Cytotoxic Isoflavans from *Eysenhardtia polystachya*

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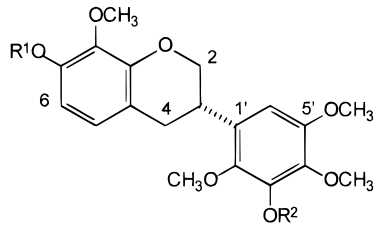
Received December 31, 1997

Two new cytotoxic isoflavans, (3*S*)-7-hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan (**1**) and (3*S*)-3′,7-dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan (**2**), were isolated from the bark and trunks of *Eysenhardtia polystachya* (Leguminosae), together with the known constituents stigmasterol, isoduartin, cuneatin, 7-hydroxy-2′,4′,5′-trimethoxyisoflavone, and 3,4-dimethoxy-8,9-(methylenedioxy)pterocarpan. The structures of **1** and **2** were elucidated on the basis of spectroscopic methods. The antimicrobial, cytotoxic, and insecticidal potential of some of these compounds were evaluated. The isoflavans **1**, **2**, and isoduartin (2′,7-dihydroxy-3′,4′,8-trimethoxyisoflavan) displayed moderate cytotoxic activity against KB cell lines.

Eysenhardtia polystachya (Ortega) Sarg. (Leguminosae: Lotoideae) is a small tree distributed throughout Mexico and Southern Texas[1] that has been used in Mexican traditional medicine as an herbal remedy for centuries.[2] Locally, it is known as "palo dulce" (sweet wood), "palo azul" (blue wood), "tlapalezpatli" (in Náhuatl language), "urza" (in Otomí language), among other common names,[3] and it has wide use for the treatment of kidney and bladder infections and as a diuretic.[4] It is also used as an antispasmodic and febrifuge.[5] During the sixteenth and seventeenth centuries, the wood of this plant was exported from Mexico to Europe, where it was known as *Lignum nephriticum*.[3,6] Infusions of the wood in water display a golden color with a bluish fluorescence, and vessels and cups made from this material were used to keep drinking water.[3] Interestingly, Robert Boyle used an aqueous extract of this wood as an acid−base indicator in the seventeenth century,[6,7] and an isoflavone has been identified as one of the fluorescent constituents.[6] Previous chemical examination of this species led to the isolation of 3,4-dimethoxy-8,9-(methylenedioxy)pterocarpan and dehydrorotenone,[8] two C-glucosyl-α-hydroxydihydrochalcones,[9] 7-hydroxy-2′,4′,5′-trimethoxyisoflavone and 9-methoxy-2,3-methylenedioxycoumestan.[6]

As part of our ongoing search for biologically active principles,[10] we describe herein the isolation and structural elucidation of two new isoflavans, (3*S*)-7-hydroxy-2′,3′,4′,5′,8 -pentamethoxyisoflavan (**1**) and (3*S*)-3′,7-dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan (**2**), and five known compounds, stigmasterol, isoduartin,[11] cuneatin,[12] 7-hydroxy-2′,4′,5′-trimethoxyisoflavone[6] and 3,4-dimethoxy-8,9-(methylenedioxy)pterocarpan.[8,13] The structures of **1** and **2** have been determined on the basis of detailed ^{1}H and ^{13}C NMR and NOE studies, including 2D experiments (^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC). The known compounds were identified by comparing their spectroscopic data with those reported. The isolates were evaluated for their antimicrobial, cytotoxic, and insecticidal activities.



	R^1	R^2
1	H	CH_3
2	H	H
3	CH_3CO	CH_3
4	CH_3CO	CH_3CO

Results and Discussion

Compound **1**, obtained as an amorphous solid, was optically active $[\alpha]_D$ +3.12 (*c* 0.320, MeOH). Its molecular formula $C_{20}H_{24}O_7$ was deduced from the $[M]^+$ ion peak at *m*/*z* 376 in the EIMS and from elemental analysis. Its IR spectrum was devoid of carbonyl absorption, and the band at 3530 cm^{-1} indicated the presence of a single hydroxyl group in the structure. Acetylation of **1** afforded the monoacetyl derivative **3**.

The ^{1}H NMR spectrum of **1** showed signals at δ 2.90 (1H, ddd, J = 16.0, 5.5, 1.0 Hz), 2.96 (1H, ddd, J = 16.0,

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S0163-3864(97)00586-7 CCC: $15.00
Published on Web 06/04/1998

Table 1. ^{13}C NMR Data (125 MHz, $CDCl_3$) for Compounds **1** and **2**[a]

C	1	2
2	70.28	70.02
3	31.84	31.19
4	31.32	31.26
4a	115.00	114.86
5	124.20	123.97
6	107.10	107.20
7	147.60	147.41
8	134.80	134.77
8a	147.12[b]	146.95
1′	128.90	129.59
2′	145.50	144.41
3′	147.15[b]	145.42
4′	141.95	138.99
5′	149.67	146.00
6′	105.23	107.61
OCH_3 C-8	60.90	60.53
OCH_3 C-2′	61.51	61.25
OCH_3 C-3′	61.00	
OCH_3 C-4′	61.89	60.79
OCH_3 C-5′	56.25	60.40

[a] Assignments were confirmed by APT, NOE, HMQC, and HMBC experiments (δ values). [b] Signals may be interchangeable.

10.5, 1.0 Hz), 3.61 (1H, dddd, J = 10.5, 10.5, 5.5, 3.5 Hz), 4.05 (1H, dd, J = 10.5, 10.5), and 4.39 (1H, ddd, J = 10.5, 3.5, 1.0 Hz) assigned to the $-CH_2CH(Ar)CH_2O-$ moiety of an isoflavan skeleton.[14−16] This skeleton was further supported by its UV spectrum (λ_{max} at 218 and 284 nm), ^{13}C NMR data (Table 1, which showed peaks for C-2, C-3, and C-4 at δ 70.28, 31.84, and 31.32, respectively), and $^1H-^1H$ COSY cross-peaks (which showed correlations between H-2/H-3 and between H-3/H-4). The ^1H NMR spectrum of **1** also revealed three aromatic hydrogen signals at δ 6.72 (1H, dd, J = 8.5, 1.0 Hz), 6.53 (1H, d, J = 8.5 Hz), and δ 6.40 (1H, s), in addition to five aromatic methoxyl signals at δ 3.95, 3.92, 3.89, 3.83, and 3.79 (3H, each s). These data indicated that **1** has tetra- and pentasubstituted benzene rings. The EIMS exhibited peaks at m/z 376 $[M]^+$, 224 (100), 209 (42), 152 (16), and 121 (14). The peaks at m/z 224 (B-ring) and 152 (A-ring), which resulted from RDA cleavage of **1**, clearly showed that the ring B has four methoxyl groups and the ring A has one hydroxyl and one methoxyl group.[17]

Cross-peaks were observed in the HMBC spectrum between the methylene protons at C-2 (δ 4.05 and 4.39) and the oxygenated quaternary carbon at δ 147.12, assigned to C-8a. The carbon-bearing the methoxyl (δ 134.8) in ring A was assigned to C-8 by its three-bond correlation with H-6 (δ 6.53). The signal at δ 6.72 was assigned to H-5, since it presented three-bond interactions with C-4, C-8a, and C-7. These observations, also supported by the observed enhancement of H-5 when the methylene on C-4 was irradiated in a NOE difference experiment, indicated that isoflavan **1** has a 7-hydroxy-8-methoxy-substituted A-ring, similar to that of isoduartin (2′,7-dihydroxy-3′,4′,8-trimethoxyisoflavan).[11]

The singlet aromatic proton at δ 6.40 on ring B showed HMBC correlations with the methoxyl-bearing carbons at δ 141.95 (C-4′), 145.50 (C-2′), and 149.67 (C-5′), and with the benzylic carbon at δ 31.84 (C-3), confirming the 2′,3′,4′,5′-tetramethoxy substitution pattern on the B ring. Identification of the NOE interactions between H-6′ and the methoxyl group at δ 3.78, linked to C-5′ (at δ 149.6, by HMBC); between the methoxyl group at δ 3.89, linked to C-2′ (δ 145.50, by HMBC) and the methoxyl group at δ 3.94, linked to C-3′ (at δ 147.12, by HMBC) allowed us to assign the signals.

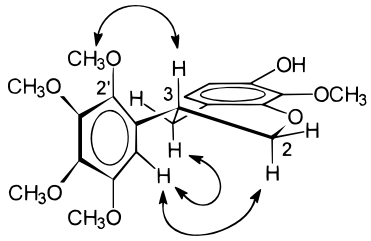

Figure 1. Preferred conformation of 1 and selected NOESY interactions.

The magnitude of the vicinal couplings between H-2α/H-3β and H-3β/H-4α ($J_{2\alpha,3\beta} = J_{3\beta,4\alpha} = 10.5$ Hz) indicated an equatorial orientation of the 3-aryl group, and the pseudochair conformation of the dihydropyran ring.[18] This conformation was in agreement with the observed long-range W-coupling between H-2β and H-4β (J = 1.0 Hz). Furthermore, NOESY interactions between H-6′/H-2α and H-6′/H-4α, as well as the interaction between H-3 and the methoxyl hydrogens at C-2′, established a preferred conformation with the methoxyl at C-2′ and H-3 in a syn spatial relationship, as depicted in Figure 1.

Finally, the CD curve of **1** in MeOH showed a negative Cotton effect in the 260−290 nm region, indicating its 3*S* configuration as previously discussed.[18−20] Thus, **1** was characterized as (3*S*)-7-hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan.

The more polar compound **2** differed from **1** by the absence of a methyl group and by the presence of an additional hydroxyl group. Acetylation of **2** afforded the diacetyl derivative **4**. Compound **2** was spectroscopically very similar to **1**, having the same structural features and the oxidation pattern on A and B rings of the isoflavan nucleus. The ^1H NMR data of **2** also showed the signals corresponding to the $-CH_2CH(Ar)-CH_2O-$ moiety at δ 2.83 (2H, m, H-4), 3.54 (1H, m, H-3), 3.94 (1H, t, J = 10.5 Hz, H-2α), and 4.35 (1H, ddd, J = 10.5, 3.0, 1.5 Hz, H-2β); three aromatic hydrogens at δ 6.66 (1H, dd, J = 8.5, 1.0 Hz), 6.49 (1H, d, J = 8.5 Hz) and 6.46 (1H, s); and a two proton broad singlet at δ 5.75, which suggested the presence of two phenolic hydrogens. The presence of four methoxyl groups was also shown by signals at δ 3.81 (3H, s), 3.88 (3H, s), 3.90 (3H, s) and 3.93 (3H, s). The MS of **2** exhibited the molecular ion at m/z 362 and the diagnostic peaks of the RDA cleavage at m/z 152 and 210, indicating that the A ring has the same substituents as **1**, while the B ring has three methoxyl and one hydroxyl groups.[14]

The carbon resonances of **2** were assigned by its HMQC, HMBC, and NOESY spectra (Table 1). The HMBC spectrum revealed cross-peaks between H-5 (δ 6.66) and C-4, C-7, and C-8a; between H-6 (δ 6.49) and C-4a and C-8; and between H-6′ and C-3, C-2′, and C-4′. In the NOESY spectrum of **2**, the aromatic singlet at H-6′ showed correlation with the methoxyl group at δ 3.93, which in turn correlated with the methoxyl group at δ 3.90, permitting the carbon resonances at δ 146.00 and 138.99 to be assigned to C-5′ and C-4′, respectively

(HMBC). These data confirmed that the oxidation pattern of **2** is similar to that of **1**, that the hydroxyl group on B ring is attached to C-3′, and that three methoxyl groups are attached to C-2′, C-4′, and C-5′. The specific rotation value of **2**, $[\alpha]_D$ +6.5 (*c* 0.2, MeOH), and the negative Cotton effect in the 260−290 nm region of its CD curve,[18−20] similar to that observed for **1**, indicated a 3*S* configuration. Thus, the structure of this compound was confirmed to be (3*S*)- 3′,7-dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan.

The natural substances **1**, **2**, isoduartin (2′,7-dihydroxy-3′,4′,8-trimethoxyisoflavan),[11] cuneatin (7-hydroxy-2′-methoxy-4′,5′-(methylendioxy)isoflavone),[12] 7-hydroxy-2′,4′,5′-trimethoxyisoflavone,[6] and 3,4-dimethoxy-8,9-(methylenedioxy)pterocarpan,[8,13] isolated from this population of *E. polystachya*, did not display antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus aureus*, *Bacillus subtilis*, *Shigella sonnei* or *Candida albicans* at concentrations up to 200 μg/mL. Although these preliminary results do not tend to support the use of the plant as an antiinfective agent, more assays are needed to evaluate the antimicrobial potential of the isolated metabolites. The above-mentioned compounds were also tested for their cytotoxic activity against P388, UISO, and KB tumor cells, and **1**, **2**, and isoduartin (2′,7-dihydroxy-3′,4′,8-trimethoxyisoflavan) exhibited moderate cytotoxic activity against the KB cell line (ED_{50} values of 3.8, 3.0, and 2.63 μg/mL, respectively). No cytotoxicity was shown by the additional natural compounds ($ED_{50} > 4$ μg/mL). In the insecticidal bioassay, the isoflavans **1**, **2**, and isoduartin did not display toxicity against the *Spodoptera frugiperda* larvae ($LC_{50} > 1000$ ng/cm^2); however, some morphological changes were observed.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 and Varian Unity Plus-500 instruments, and the chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Samples for NOE experiments were degassed and sealed under argon. Standard pulse sequences were used for COSY, NOEdiff, DEPT, HMQC, and HMBC experiments. Infrared spectra were recorded with a Nicolet Magna IR TM 750 and Perkin-Elmer 283B instruments. MS data were recorded with a JEOL JMS-AX 505 HA mass spectrometer. Electron impact mass spectra (EIMS) were obtained at 70 eV ionization energy. CD spectra were measured with a JASCO J-720 instrument in MeOH flushed with N_2. TLC: Merck silica gel 60 F254 plates (0.25 mm), spots and bands were detected by UV irradiation (254 and 365 nm).

Plant Material. The bark and trunks of *E. polystachya* were collected near Xalapa, Veracruz, México, and identified by Profs. Clara H. Ramos and Esteban M. Martínez. Voucher specimens (no. CHR 739) are deposited at the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation. The air-dried and finely powdered bark and trunks of *E. polystachya* (3.2 kg) were extracted with $CHCl_3$−MeOH (1:1) three times at room temperature. Filtration and removal of solvent in vacuo afforded 195 g of crude extract, which was chromatographed on a silica gel column (800 g) eluting with a *n*-hexanes−EtOAc gradient system and then with acetone to give six main fractions (F-1−F-6). F-1 (3.4 g) mainly contained fatty materials, while the last fraction (F-6, eluted with acetone, 56 g) was a complex mixture. F-2 (4.6 g) was rechromatographed with *n*-hexanes−EtOAc gradient to give stigmasterol (70 mg). F-3 (2.28 g) was rechromatographed on silica gel, using *n*-hexane−EtOAc gradient elution system, to give **1** (133 mg), **2** (190 mg), and 2′,7-dihydroxy-3′,4′,8-trimethoxyisoflavan (isoduartin, 65 mg, mp 105−108 °C [lit.[11] oil]). F-4 (4.5 g) was rechromatographed on silica gel CC and eluted with *n*-hexanes−EtOAc gradient to afford 3,4-dimethoxy-8,9-(methylenedioxy)pterocarpan (112 mg), mp 253−254 °C [lit.[13] 245−247 °C]. F-5 (4.6 g) was purified by silica gel column chromatography (*n*-hexanes−EtOAc, 1:1), fraction 5 from this rechromatography afforded 7-hydroxy-2′-methoxy-4′,5′-(methylendioxy)-isoflavone (cuneatin,[12] 23 mg) [mp 236−237 °C; IR, ^{1}H NMR and MS data of cuneatin were not previously reported,[12] but were in agreement with its structure: IR ($CHCl_3$) ν_{max} 3500, 2927, 2852, 1729, 1624, 1464, 1378, 1275, 1247 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO, 200 MHz) δ 10.08 (1H, s, O*H*), 8.10 (1H, d, J = 8.7 Hz, H-5), 7.92 (1H, s, H-2), 6.85 (1H, d, J = 2.3 Hz, H-8), 6.83 (1H, dd, J = 8.7, 2.3, H-6), 6.79 (1H, d, 1.0, H-6′), 6.72 (1H, d, J = 1.0 Hz, H-3′), 5.90 (2H, s, OC*H*$_2$O), 3.93 (3H, s, OC*H*$_3$); EIMS *m*/*z* (rel int) 312 [M]$^+$ (100), 176 (24), 131 (15), 70 (16), 63 (18)], and fractions 12−14 gave 7-hydroxy-2′,4′,5′-trimethoxyisoflavone (183 mg): mp 239−240 °C [lit.[6] mp 234−237 °C].

(3*S*)-7-Hydroxy-2′,3′,4′,5′,8-pentamethoxyisoflavan (1): amorphous powder; mp 125−126 °C; R_f 0.46 (*n*-hexanes−EtOAc 7:3); $[\alpha]_D$ +3.12 (c 0.320, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.91), 284 (2.89) nm; CD (*c* 0.0136 mg mL^{-1}, MeOH): $[\theta]_{210}$ −2.699, $[\theta]_{226}$ 0.4130, $[\theta]_{256}$ −0.8567, $[\theta]_{265.5}$ −0.6865; IR ($CHCl_3$) ν_{max} 3530, 2939, 2840, 1603, 1494, 1193, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.72 (1H, dd, $J_{5,6}$ = 8.5, $J_{4\alpha,5}$ = 1.0 Hz, H-5), 6.53 (1H, dd, $J_{5,6}$ = 8.5 Hz, H-6), 6.40 (1H, s, H-6′), 5.80 (1H, brs, O*H*), 4.39 (1H, ddd, $J_{2\beta,2\alpha}$ = 10.5, $J_{2\beta,3\beta}$ = 3.5, $J_{2\beta,4\beta}$ = 1.0 Hz, H-2β), 4.05 (1H, dd, $J_{2\beta,2\alpha}$ = $J_{2\alpha,3\beta}$ 10.5 Hz, H-2α), 3.95 (3H, s, C*H*$_3$O−C-3′), 3.92 (3H, s, C*H*$_3$O−C-8), 3.89 (3H, s, C*H*$_3$O−C-4′), 3.83 (3H, s, C*H*$_3$O−C-2′), 3.79 (3H, s, C*H*$_3$O−C-5′), 3.61 (1H, dddd, J = 10.5, 3.5, 5.5, 10.5 Hz, H-3), 2.96 (1H, ddd, $J_{4\alpha,4\beta}$ = 16.0, $J_{4\alpha,3\beta}$ = 10.5, $J_{4\alpha,5}$ = 1.0 Hz, H-4α), 2.90 (1H, ddd, J = 16.0, $J_{3\beta,4\beta}$ = 5.5, $J_{2\beta,4\beta}$ = 1.0 Hz, H-4β); ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; EIMS *m*/*z* (rel int) 376 [M]$^+$ (73), 224 (100), 209 (42), 152 (16), 151 (38), 121 (14); *anal.* C 63.65%, H 6.68%, calcd for $C_{20}H_{24}O_7$, C 63.82%, H 6.43%.

(3*S*)-3′,7-Dihydroxy-2′,4′,5′,8-tetramethoxyisoflavan (2): amorphous powder; mp 97−98 °C; R_f 0.40 (*n*-hexanes−EtOAc 7:3); $[\alpha]_D$ +6.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (3.91), 281 (3.75) nm; CD (*c* 0.006 mg mL^{-1}, MeOH) $[\theta]_{212}$ −8.2175, $[\theta]_{233}$ −2.2300, $[\theta]_{256}$ −0.0800, $[\theta]_{283}$ −0.3197; IR ($CHCl_3$) ν_{max} 3530, 2939, 2840, 1603, 1494, 1193, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.66 (1H, dd, $J_{5,6}$ = 8.5, $J_{4\alpha,5}$ = 1.0 Hz, H-5), 6.49 (1H, d, $J_{5,6}$ = 8.5 Hz), 6.46 (1H, s, H-6′), 5.75 (2H, s, O*H*), 4.35 (1H, ddd, $J_{2\alpha,2\beta}$ = 10.5 Hz, $J_{2\beta,3\beta}$ = 3.0, $J_{2\beta,4\beta}$ = 1.5 Hz, H-2β), 3.94 (1H, dd, $J_{2\alpha,2\beta}$ = $J_{2\alpha,3\beta}$ = 10.5 Hz, H-2α), 3.93 (3H, s, CH$_3$O-C-5′), 3.90 (3H, s,

CH_3O-C-4′), 3.88 (3H, s, CH_3O-C-8), 3.81 (3H, s, CH_3O-C-2′), 3.54 (1H, m, H-3), 2.83 (2H, m, H-4); ^{13}C NMR (125 MHz, $CDCl_3$) see Table 1; EIMS *m*/*z* (rel int) 362 $[M]^+$ (47), 210 (100), 195 (47), 183 (58), 152 (30), 138 (10), 123 (20).

(3*S*)-2′,3′,4′,5′,8-Pentamethoxy-7-*O*-acetylisoflavan (3). Acetylation of **1** (45 mg) with Ac_2O (1 mL) and pyridine (0.5 mL) at room temperature for 2 h afforded **3** (23.8 mg) after usual workup and TLC purification (*n*-hexanes−EtOAc 85:15). **3**: mp 79−80 °C; R_f 0.31 (*n*-hexanes−EtOAc 7:3); IR ($CHCl_3$) 2939, 1760, 1491, 1238, 1047 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.81 (1H, d, J = 8.4 Hz, H-5), 6.59 (1H, d, J = 8.4 Hz, H-6), 6.31 (1H, s, H-6′), 4.44 (1H, ddd, $J_{2\alpha,2\beta}$ = 10.5 Hz, $J_{2\beta,3\beta}$ = 3.5, $J_{2\beta,4\beta}$ = 1.5 Hz, H-2β), 4.07 (1H, dd, $J = J_{2\alpha,2\beta}$ = 10.5 Hz, $J_{2\alpha,3\beta}$ = 10.5 Hz, H-2α), 3.95 (3H, s, CH_3O), 3.88 (3H, s, CH_3O), 3.86 (3H, s, CH_3O), 3.84 (3H, s, CH_3O), 3.79 (3H, s, CH_3O), 3.65 (1H, m, H-3), 3.01 (2H, m, H-4), 2.31 (3H, s, CH_3CO); EIMS *m*/*z* (rel int) 418 $[M]^+$ (65), 416 (78), 403 (35), 488 (55), 375 (80), 224 (100), 197 (81), 184 (85), 43 (80).

(3*S*)-2′,4′,5′,8-Tetramethoxy-3′,7-*O*-diacetylisoflavan (4). Compound **2** (65 mg) was acetylated with Ac_2O (1.5 mL) and pyridine (0.5 mL) to afford **4** (39 mg) as an amorphous solid. **4**: mp 56−58 °C; R_f 0.22 (*n*-hexanes−EtOAc 7:3); IR ($CHCl_3$) 2938, 1762, 1488, 1462, 1370, 1090, 1049 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.81 (1H, d, J = 8.3 Hz, H-5), 6.59 (1H, d, J = 8.3 Hz, H-6), 6.55 (1H, s, H-6′), 4.42 (1H, ddd, $J_{2\alpha,2\beta}$ = 10.2 Hz, $J_{2\beta,3\beta}$ = 3.8 Hz, $J_{2\beta,4\beta}$ = 1.5 Hz, H-2β), 3.98 (1H, dd, $J_{2\alpha,2\beta} = J_{2\alpha,3\beta}$ = 10.2 Hz, H-2α), 3.93 (3H, s, CH_3O), 3.88 (6H, s, 2CH_3O), 3.85 (3H, s, CH_3O), 3.61 (1H, m, H-3′), 2.96 (2H, m, H-4), 2.33 (3H, s, CH_3CO), 2.31 (3H, s, CH_3CO); EIMS *m*/*z* (rel int) 446 $[M]^+$ (70), 444 (68), 431 (80), 416 /75), 414 (70), 386 (81), 252 (100), 194 (85), 43 (90).

Evaluation of Biological Activity. Cytotoxic Activity. The KB (nasopharingeal carcinoma), P388 (murine leukemia), and SQC-1 UISO (Squamous cell cervix carcinoma) cell lines were maintained in RPMI culture medium with 10% fetal bovine serum (FBS). All cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at a log phase of their growth cycle were treated in triplicate at various concentrations of the natural compounds (0.5−100 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by protein analysis. Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percent of viable cells.[21]

Antimicrobial Activity. Evaluations were performed with cultures of *E. coli* (ATCC 8937), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *S. sonnei* (ATCC 11060), and *C. albicans* (ATCC 10231). The bacteria were maintained in trypticase soy agar (TSA), and the yeast on Sabourand's dextrose agar (SDA). The screening method was performed in duplicates and based on disk assay procedures.[22]

Insecticidal Activity. This activity was tested by ingestion of solutions of the compounds **1**, **2**, and isoduartin by target insect neonate larvae following the artificial diet feeding.[23] We used neonate larvae of the fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), an important pest in Mexico. The concentrations used ranged from 100 to 1000 ng/cm^2 of surface diet. The experiments were run in triplicate. Concentration−response curves were analyzed by the log dose/probit method to determine the doses that kill half of the organisms (LC_{50}).

Acknowledgment. We thank Rocío Patiño, Luis Velasco, and Javier Pérez-Flores (Instituto de Química de la UNAM) and Atilano Gutiérrez (Universidad Autónoma Metropolitana) for technical assistance. We also thank Dr. Masaaki Shibuya (Faculty of Pharmaceutical Sciences, University of Tokyo) for spectroscopic data of isoduartin and Profs. Clara H. Ramos (Facultad de Ciencias, UNAM) and Esteban M. Martínez (Instituto de Biología, UNAM) for collection and identification of the plant material. This work was supported in part by UNAM (PADEP project No. 5373 and DGAPA-IN 212195) and CONACyT (Project No. 3419P-N9607 and Grant No. 940040).

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NP970586B