DIHYDROLICOISOFLAVONE, A NEW ISOFLAVANONE FROM *SWARTZIA POLYPHYLLA*

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ABSTRACT.—Fractionation of an ethanolic extract of *Swartzia polyphylla*, guided in part by an assay for inhibition of protein kinase C, led to the isolation of the known flavonoids biochanin A, dihydrobiochanin A, ferreirin, dalbergioidin, and naringenin, and one new prenylated isoflavanone, dihydrolicoisoflavone [1].

Swartzia polyphylla A.DC. (Leguminosae) is a tree found in the Amazonian region of Peru where the wood is used in construction, and alcohol extracts of the duramen (heartwood) are used in healing (1). A recent report on *S. polyphylla* described the isolation of isoflavanones and their antibacterial activity (2). Several other *Swartzia* species from Africa and South America have been previously investigated, and have yielded molluscicidal steroidal saponins (3,4) and pterocarpans with potential antifungal activity (5–7).

In an evaluation of EtOH extracts of various plants for inhibition of PKC activity, an EtOH extract of the dried, ground heartwood of *Swartzia polyphylla* showed significant inhibition (IC$_{50}$=31 µg/ml) of PKC activity. Protein kinase C (PKC) is a Ca^{+2}- and phospholipid-dependent protein kinase, which is involved in signal transduction, cellular proliferation, and cellular differentiation (8). PKC is activated by diacylglycerol and the tumor-promoting phorbol esters, and it has been suggested that the uncontrolled production of an active form of PKC may promote carcinogenesis. PKC has also been implicated in the *trans*-activation of HIV-1, and depletion of PKC reduces HIV-1 activation without affecting the synthesis of the *tat* protein (9). Some naturally occurring compounds, such as verbascoside (10) and 11-hydroxystauro–sporine (11), which inhibit PKC activity, have also demonstrated antineoplastic activity, indicating that assays for inhibi-

tion of PKC activity may be useful in finding new antineoplastic agents and anti-HIV agents from natural sources. The extract of *S. polyphylla* also demonstrated antibacterial and antifungal activity.

The EtOH extract of *S. polyphylla* was fractionated by standard solvent partitioning and chromatographic techniques, guided by assays for inhibition of protein kinase C, resulting in the isolation of six flavonoids. The major component isolated was the well-known isoflavone, biochanin A. Of the five remaining flavonoids, three were the known isoflavanones (ferreirin, dalbergioidin, and dihydrobiochanin A) and one was the well-known flavanone (naringenin). The sixth compound, however, was a new prenylated isoflavanone, dihydrolicoisoflavone [1].

High-resolution eims of 1 gave a parent ion at m/z 356.1254 [M]$^+$ (C$_{20}$H$_{20}$O$_6$ requires 356.1259), establishing the molecular formula. The ^{1}H-nmr spectrum of 1 was typical of an isoflavanone structure. Two aromatic pro-


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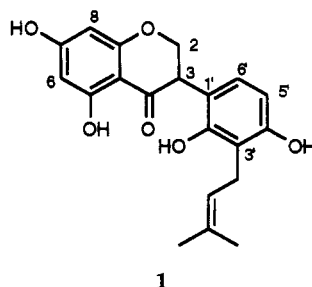
tons resonated at 6.38 ppm (1H, d, J=8.4 Hz) and 6.87 ppm (1H, d, J=8.4 Hz). A COSY experiment, in addition to their coupling constants, indicated that these two protons were ortho to one another and were not coupled to any other protons. A two-proton resonance at 5.91 ppm, due to two overlapping doublets (J=1.4 Hz), could be assigned to the C-6 and C-8 protons of the A ring. The resonances for the C-2 and C-3 protons appeared as three doublets of doublets, integrating for one proton each, at 4.12, 4.54, and 4.64 ppm, characteristic of an isoflavanone structure. Four remaining resonances, a one-proton multiplet at 5.18 ppm, a two-proton doublet at 3.36 ppm, and two methyl singlets at 1.60 ppm and 1.71 ppm, suggested a prenyl moiety. The COSY spectrum confirmed this assignment. The methine proton at 5.18 ppm was coupled to the methylene proton at 3.36 ppm. Both of these resonances also showed coupling to the two methyl resonances at 1.60 ppm and 1.71 ppm. This coupling pattern is indicative of a dimethallyl structure, and the chemical shift of the methylene protons (3.36 ppm) indicated that the prenyl group was bonded to an aromatic ring.

The fragmentation pattern from the mass spectrum confirmed that the dimethallyl moiety was substituted on the C ring. Flavonoids typically fragment in a retro-Diels-Alder fashion. The low-resolution eims exhibited two major fragment ions at m/z 204, due to the C ring with its substituents plus carbons C-2 and C-3, and at m/z 153, due to the A ring. To account for the m/z 204 fragment, the C ring must contain two hydroxyls and the prenyl moiety as substituents.

Combining the ^{1}H-nmr data with data from the mass spectrum, the C ring must be tetrasubstituted, with two hydroxyls, one prenyl group, and the AB ring system. There are five possible permutations for a C ring with two hydroxyls and a prenyl group, which allow

the remaining two protons to be ortho to one another, but only two have a 4' hydroxyl, biogenetically the first site of oxygenation. Therefore, the dimethallyl structure must be substituted on the C-2' or the C-3' position, with the second hydroxyl group occupying the other position.

To confirm the substitution pattern in the C ring, **1** was treated with formic acid at 80° for 25 min to form a dihydropyran moiety from the prenyl group and the adjacent hydroxyls (12). If the second hydroxyl was at C-2' with the prenyl group at C-3', two cyclized products would be expected. If the second hydroxyl was at C-3' with the prenyl group at C-2', only one product would be expected. Two products, **2** and **3**, were formed in this reaction and were identified by ^{1}H-nmr techniques. Both products gave similar ^{1}H-nmr spectra, which included the resonances for the isoflavanone portion of the structure. The major differences, in both cases, were the shifts of the methyl singlets to the region of 1.2–1.3 ppm, the disappearance of the vinyl proton resonance and the appearance of a new, two-proton resonance at 1.7–1.8 ppm, and the shift of the benzylic methylene resonance from 3.36 ppm to 2.6–2.7 ppm, all indicating that both **2** and **3** contained a dihydropyran moi-

2

3
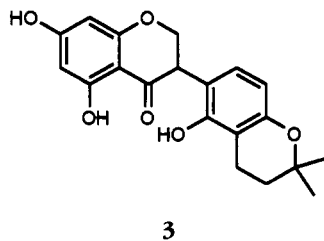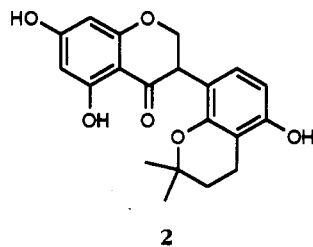
ety. This confirmed the structure of dihydrolicoisoflavone as **1**.

Although all six flavonoids were isolated from active fractions, the only compound active in the protein kinase C inhibition bioassay was biochanin A (using a criterion of an IC_{50} <50 μg/ml for activity). Further investigation of other active fractions from this plant are in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-Nmr spectra were recorded in Me_2CO-d_6 on a General Electric QE-300 spectrometer at 300 MHz (^{1}H) or 75 MHz (^{13}C) using residual Me_2CO as an internal standard. ^{13}C-Nmr assignments were based on literature data (13) and heteronuclear correlated spectra. Ir spectra were measured on a Perkin-Elmer model 1600 Ft-ir spectrometer. Specific rotations were measured on a Perkin-Elmer model 141 polarimeter. Lreims were measured on a Hewlett-Packard model 5988A mass spectrometer and hreims were obtained at Philip Morris USA on a JEOL SX 102/102 four sector tandem mass spectrometer. PKC inhibition assays were carried out at Sphinx Pharmaceuticals Corporation in Durham, North Carolina.

PLANT MATERIAL.—The plant material was collected from the Amazon region of Peru in 1990, by Dr. Franklyn Ayala Flores, Director of the Herbarium Amazonense at the Universidad Nacional de la Amazonia Peruana, where voucher specimens are preserved.

EXTRACTION AND ISOLATION.—The dried, ground heartwood of *Swartzia polyphylla* (1.135 kg) was percolated in a Soxhlet extractor with 11 liters of 95% EtOH for 24 h. The resulting extract was concentrated *in vacuo* to give a dark tar (199.0 g). This material was partitioned between CH_2Cl_2 (3×500 ml) and H_2O (500 ml). The material that would not dissolve in either phase was active, and fractionation of this material by cc, guided by bioassays, led to the isolation of large amounts of biochanin A and small amounts of the other flavonoids. Examination of the CH_2Cl_2-soluble material by tlc showed additional amounts of the minor flavonoids. Thus, 2 g of the CH_2Cl_2 layer were subjected to cc in CH_2Cl_2-CH_3CN-H_2O (90:9.7:0.3). Fractions were examined by tlc, and similar fractions were combined. Those fractions containing flavonoids were subjected to prep. tlc runs using Si gel 60 developed with $MeOH$-CH_2Cl_2 (95:5) to isolate all six flavonoids in amounts sufficient for compound identification. Known flavonoids were identified from eims and ^{1}H-nmr data.

Dihydrolicoisoflavone [1].—Amorphous white solid: $[\alpha]^{20}D$ +157° (c=3.5×10^{-4} g/ml, EtOH); ir (KBr) ν max 3394, 2918, 1638, 1450 cm^{-1}; uv (EtOH) λ max (log ϵ) 225 (4.47), 280 nm (4.32); ^{1}H nmr (Me_2CO-d_6) δ 1.60 (3H, s, Me), 1.71 (3H, s, Me), 3.36 (2H, d, J=6.7 Hz, Ar-CH_2), 4.12 (1H, dd, J=5.0 and 7.5 Hz, H-3), 4.54 (1H, dd, J=5.0 and 11.4 Hz, H-2), 4.64 (1H, dd, J=7.6 and 11.4 Hz, H-2), 5.18 (1H, m, -CH=C(CH$_3$)$_2$), 5.91 (2H, d, J=1.4 Hz, H-6, H-8), 6.38 (1H, d, J=8.4 Hz, H-5'), 6.87 (1H, d, J=8.4 Hz, H-6'); ^{13}C nmr (Me_2CO-d_6) δ 17.5 (CH$_3$), 22.87 (C-1''), 25.47 (C-4''), 46.27 (C-3), 70.60 (C-2), 95.37 (C-8), 96.62 (C-6), 102.80 (C-8a), 108.06 (C-5'), 114.93 (C-3'), 116.60 (C-1'), 123.35 (CH$_2$-CH), 126.64 (C-6'), 131.32 (CH=C), 154.37 (C-2'), 156.04 (C-4'), 163.97 (C-5a), 165.37 (C-5), 167.42 (C-7), 198.09 (C-4); eims m/z 356 [M]$^{+}$ 338, 204, 153; hreims m/z 356.1254 [M]$^{+}$ ($C_{20}H_{20}O_6$ requires 356.1259).

CYCLIZATION OF **1**.—Dihydrolicoisoflavone **1** was heated at 80° for 25 min in HCO_2H (1.0 ml). The mixture was cooled to room temperature and allowed to stand for 2 h. H_2O (5 ml) was added and the solution was extracted with CHCl$_3$ (3×10 ml). The combined CHCl$_3$ layers were washed with aqueous NaHCO$_3$ (5 ml) and H_2O (5 ml), dried over anhydrous Na_2SO_4, and evaporated. The residue was subjected to prep. tlc on Si gel 60 eluted with CH_2Cl_2-CH_3CN-H_2O (90:9.7:0.3) to give 1.1 mg of **2** and 1.4 mg of **3**. ^{1}H nmr of **2** (Me_2CO-d_6) δ 1.21 (3H, s, Me), 1.22 (3H, s, Me), 1.76 (2H, m, CH_2-CH_2Ar), 2.64 (2H, m, CH_2-CH_2Ar), 4.12 (1H, dd, J=5.7 and 10.9 Hz, H-3), 4.35 (1H, dd, J=5.7 and 10.8 Hz, H-2), 4.52 (1H, dd, J=10.9 and 10.9 Hz, H-2), 5.92 (1H, d, J=2.1 Hz, H-8), 5.95 (1H, d, J=2.1 Hz, H-6), 6.35 (1H, d, J=8.2 Hz, H-5'), 6.80 (1H, d, J=8.2 Hz, H-6'). ^{1}H nmr of **3** (Me_2CO-d_6) δ 1.19 (3H, s, Me), 1.27 (3H, s, Me), 1.79 (2H, br t, J=6.8 Hz, CH_2-CH_2Ar), 2.68 (2H, br t, J=6.80 Hz, CH_2-CH_2Ar), 4.20 (1H, dd, J=5.2 and 8.6 Hz, H-3), 4.54 (1H, dd, J=5.2 and 11.2 Hz, H-2), 4.67 (1H, dd, J=8.6 and 11.2 Hz, H-2), 5.94 (2H, br s, H-6, H-8), 6.29 (1H, d, J=8.5 Hz, H-5'), 6.95 (1H, d, J=8.5 Hz, H-6').

ACKNOWLEDGMENTS

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