Notes

An Antiplasmodial Lignan from *Euterpe precatoria*

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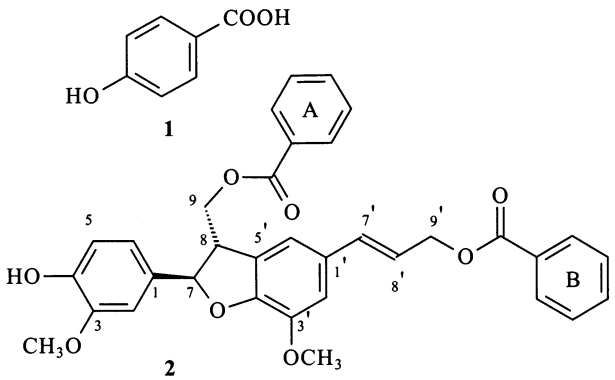
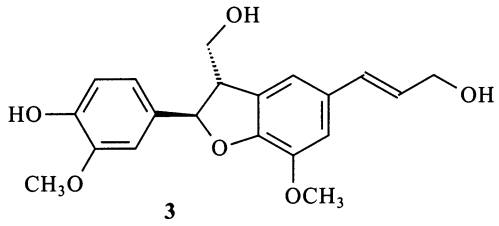
As a part of a study on new antiplasmodial natural products, a new 8−5′ linked lignan dehydrodiconiferyl dibenzoate (**2**) and *p*-hydroxybenzoic acid (**1**) were isolated from the roots of the palm *Euterpe precatoria*. In contrast to compound **1**, compound **2** showed a moderate antiplasmodial activity.

The palm *Euterpe precatoria* Mart. (Aracaceae) is widely distributed in the Western Amazon Basin including the Peruvian Amazon region. The trunks are used for construction, and the young undeveloped leaves known as palm heart are either eaten locally or canned for commerce. Heavy exploitation has depleted *E. precatoria* populations near many populated areas, but in Peru the species remains abundant in extensive more distant areas.

In 1997, a survey on plants used against the parasitic diseases malaria and leishmaniasis was performed throughout the Loreto Department comprising all of northeastern Peru and predominantly covered with little disturbed Amazon lowland rainforest. The study focused on Ribereños (detribalized people currently speaking Spanish and constituting the main population segment) but also included representatives of three Amerindian groups: the Chayahuitos, the Cocomas, and the Ticunas. Individual families were questioned about their use and knowledge of plants supposed to cure or relieve malaria and leishmaniasis. In the case of malaria the most frequently mentioned useful plant was *E. precatoria*. Ten percent of all used records referred to this species, corresponding to positive indications from 102 different families. Only the roots of *E. precatoria* were used. A concentrated decoction for oral use was prepared by mixing the comminuted roots with water and boiling for several hours.[1]

The potent antiplasmodial activity of the ethyl acetate extract of comminuted roots prompted the current investigation to find a possible lead structure for development of a new antimalarial drug. The extract showed no activity against the leishmania parasites in vitro, which might be correlated to the absence of positive reports on use of the decoction against leishmaniasis. A previous study on *E. precatoria* has focused on the nutritional value of the palm oil.[2] The dominant constituent in an active fraction, obtained from the ethyl acetate extract of the roots ($IC_{50} \approx 25$ μg/mL), was shown to be *p*-hydroxybenzoic acid (**1**) by ^{1}H NMR spectroscopy. This compound was shown to be inactive. Further fractionation using HPLC afforded compound **2** in a yield of 0.0008% of dried plant material. A molecular formula $C_{34}H_{30}O_8$ was revealed by HRMS. The presence of a strong peak in the MS at 105, the intensities of the pattern of the signals in the region 7.4−8.1 ppm in the ^{1}H NMR spectrum, and the two carbonyl signals at 167.0 and 167.1 strongly indicated the presence of two benzoate residues in the molecule. The presence of two methoxy groups as revealed by the ^{1}HNMR spectrum implied the presence of 18 carbon atoms in the skeleton. Lignans and neolignans are formed by dimerization of two molecules of phenylpropanoids, affording carbon skeletons with 18 carbons. Comparison of ^{1}H and ^{13}C NMR spectra with those published for dehydrodiconiferyl alcohol and its esters[3−10] indicated that compound **2** was the dibenzoate of **3**. The two substituents at the dihydrofuran ring are





generally *trans* oriented in naturally occurring dehydrodiconiferyl alcohol (**3**) and its derivatives.[6,11,12] A NOESY spectrum of **2** revealed a pronounced coupling between the two protons at C-9 and C-7, suggesting a *trans* configuration in **2**. Dehydrodiconiferyl alcohol (**3**), as well as its glycosides and esters, has been isolated from different

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species spanning all groups of vascular plants, and **3** is thought to be part of a universal defense system.[13] The alcohol **3** has been isolated in the dextrorotatory form (+)-2*S*,3*R*[14,15] and as the racemic mixture.[8,16] Some plant species forming **3** as a racemic mixture glycosidate the enantiomers at different oxygens, proving that they are able to differentiate the isomers,[17] whereas other species glycosidate the isomers at the same oxygen.[17−19] The absence of optical rotation and missing CD spectrum proved that **2** was a racemic mixture.

The biosynthesis of **3** is assumed to involve an oxidative phenolic coupling of the radical formed by one-electron oxidation of coniferyl alcohol. Two enzymes have been shown to catalyze the formation of pinoresinol, an 8−8′ linked lignan. One enzyme catalyzes the radical formation and a dirigent protein catalyzes the regioselective and stereoselective coupling of the radicals.[20,21] In the absence of a dirigent enzyme the product distribution is under kinetic control[20,21] and racemic mixtures are formed, explaining why reactions catalyzed with horseradish peroxidase afford series of racemic mixtures.[8] In contrast to pinoresinol there is no explicit description of the biochemical pathway and the enzymes involved in the formation of the 8−5′ lignan in the literature.[13] The presence of racemic dehydrodiconiferyl benzoate (**2**) in *E. precatoria* indicates the absence of a dirigent enzyme in this species.

An IC_{50} value of 12 μM was found when **2** was tested against the chloroquine-sensitive 3D7 *Plasmodium falciparum*, which discloses **2** as a moderate potent antiplasmodial agent.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian XL-400 spectrometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C NMR spectra, respectively. COSY, NOESY, HMBC, and HMQC spectra were recorded with standard pulse set up in the Varian program. Acetone-d_6 and acetonitrile-d_3 were used as solvent and TMS as internal standard. Electron impact mass spectra were obtained on a JEOL JMS-HX/HX 110A spectrometer at 70 eV using the direct inlet system. The optical rotations was determined on a Perkin-Elmer 241 polaritmeter, the circular dichroism (CD) spectrum was recorded on a modified JASCO J-710 spectropolarimeter, and the UV spectrum was recorded on a Shimadzu UV 265. Vacuum liquid chromatography (VLC) was performed using Merck LiChroprep RP-8 (40−63 μm). Fractions were monitored by TLC aluminum sheets, Merck Si gel 60 F_{254} impregnated with KH_2PO_4,[22] and Merck RP-18 F_{254S}, using UV light to visualize the spots. HPLC was performed over LUNA 5μ C18(2) (Phenomenex column 150 × 21.2 mm), UV detection at 220 nm. All solvents were freshly distilled except acetonitrile (LAB SCAN far UV). The solvents were degassed by exposure to vacuum for 1 min. The assay for antileishmanial activity is described elsewhere.[23]

Plant Material. *E. precatoria* was collected in September 1997 in the village Maniti, located in the Indiana district of the Loreto Department, Peru, by A. H. Gonzales. A voucher specimen (Andrea Gonzales H. no. 27) was deposited at El Herbarium Amazonense (AMAZ) de la Facultad de Ciencias Biológicas de la UNAP, Iquitos.

Extraction and Isolation. Roots were air-dried and comminuted, and the material (660 g) was extracted three times with 1:1 CH_2Cl_2−MeOH (3 × 3 L) each time for 18 h at room temperature. After filtration and concentration in vacuo the residue was dissolved in 9:1 MeOH−H_2O (450 mL) and washed with petroleum ether, and methanol was subsequently removed in vacuo. The aqueous layer was added to 300 mL of water and extracted with 4 × 150 mL of ethyl acetate. The aqueous phase was concentrated to give 13.2 g of a gum, and the ethyl acetate phase afforded 3.9 g of a gum. The ethyl acetate fraction showed high antiplasmodial activity, and the water fraction showed no activity. The solvent was removed in vacuo. A solution of the residue dissolved in MeOH−H_2O (9:1 450 mL) was washed with petroleum ether, and methanol was subsequently removed.

Isolation of *p*-Hydroxybenzoic Acid (1). A 254 mg amount of the ethyl acetate phase was chromatographed over Sephadex LH-20 (d = 20 mm, 20 mg, packed in MeOH) with MeOH. The fraction eluted from 55−80 mL was evaporated to give 105.6 mg, which was chromatographed over SiO_2 by VLC (d = 55 mm, gelbed = 30 mm) using CH_2Cl_2 with increasing amounts of EtOH: CH_2Cl_2 (30 mL), CH_2Cl_2−EtOH (98:2, 155 mL), CH_2Cl_2−EtOH (96:4, 85 mL), CH_2Cl_2−EtOH (96:4, 50 mL), CH_2Cl_2−EtOH (96:4, 20 mL). Concentration of the 20 mL fraction eluted with CH_2Cl_2−EtOH (96:4) afforded 3.2 mg of *p*-hydroxybenzoic acid (**1**).

Isolation of Dehydrodiconiferyl Alcohol Dibenzoate (2). A 3.4 g amount of the ethyl acetate phase was chromatographed by VLC RP-18 (d = 7 cm, gelbed = 3 cm) using the following eluents: H_2O (100 mL), H_2O−EtOH (90:10, 100 mL), H_2O−EtOH (80:20, 300 mL), H_2O−EtOH (70:30, 300 mL), H_2O−EtOH (60:40, 100 mL), H_2O−EtOH (50:50, 100 mL), H_2O−EtOH (40:60, 100 mL), H_2O−EtOH (30:70, 100 mL), H_2O−EtOH (20:80, 100 mL). The last three fractions from 1000 to 1300 mL of H_2O−EtOH (40:60 → 20:80) were pooled to give an active fraction of 408 mg. This fraction was chromatographed by HPLC with MeOH−H_2O (9:1) to give an active fraction (110 mg) eluting between 0 and 22 min (flow = 9 mL/min). Compound **2** (5 mg) was subsequently isolated from this fraction by further chromatographing by HPLC using CH_3CN−H_2O (7:3) as an eluent, eluting between 20 and 22 min (flow 9 mL/min).

Dehydrodiconiferyl Alcohol Dibenzoate (2). *E*-3-[3-Benzoyloxymethyl-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl]propenyl benzoate: colorless oil; UV (CH_3CN, c 1.7) λ_{max} (log ϵ) 250 (1.35); ^{1}H NMR (acetone-d_6 400 MHz) δ 3.77 (3H, s, OMe), 3.90 (3H, s, OMe′), 3.99 (1H, q, J = 7.2, 7.1, 5.4 Hz, H-8), 4.61 (1H, dd, J = 11.0, 7.4 Hz, H-9b), 4.75 (1H, dd, J = 11.0, 5.2 Hz, H-9a), 4.95 (2H, dd, J = 5.2, 1.3 Hz, H-9′), 5.68 (1H, d, J = 6.9 Hz, H-7), 6.38 (1H, dt, J = 15.7, 6.6 Hz, H-8′), 6.78 (1H, d br, J = 15.8 Hz, H-7′), 6.83 (1H, d, J = 8.1 Hz, H-5), 6.95 (1H, dd, J = 1.9, 8.1 Hz, H-6), 7.08 (1H, d, J = 2.3 Hz, H-2), 7.11 (1H, s br, H-2′), 7.17 (1H, s br, H-6′), 7.48 (2H, t, J = 7.6 Hz, H-*meta* in benzoyl A), 7.53 (2H, t, J = 7.6 Hz, H-*meta* in benzoyl B), 7.63 (2H, m, H-*para* in benzoyl A and B), 7.94 (2H, dd, J = 8.4, 1.4 Hz, H-*ortho* in benzoyl A), 8.06 (2H, dd, J = 1.4, 8.4 Hz, H-*ortho* in benzoyl B); ^{13}C NMR (acetonitrile-d_3 100 MHz,) δ 50.81 (C-8), 56.44 (OMe), 56.48 (OMe′), 66.32 (C-9′), 66.62 (C-9), 89.58 (C-7), 110.82 (C-2), 111.99 (C-2′), 115.59 (C-5), 116.25 (C-6′), 120.22 (C-6), 122.35 (C-8′), 129.23 (C-5′), 129.54 and 129.60 (C-*meta* in benzoyl A and B), 130.26 and 130.28 (C-*ortho* in benzoyl A or B), 130.89 (C-1′), 131.35 and 131.50 (C-*ipso* in benzoyl A and B), 133.47 (C-1), 134.11 and 134.22 (C-*para* in benzoyl A and B), 134.76 (C-7′), 145.41 (C-3′), 147.31 (C-4), 148.34 (C-3), 149.29 (C-4′), 166.98 (C═O in benzoyl A), 167.05 (C═O in benzoyl B); EIMS m/z 567(8), 566 (16) $[M]^+$, 444(51), 122(100), 105(82); HREIMS m/z 566.1956 (calcd for $C_{34}H_{30}O_8$ 566.1941).

Assay for Antiplasmodial Activity. A modification of the radioisotope method originally described by Desjardin et al.[25] was used to monitor the antiplasmodial activity of the fractions and the isolated compound. A chloroquine-sensitive strain of *Plasmodium falsiparum* (3D7) was used, where the uptake of radioactive [^{3}H] phenylalanine is used as an index of growth. The assay is described in detail elsewhere.[25]

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