23 1H d 8.5	6.85 1H br d 7.8	6.91 1H dd 2.0, 8.4
H-6′	6.87 1H dd 1.4, 8.0	7.22 1H br d 8.8	7.33 1H d 8.5	7.22 1H br d 7.8	7.31 1H d 8.4
2′-OMe		3.73 3H s		3.70 3H s	3.69 3H s
H-2″		7.44 1H br s		7.54 1H d 8.2	
H-3″	6.84 1H br s		6.86 1H br s	7.22 1H d 8.2	6.87 1H d 2.0
H-4″					
H-5″	6.81 1H br d 8.1	7.29 1H br d 8.0	6.85 1H br d 7.7	7.22 1H d 8.2	6.93 1H dd 2.0, 8.1
H-6″	7.36 1H br d 8.1	7.03 1H br d 8.0	7.39 1H br d 7.7	7.54 1H d 8.2	7.54 1H d 8.1
2″-OMe	3.73 3H s		3.72 3H s		3.71 3H s

[a] Measurements were performed in C_5D_5N at 400 MHz, 337 K.

Table 2. ^{13}C-NMR Chemical Shifts (ppm, multiplicity) for Compounds **1**−**5**[a]

assignment	compound **1**	compound **2**	compound **3**	compound **4**	compound **5**
C-2	161.03 s	161.00 s	161.01 s	161.00 s	161.04 s
C-3	113.11 d	113.12 d	113.14 d	113.21 d	113.02 d
C-4	143.88 d	143.87 d	143.88 d	143.81 d	143.85 d
C-5	129.40 d	129.41 d	129.37 d	129.48 d	129.45 d
C-6	124.24 s	124.37 s	124.17 s	124.36 s	124.55[c] s
C-7	158.61[b] s	158.30 s	158.51 s	158.43 s	158.86[d] s
C-8	103.57 d	103.69 d	103.59 d	103.79 d	103.57 d
C-9	154.57 s	154.46 s	154.59 s	154.51 s	154.59 s
C-10	113.29 s	113.27 s	113.33 s	113.36 s	113.21 s
C-11	32.07 d	32.32[c] d	31.95 d	32.61[c] d	32.84 d
C-12	124.01 d	123.99 d	123.93[b] d	124.71 d	124.55[c] d
C-13	134.43 s	134.26 s	134.51 s	133.95 s	134.31 s
C-14	35.25[b] t	33.49[b] t	35.7[b] t	32.6[b,c] t	33.67[b] t
C-15	39.09 d	32.32[c] d	38.92 d	32.61[c] d	31.94[b] d
C-16	41.41 d	39.90 d	41.21 d	39.67 d	39.06 d
C-17	74.18[b] d	79.34 d	74.21[b] d	79.43 d	73.63[b] d
C-18	23.43 q	23.40 q	23.41 q	23.39 q	23.44 q
C-1′	136.48 s	123.3[c] s	135.3[c] s	123.3[c] s	123.5[c] s
C-2′	116.24 d	158.88 s	129.37 d	158.75 s	158.97[d] s
C-3′	147.25 s	100.54 d	116.33 d	100.45 d	100.42 d
C-4′	145.71 s	158.91 s	157.63 s	158.92 s	158.82[d] s
C-5′	116.54 d	108.15 d	116.33 d	108.08 d	108.07 d
C-6′	119.40[c] d	128.82[b] d	129.37 d	128.64[b] d	128.62 d
2′-OMe		55.31 q		55.19 q	55.34 q
C-1″	119.40[c] s	131.87[b] s	119.43 s	130.65 s	119.08 s
C-2″	158.70 s	115.66[b] d	158.57[b] s	129.41 d	159.04[d] s
C-3″	100.51 d	147.31 s	100.52 d	116.27 d	100.26 d
C-4″	160.34 s	147.37 s	160.33 s	159.24 s	160.42 s
C-5″	108.43 d	116.38 d	108.42 d	116.27 d	108.40 d
C-6″	128.73[b] d	119.15[b] d	128.66[b] d	129.41 d	129.61[b] d
2″-OMe	55.45 q		55.39 q		55.51 q

[a] Measurements were performed in C_5D_5N at 100 MHz, 337 K. [b] Signals bearing this superscript were significantly broadened in the spectrum. [c] Signals bearing this superscript were overlapped or superimposed on solvent signals. [d] Assignments for values bearing this superscript may be reversed.

Complete assignments of the ^{1}H- and ^{13}C-NMR signals of palodesangrens A−E (**1**−**5**) are shown in Tables 1 and 2. The known coumarins xanthyletin,[5] (*R*)-aegelinol,[6] and 7-demethylsuberosin[2] were identified by comparison of the physical data with those described in the literature.

Biosynthetically, palodesangrens appear to arise by condensation of chalcone derivatives with a prenylcoumarin via a Diels−Alder-type addition reaction. Compounds of this class have been isolated from plants belonging to the Moraceae family, for example, kuwanons from the mulberry tree *Morus alba* L.[7] The kuwanons and other related Diels−Alder adducts isolated from Moraceous plants came from prenylchalcone derivatives, prenylflavonoid derivatives, and prenylbenzofuran derivatives.[8−10] Biosynthetic studies on chalcomoracin, which was produced in *M. alba* cell cultures, confirmed that the cycloaddition reaction in the cell

Table 3. Inhibitory Activities of Compounds **1**−**5** against Testosterone 5α-Reductase and DHT−Receptor Binding at Various Concentrations[a]

	5α-reductase (inhibitory rate; %)[b]			DHT−receptor binding (inhibitory rate; %)[c]		
compound	50 μg/mL	100 μg/mL	200 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
1	0.3	4.8	5.2		−30.4	
2	0.2	8.4	19.8		−8.6	
3	5.4	28.8	59.2	62.5	73.3	78.6
4	3.4	44.1	69.2	56.5	57.5	61.5
5	8.6	49.9	72.3	60.3	70.9	62.0

[a] Data were determined as described in the Experimental Section. [b] Positive control standard, oxendlone, inhibited 5α-reductase completely at 50 μg/mL. [c] Oxendlone showed inhibition of DHT−receptor binding at a rate of 51.0% at 7.5 ng/mL and 69.5% at 15 ng/mL.

cultures was enzymatic.[11−13] Compounds that may be natural [4 + 2]-cycloadducts[14] have been isolated not only from Moraceous plants but also from Rutaceous plant species (e.g., paraensidimerins from *Euxylophora paraenis* and vepridimerines from *Vepris louisii*),[15,16] the Australian Lauraceous plant *Endiandra introrsa* (endiandric acids),[17] the South American Celastraceous plants (e.g., xuxuarines from *Maytenus chuchuhuasca* and cangorosins from *Maytenus ilicifolia*),[18,19] the fungus *Aspergillus terreus* (mevinolin),[20] the fungus *Chaetomium globosum* (chaetoglobosins),[21,22] the Okinawan marine sponges (e.g., manzamines from *Haliclona sp.* and keramaphidins from *Amphimedon sp.*),[23,24] and so on. Recently the first enzyme that catalyzes a Diels−Alder reaction has been partially purified from a cell-free extract of the pathogenic fungus *Alternaria solani*, and the enzyme, a bifunctional enzyme catalyzing both oxidation and Diels−Alder reaction, was able to catalyze the [4 + 2] cycloaddition of prosolanapyrones to the phytotoxic solanapyrones.[25] The possibility that the palodesangrens are artifacts produced during the isolation process was ruled out by an additional gentle extraction experiment described in the Experimental Section. This indicates that the compounds really exist in the folk medicine (dried material). Furthermore, the experimental evidence of the enzymatic cycloaddition reaction in the case of *M. alba*, which belongs to the same Moraceous family as *B. rubescens*, and the case of *A. solani*, which showed the presence of a Diels−Alder-ase in nature, are constituent with the possibility of enzymatic [4 + 2] Diels−Alder-cycloaddition production of palodesangrens in the medicinal plant. In any case, palodesangrens are the first natural Diels−Alder adducts arising from reaction of chalcone and prenylcoumarin derivatives.

Testosterone, a hormone essential for the growth of male secondary sexual characteristics, is also responsible for androgen-dependent diseases like prostatic hypertrophy, prostate cancer, male pattern baldness, hirsutism, acne, and so on.[26,27] Testosterone is converted to DHT by the enzyme 5α-reductase,[28] located in the cytoplasm of prostate cells. The DHT binds with an androgen receptor to form a DHT−receptor complex[29] that causes the diseases. To investigate the possibility of palodesangrens as antiandrogen agents, inhibitory assays for the 5α-reductase and for formation of the DHT−receptor complex were carried out. As a result, as listed in Table 3, palodesangrens C (**3**), D (**4**), and E (**5**) showed moderate activity against the 5α-reductase, and effective action against DHT−receptor binding.

Experimental Section

General Experimental Details. Extraction was made below 50 °C, and the extract and all fractions were concentrated in vacuum below 40 °C. Si gel open column chromatography was performed on Si gel 60 (Merck). MPLC was performed with a CIG column system (22 mm i.d. × 300 mm or 22 mm i.d. × 100 mm; Kusano Scientific Co., Tokyo) packed with 10 μm or 5 μm of Si gel and/or octadecyl Si gel (ODS). HPLC was performed with an Inertsil PREP-ODS column (5 mm i.d. × 250 mm for analysis, 20 mm i.d. × 250 mm for preparative; GL Science Inc., Tokyo) packed with 10 μm of ODS. TLC was conducted on precoated Si gel 60 F_{254} (Merck) and/or RP-18 F_{254} s (Merck), and the spots were detected by heating after spraying with 10% H_2SO_4. Melting points were determined on a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2g^{-1}. FABMS were obtained on a JEOL AX-505H spectrometer. UV and IR spectra were taken with a Hitachi U-2000 spectrophotometer and a JASCO FT/IR-5300 spectrophotometer, respectively. 1D-, 2D- ^{1}H- and ^{13}C-NMR spectra were recorded on a Unity Plus 400 spectrometer (Varian) at 300 K using Varian standard pulse sequences. NMR coupling constants (*J*) are given in Hz. Phase-sensitive NOESY experiments were conducted with a mixing time of 750 ms. A 150-msec delay was used to optimize one-bond correlation in HSQC spectra and suppress them in HMBC spectra, and the evolution delay for long-range couplings in HMBC spectra was set to 63 ms.

Plant Material. The bark of *Brosimum rubescens* Taubert (2.4 kg), commonly known as "palo de sangre" in Peru, was purchased in the market at Iquitos, Peru, in 1992. The botanical identification was made by Dr. Franklin Ayala Flores (Herbarium Amazonense, Universidad Nacional Amazoniana del Peru, Iquitos, Peru). A voucher specimen has been deposited at the National Institute of Health Sciences, Japan.

Extraction and Isolation. The bark of *B. rubescens* (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-soluble fraction (25.2 g) was subjected to Si gel column chromatography using an *n*-hexane−EtOAc gradient system (9:1−0:1) followed by an EtOAc−MeOH gradient system (9:1−0:1) to give 12 fractions (fractions I−XII). Xanthyletin (ca. 1 g) was crystallized from an *n*-hexane−EtOAc solution of fraction III. Xanthyletin (20 mg) was also obtained from fraction IV, which was separated by Sephadex LH-20 column chromatography with *n*-hexane−CH_2Cl_2−MeOH (4:5:1) followed by Si gel MPLC with *n*-hexane−EtOAc (7:3). Fraction V was subjected to Sephadex LH-20 column chromatography with *n*-hexane−CH_2Cl_2−MeOH (4:5:1), and the derived fractions were further separated by Si gel MPLC with *n*-hexane−EtOAc (7:3) to afford xanthyletin (4 mg), (*R*)-aegelinol (25 mg), and 7-demethylsuberosin (8 mg), and by ODS MPLC with MeOH−H_2O (3:1) to give the mixture of **3** and **4**. Compounds **3** (20 mg) and **4** (18 mg) were finally

separated by ODS HPLC with CH_3CN-H_2O (1:1). Fraction VI was subjected to Sephadex LH-20 column chromatography (*n*-hexane−CH_2Cl_2−MeOH, 4:5:1), then to Si gel MPLC with *n*-hexane−EtOAc (7:3) to obtain xanthyletin (4 mg) and (*R*)-aegelinol (44 mg), and to ODS MPLC with MeOH−H_2O (7:3) to afford of **1** and **2**. Compounds **1** (25 mg) and **2** (45 mg) were further purified by ODS HPLC with CH_3CN-H_2O (4:6). Remaining fractions from fractions V and VI were further separated by ODS MPLC (CH_3CN-H_2O, 1:1) followed by Toyopearl HW-40 column chromatography (EtOH−H_2O, 1:1), then by ODS HPLC (CH_3CN-H_2O, 45:55) to afford compound **5** (13 mg).

Palodesangren A (1): obtained as a colorless amorphous solid (25 mg); mp 195−200 °C; $[\alpha]^{25}_D$ −11.3° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 287 (3.84), 334 (4.08) nm; IR (KBr) ν_{max} 3386, 1701, 1624, 1561, 1508, 1396, 1280, 1198, 1136, 1035, 959, 826 cm^{-1}; ^{1}H-NMR (50 °C, C_5D_5N, 400 MHz) data, see Table 1; ^{13}C-NMR (50 °C, C_5D_5N, 100 MHz) data, see Table 2; FABMS *m/z* (%) $[M + Na]^+$ 521 (7), $[M + H]^+$ 499 (12), $[M]^+$ 498 (7), 482 (3), 413 (6), 391 (5), 271 (10); HRFABMS *m/z* $[M + H]^+$ 499.1752 (calcd for $C_{30}H_{27}O_7$, 499.1757).

Palodesangren B (2): obtained as a colorless amorphous solid (45 mg); mp 174−177 °C; $[\alpha]^{25}_D$ +7.7° (*c* 0.65, MeOH); UV (MeOH) λ_{max} (log ϵ) 250 (3.84), 287 (3.99), 334 (4.24) nm; IR (KBr) ν_{max} 3344, 1711, 1624, 1561, 1508, 1396, 1284, 1196, 1139, 1036, 959, 827 cm^{-1}; ^{1}H-NMR (50 °C, C_5D_5N, 400 MHz) data, see Table 1; ^{13}C-NMR (50 °C, C_5D_5N, 100 MHz) data, see Table 2; FABMS *m/z* (%) $[M + Na]^+$ 521 (4), $[M + H]^+$ 499 (20), 482 (2), 413 (1), 375 (2), 271 (13); HRFABMS *m/z* $[M + H]^+$ 499.1748 (calcd for $C_{30}H_{27}O_7$, 499.1757).

Palodesangren C (3): obtained as a colorless amorphous solid (20 mg); dec around 200 °C; $[\alpha]^{25}_D$ +5.6° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.37), 249 (3.64), 260 (3.65), 286 (3.83), 333 (4.14) nm; IR (KBr) ν_{max} 3376, 1711, 1620, 1561, 1514, 1468, 1395, 1279, 1198, 1138, 1034, 959, 829 cm^{-1}; ^{1}H-NMR (50 °C, C_5D_5N, 400 MHz) data, see Table 1; ^{13}C-NMR (50 °C, C_5D_5N, 100 MHz) data, see Table 2; FABMS *m/z* (%) $[M + Na]^+$ 505 (5), $[M + H]^+$ 483 (60), 482 (20), 467 (2), 255 (100); HRFABMS *m/z* $[M + H]^+$ 483.1827 (calcd for $C_{30}H_{27}O_6$, 483.1807).

Palodesangren D (4): obtained as a colorless amorphous solid (18 mg); dec around 200 °C; $[\alpha]^{25}_D$ +2.5° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.38), 249 (3.69), 260 (3.68), 285 (3.87), 332 (4.19) nm; IR (KBr) ν_{max} 3397, 1701, 1685, 1624, 1560, 1508, 1458, 1395, 1282, 1198, 1138, 1035, 957, 826 cm^{-1}; ^{1}H-NMR (50 °C, C_5D_5N, 400 MHz) data, see Table 1; ^{13}C-NMR (50 °C, C_5D_5N, 100 MHz) data, see Table 2; FABMS *m/z* (%) $[M + Na]^+$ 505 (7), $[M + H]^+$ 483 (68), 482 (25), 255 (78); HRFABMS *m/z* $[M + H]^+$ 483.1827 (calcd for $C_{30}H_{27}O_6$, 483.1807).

Palodesangren E (5): obtained as a colorless amorphous solid (13 mg); dec around 200 °C; $[\alpha]^{25}_D$ −5.2° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.41), 249 (3.75), 260 (3.73), 285 (3.90), 333 (4.19) nm; IR (KBr) ν_{max} 3416, 1709, 1620, 1561, 1508, 1466, 1397, 1279, 1198, 1140, 1036, 959, 830 cm^{-1}; ^{1}H-NMR (50 °C, C_5D_5N, 400 MHz) data, see Table 1; ^{13}C-NMR (50 °C, C_5D_5N, 100 MHz) data, see Table 2; FABMS *m/z* (%) $[M + Na]^+$ 535 (13), $[M + H]^+$ 513 (42), 512 (18), 483 (6), 285 (75); HRFABMS *m/z* $[M + H]^+$ 513.1930 (calcd for $C_{31}H_{29}O_7$, 513.1913).

Xanthyletin: obtained as colorless crystals (ca. 1.1 g); mp 128−129 °C; identification made by comparison with literature data.[5]

(*R*)-Aegelinol: obtained as a colorless amorphous solid (69 mg); mp 179−180 °C; $[\alpha]^{25}_D$ −119.9° (*c* 0.83, MeOH); identification made by comparison with literature data.[6]

7-Demethylsuberosin: obtained as colorless crystals (8 mg); mp 133−135 °C; identification made by comparison with literature data.[2]

HPLC Analysis of Palodesangrens in the CH_2Cl_2 Extract. The same dried material (1 g) used in the original extraction experiment was milled and extracted with 10 mL of CH_2Cl_2 by a sonication for 30 min at room temperature. The extract was filtered, concentrated *in vacuo* at room temperature, and dissolved in 1 mL of MeOH−H_2O (9:1). The solution was passed through a SEP-PAK C18 cartridge (Waters Associates) and concentrated *in vacuo* at room temperature. An aliquot was then subjected to HPLC (detected at 333 nm; method A: CH_3CN-H_2O, 45:55; method B: MeOH−H_2O, 65:35). Using these two HPLC methods, palodesangrens can be separated from each other (Method A: t_{R-A} = 22.8 min, t_{R-B} = 24.7 min, t_{R-C} = 41.1 min, t_{R-D} = 39.8 min t_{R-E} = 41.7 min; method B: t_{R-A} = 23.5 min, t_{R-B} = 25.1 min, t_{R-C} = 40.2 min, t_{R-D} = 39.1 min t_{R-E} = 37.3 min). The analysis of the CH_2Cl_2 extract showed the presence of all of these components in the extract.

Inhibitory Assay for 5α-Reductase. Ventral prostates taken from male Wistar rats (10−12 weeks old) were homogenized with 5 volumes (W/V) of 50 mM Tris−HCl buffer (buffer A; pH 7.4, containing 1.5 mM EDTA, 1 mM DTT, 10 mM Na_2MoO_4, and 10% glycerol). The homogenate was centrifuged at 700 × *g* for 10 min, and the supernatant was used as an enzyme solution. A reaction mixture, consisting of 250 μL of the enzyme solution, 10 μL of [^{3}H]-testosterone EtOH solution (2 μCi), 150 μL of buffer A containing 3.3 mM NADPH, and 100 μL of test sample solution, was incubated for 60 min at 37 °C. The reaction was terminated by addition of 2 mL of $CHCl_3$−MeOH (2:1) solution, and the mixture was centrifuged at 3000 rpm for 10 min. The lower layer was separated and concentrated, and the residue was then dissolved in 50 μL of MeOH, and was separated into three bands (testosterone, dihydrotestosterone, and androstandiol) by TLC ($CHCl_3$−MeOH, 100:1). The radioactivity of each band was measured by a radiochromatoanalyzer. The inhibitory activity of each test sample was calculated as follows; Inhibitory Activity (%) = $(M_{control} - M_{sample} / M_{control}) \times 100$, where M is the metabolic ratio: M (%) = (D + A) / (T + D + A) × 100, (T, counts of testosterone; D, counts of DHT; A, counts of androstandiol). Positive control standard oxendlone,[30] a synthetic antiandrogen agent for the treatment of prostatic hypertrophy, inhibited completely at 50 μg/mL.

Inhibitory Assay for Formation of the DHT−Receptor Complex. Male Wistar rats, 10−12 weeks old, were castrated under ether anesthesia. One day after castration, the ventral prostates were isolated and homogenized with 4 volumes (w/v) of buffer A. The homogenate was ultracentrifuged at 105 000 × *g* for 60

min at 4 °C. The receptor solution (cytosol fraction) was obtained as the supernatant fluid. A reaction mixture consisting of 100 μL of the receptor solution, 1 nM [^{3}H] DHT, 400 nM DHT, 5 μL of test sample solution, and buffer A (total 250 μL) was incubated for 16 h at 4 °C. After incubation, free and receptor-complexed [^{3}H] DHT were separated by addition of 250 μL of dextran-coated charcoal suspension containing 5% (W/V) charcoal and 0.5% (W/V) dextran in buffer A. After standing for 10 min at 4 °C, the mixture was centrifuged at 700 × *g* for 10 min at 4 °C. The radioactivity of the supernatant was measured with a liquid scintillation counter, to determine the specific binding. The percent inhibition of the formation of DHT−receptor complex was calculated as follows; Inhibitory Activity (%) = $(B_{control} - B_{sample} / B_{control}) \times 100$, where B is the specific [^{3}H] DHT binding (fmol/mL protein). Oxendlone,[30] a positive control, showed inhibition of complex formation at a rate of 51.0% at 7.5 ng/mL and 69.5% at 15 ng/mL.

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