Isoflavonoids from *Erythrina poeppigiana*: Evaluation of Their Binding Affinity for the Estrogen Receptor[⊥]

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Five new isoflavones, named 5,4′-dihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)isoflavone (**1**), 5,2′,4′-trihydroxy-7-methoxy-5′-(3-methylbuten-2-yl)isoflavone (**2**), 5,4′-dihydroxy-7-methoxy-3′-(3-methyl-2-hydroxybuten-3-yl)isoflavone (**3**), 3′-formyl-5,4′-dihydroxy-7-methoxyisoflavone (**4**), and 5-hydroxy-3″-hydroxy-2″,2″-dimethyldihydropyrano[5″,6″:3′,4′]isoflavone (**5**), as well as six known compounds, wighteone (**6**), 3′-isoprenylgenistein (**7**), isolupabigenin (**8**), alpinumisoflavone (**9**), erypoegin D (**10**), and crystacarpin (**11**), were isolated from *Erythrina poeppigiana.* The structures of the isolated compounds were elucidated on the basis of chemical and spectroscopic analysis. The affinity of these compounds for the estrogen receptors ERα and ER*β* was evaluated using a receptor binding assay. While isoprenyl and dimethylpyrano substituents in ring A reduced the affinity of binding to ER*β* ca. 100-fold compared to genistein, the isoprenyl substituent in ring B was better accommodated, allowing **7** to bind with ca. 10-fold lower affinity than genistein.

Erythrina (Fabaceae) is a pantropical genus consisting of some 113 species.[1] Many *Erythrina* species have been reported as being of use in the folk empirical system of medicine as an abortive agent[2] and for the treatment of various diseases, including amenorrhea,[2] malaria,[3] microbial infections,[4] jaundice,[5] agitation,[6] insomnia,[6] unspecified infections,[7] and inflammation.[8] The published data connecting *Erythrina* species with menopause-related illnesses refer mostly to the activity of its different total extracts,[9−12] while the data reporting the estrogenicity of their constituents are limited.[13]

Erythrina poeppigiana (Walp.) O.F. Cook[14] is a large tree distributed in South America, Africa, and Asia that grows up to 25 m high and 1 m in diameter and has red flowers and a gray-brown bark. Previous phytochemical analysis of this plant has revealed the presence of erythrina alkaloids[15] and isoflavonoids.[16] In particular, it is rich in isoflavones,[17] coumestans,[18] arylbenzofurans,[19] and specifically isoprenylated derivatives thereof.[20−22] However, most of the previous phytochemical studies have concerned plant populations that originated from Asia, in contrast with the present study dealing with plant material from Bolivia in South America.

Isoflavones, coumestans, and arylbenzofurans have been reported to bind to the two isotypes of estrogen receptor (ER), alpha (ERα) and beta (ER*β*), and to exhibit estrogenic or antiestrogenic properties.[23−25] The estrogenic activity of the isoflavone genistein, in particular, has been well documented, and the molecular determinants of its high binding affinity and selectivity for ER*β* have been studied in detail.[26−28] Hydrogen bond formation between the phenolic OH-4′ and Glu-305 and Arg-346 (Glu-353 and Arg-394 in ERα), the presence of a second hydroxy (OH-7) at a distance of 12.1 Å (11.8 Å in 17*β*-estradiol) that allows for hydrogen bond formation between OH-7 and His-475 (His-524 in ERα), and the presence of an OH-5 reportedly provide for the much higher affinity and selectivity of genistein for ER*β* compared to daidzein (which lacks the OH-5).[24,26,27] Various isoprenylated derivatives of isoflavones, coumestans, and arylbenzofurans were also found to bind to the ER and to exhibit estrogenic or antiestrogenic properties.[24,25,29]

Results and Discussion

Aiming to discover novel estrogenic natural products based on long-established traditional knowledge, phytochemical investigation of *E. poeppigiana* collected in Bolivia was undertaken. In the present study, five new isoflavones with methyl ether groups at C-7 were isolated from the stem bark of *E. poepigiana* and structurally characterized. These are 5,4′-dihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)isoflavone (**1**), 5,2′,4′-trihydroxy-7-methoxy-5′-(3-methylbuten-2-yl)isoflavone (**2**), 5,4′-dihydroxy-7-methoxy-3′-(3-methyl-2-hydroxybuten-3-yl)isoflavone (**3**), 3′-formyl-5,4′-dihydroxy-7-methoxyisoflavone (**4**), and 5-hydroxy-7-methoxy-(3″,4″-dihydro-3″-hydroxy)-2″,2″-dimethylpyrano[5″,6″:3′,4′]isoflavone (**5**). The new isoflavones were isolated together with the known compounds wighteone (**6**),[30] 3′-isoprenylgenistein (**7**),[31] isolupabigenin (**8**),[32] alpinumisoflavone (**9**),[33] erypoegin D (**10**),[34] and crystacarpin (**11**).[35] The structures of the compounds were determined by interpretation of their spectroscopic data. It is worth noting that the Bolivian collection seems to be richer in isoflavone C-7 methyl ethers compared to the Asian collections, which may be of chemotaxonomic value.

Compound **1** is the 7-methyl ether of 3′-isoprenylgenistein, was isolated as an amorphous, yellow solid, and exhibited a UV spectrum characteristic of an isoflavone[36] with a maximum at 264 nm. The APCIMS of compound **1** exhibited a pseudomolecular ion peak at *m/z* 353 $[M + 1]^+$, and its molecular formula was determined as $C_{21}H_{20}O_5$ by HREIMS. The ^{1}H NMR spectrum indicated the presence of an ABX system consisting of a multiplet (H-3′ and H-6′) at 7.22 ppm and a doublet (H-5′, $J = 8.8$, Hz) at 6.86 ppm. The carbon atoms of the aforementioned protons were found to resonate at 128.2 (C-6′), 115.9 ppm (C-5′), and 130.5 ppm (C-2′), respectively. Two additional signals were observed in the ^{1}H NMR spectrum at 6.38 (H-6) and 6.40 ppm (H-8), while C-6 and C-8 resonated at 98.2 and 92.4 ppm, respectively (HMQC spectrum). Also, the characteristic singlet of the deshielded H-2 at 7.85 ppm was observed with the corresponding carbon atom resonating at 152.7 ppm. The methoxy protons were observed as a singlet at 3.87 ppm and the corresponding carbon atom at 55.8 ppm.

[⊥] This paper is dedicated to the memory of our late colleague Prof. Zacharias Tanee Fomum.

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Chart 1

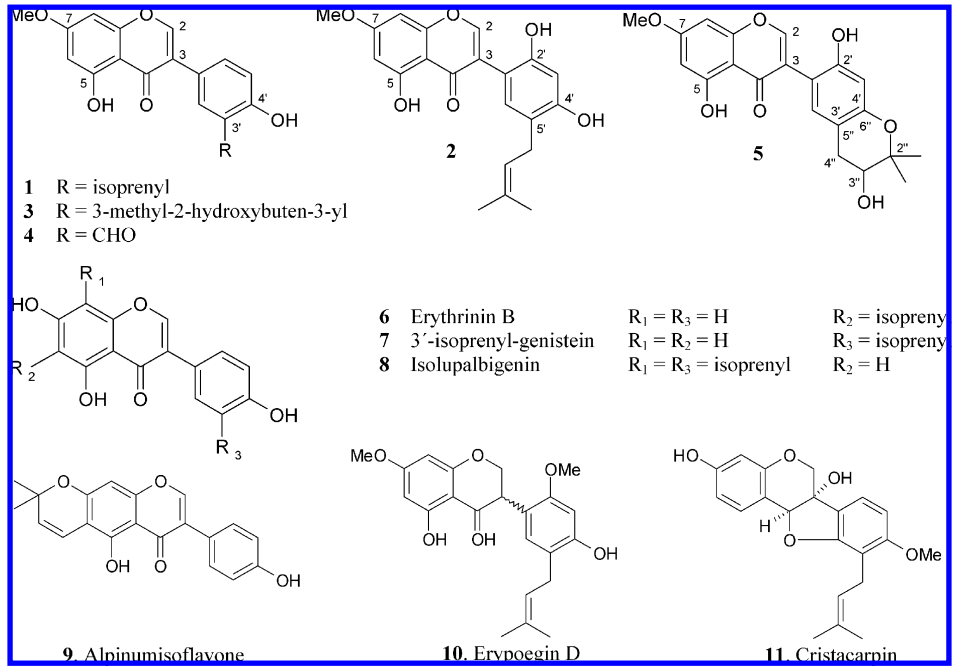

Table 1. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds **1**−**5**

	δ_H (J in Hz)				
position	**1**	**2**	**3**	**4**	**5**
2	7.85, s	7.98, s	7.85, s	7.93, s	7.85, s
3					
4					
4α					
5					
6	6.38, d (2.2)	6.45, d (2.0)	6.38, d (2.3)	6.44, d (2.0)	6.38, d (1.9)
7					
8	6.40, d (2.2)	6.48, d (2.0)	6.40, d (2.3)	6.41, d (2.0)	6.40, d (1.9)
8α					
1′					
2′	7.22, m		7.26, d (2.3)	7.83, d (2.0)	7.28, m
3′		6.56, s			
4′					
5′	6.86, d (8.8)		7.00, d (8.2)	7.08, d (8.4)	6.89, d (8.5)
6′	7.22, m	6.86, s	7.28, dd (8.2, 2.3)	7.67, dd (8.4, 2.0)	7.23, m
1″	3.39, d (7.0)	3.31, d (7.3)			
1″α			3.03, dd (14.9, 8.9)		
1″β			2.83, dd (14.9, 2.0)		
2″	5.34, t (7.0)	5.30, t (7.3)	4.44, d (8.9)		
Me-2″					1.39, s
					1.34, s
3″					3.84, m
4″	1.77, s	1.77, s			
4″α			5.03, s		3.13, dd (17.0, 2.4)
4″β			4.90, s		2.84, dd (17.0, 3.5)
5″	1.78, s	1.77, s	1.84, s		
6″					
OCH_3	3.87, s	3.90, s	3.87, s	3.89, s	3.87, s
CHO				9.96, s	
OH-5	12.88, s	12.31	12.87	12.68	12.84
OH-4′	5.30, s		5.30	11.11	

The HMBC spectrum revealed a correlation of the methoxy protons with C-7 (^3J), confirming its position (Table S1, Supporting Information). The isoprenyl side chain was established by the appearance of a methine group at 5.34 ppm (H-2″, J = 7.0 Hz), which appeared as a triplet and displayed a ^{1}H−^{1}H COSY correlation to the downfield methylene at 3.39 ppm (H-1″, J = 7.0 Hz), and long-range correlations with two methyl groups at 1.77 (H-4″) and 1.78 ppm (H-5″). The ^{13}C, HMQC, and HMBC NMR spectra confirmed the presence of this specific side chain (Table 2). In addition, the HMBC spectrum revealed the correlations of H-1″ with C-3′ and C-4′, thus indicating the position of the isoprenyl moiety on the basic skeleton (Table S1, Supporting Information). Hence, the structure of **1** was assigned as 5,4′-dihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)isoflavone.

Compound **2** was also isolated as an amorphous, yellow solid with UV maxima at 264 and 310 nm. The ESIMS showed a pseudomolecular ion peak at m/z 369 $[M + 1]^+$, and its molecular formula was calculated as $C_{21}H_{20}O_6$. The NMR spectra of **2** were typical of an isoflavone trisubstituted in the B ring. In the ^{1}H NMR spectrum were signals corresponding to the H-6 (δ 6.45, J = 2.0

Table 2. ^{13}C NMR Spectroscopic Data (150 MHz, $CDCl_3$) for Compounds **1**−**5**

	δ_C, mult.				
position	**1**	**2**	**3**	**4**	**5**
2	152.7, CH	155.0, CH	152.7, CH	152.6, CH	152.5, CH
3	123.9, qC	123.4, qC	123.8, qC	122.3, qC	123.2, qC
4	180.9, qC	182.0, qC	180.9, qC	180.2, qC	180.8, qC
4α	106.3, qC	105.6, qC	106.2, qC	105.5, qC	106.2, qC
5	162.7, qC	162.3, qC	162.7, qC	162.4, qC	162.6, qC
6	98.2, CH	98.9, CH	98.2, CH	98.1, CH	98.2, CH
7	165.5, qC	166.2, qC	165.5, qC	165.2, qC	165.5, qC
8	92.4, CH	92.5, CH	92.4, CH	92.4, CH	92.4, CH
8α	157.9, qC	157.8, qC	157.9, CH	157.7, qC	157.9, qC
1′	122.7, qC	111.9, qC	122.5, qC	122.3, qC	122.8, qC
2′	130.5, CH	155.5, CH	132.0, CH	134.1, CH	130.7, CH
3′	127.1, qC	106.8, qC	125.8, qC	120.4, qC	118.9, qC
4′	154.7, qC	156.5, qC	156.2, qC	161.5, qC	153.1, qC
5′	115.9, CH	120.0, CH	117.6, CH	117.9, CH	117.6, CH
6′	128.2, CH	130.7, qC	128.9, CH	137.2, CH	128.3, CH
1″	29.8, CH_2	28.9, CH_2	38.1, CH_2		
2″	121.5, CH	121.8, CH	78.3, CH		69.5, qC
Me-2″					22.2, CH_3
					24.6, CH_3
3″	135.0, qC	134.8, qC	146.5, qC		77.0, CH
4″	25.8, CH_3	25.8, CH_3	111.4, CH_2		31.3, CH_2
5″	17.9, CH_3	17.9, CH_3	18.1, CH_3		
6″					
OCH_3	55.8, CH_3	55.9, CH_3	55.8, CH_3	55.8, CH_3	55.8, CH_3
CHO				196.2, CH	

Hz) and H-8 (δ 6.48, $J = 2.0$ Hz) protons of the A ring, the typical singlet of H-2 (δ 7.98, s) of the C ring, the signals of the H-3′ (δ 6.86, s) and H-6′ (δ 6.56, s) protons of the B ring, and signals of an isoprenyl moiety (3.31, 5.30, and 1.77 ppm). Moreover, the methoxy group protons were evident at 3.90 ppm, and the position of this group was revealed from the HMBC spectrum by the correlation with C-7. The HMBC spectrum also helped determine the position of the isoprenyl moiety at C-5′, with correlations of H-1″ with C-5′ (^2J), C-4′ (^3J), and C-6′ (^3J) observed (Table S1, Supporting Information). Accordingly, the structure of **2** was assigned as 5,3′,4′-trihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)-isoflavone.

Compound **3** was found to be a derivative of **1** in which the isoprenyl moiety was replaced with a 3-methyl-2-hydroxybuten-3-yl chain. The UV spectrum was in accordance with those of the preceding compounds, and a peudomolecular ion peak at *m/z* 369 was clear in the APCIMS. The HREIMS confirmed the molecular formula of $C_{21}H_{20}O_6$ for **3**. The NMR spectra of the compound exhibited typical peaks of an isoflavone. The only differences concerned the side chain, where the geminal protons of the C-4″ terminal double bond resonated at 5.03 and 4.90 ppm as two singlets, while the protons of the C-1″ methylene group resonated separately as two double doublets at 3.03 ppm (H-1″a, $J = 8.9$ and 14.9 Hz) and 2.83 ppm (H-1″b, $J = 2.0$ and 14.9 Hz). The oxymethine proton of the moiety was observed as a doublet ($J = 8.9$ Hz) at 4.44 ppm, and the methyl group protons (H-5″) resonated at 1.84 ppm. The cross-peaks evident in the COSY and LRCOSY spectra were used in the identification of the side chain. More specifically, a ^4J correlation between the C-5″ methyl group protons and the C-4″ protons was observed (LRCOSY) along with a ^3J (COSY) and a ^5J (LRCOSY) correlation of H-1″ with H-2″ and H-5′, respectively. Therefore, the structure of **3** was established as 5,4′-dihydroxy-7-methoxy-3′-(3-methyl-2-hydroxybuten-3-yl)isoflavone.

Compound **4** is a formyl derivative of the 7-methyl ether of genistein. The APCIMS showed a pseudomolecular ion at *m/z* 311 $[M - 1]^-$, consistent with the molecular formula, $C_{17}H_{12}O_6$ (HRESIMS), with UV−vis spectra characteristic for an isoflavone nucleus. Comparison of the ^1H (Table 1) and ^{13}C (Table 2) NMR spectra of **4** with those of **1** and **3** revealed identical oxygenation at C-7, C-5, and C-4′. However, signals corresponding to a side chain were completely absent, even though C-3′ was found to be substituted, and in the ^1H NMR spectrum an ABX spin system was observed. In the same spectrum, a singlet at low field (δ 9.96) was present, while the corresponding carbon atom resonated at 196.2 ppm (HSQC), values characteristic of an aldehyde moiety. The HMBC spectrum revealed a correlation of the aldehyde moiety proton with C-4′ (^3J) and C-3′ (^2J), confirming this functional group position (Table S1, Supporting Information). Thus, the structure of **4** was assigned as 3′-formyl-5,4′-dihydroxy-7-methoxyisoflavone.

Compound **5** was assigned as a dimethyldihydropyran derivative of **1**. It was isolated as an amorphous, yellow solid with UV maxima at 266 and 279 nm. The ESIMS revealed a pseudomolecular ion at *m/z* 367 $[M - 1]^-$. HRESIMS allowed for the molecular formula to be calculated as $C_{21}H_{20}O_6$. Due to its structural resemblance, **5** exhibited similar NMR spectra to the above-mentioned metabolites **1**−**4**. The substitution pattern of the basic isoflavone skeleton was evident from the ^1H NMR spectrum (Table 1), which showed the presence of a pair of *meta*-coupled aromatic protons at δ 6.38 (d, $J = 1.9$ Hz) and δ 6.40 (d, $J = 1.9$ Hz) and an ABX aromatic spin system at δ 6.89 (d, $J = 8.5$ Hz), 7.23 (m), and 7.24 (m). In the same spectrum, additional signals were consistent with the presence of an extra ring. Specifically, a pair of geminal protons resonated at 3.13 (H-4″a, $J = 2.0$ and 17.0 Hz) and 2.84 (H-4″b, $J = 3.5$ and 17.0 Hz) ppm as two double doublets, an oxygenated signal resonated at 3.84 ppm as a multiplet, and two methyl protons resonated at 1.39 and 1.34 ppm as singlets. The chemical shift values of the corresponding carbon atoms of the aforementioned protons obtained from the HSQC and HMBC spectra were very useful for the structural determination of **5** (Table 2). The allocation of the dimethyldihydropyran ring in the B ring, between C-3′ and C-4′, was determined by the characteristic correlations of the H-4″ methylene protons with C-3′ (HMBC) and the long-range correlation with H-2′ (LRCOSY). Accordingly, the structure of **5** was assigned as 5-hydroxy-3″-hydroxy-2″,2″-dimethyldihydropyrano[5″,6″:3′,4′]isoflavone.

ER-binding affinity of **1**−**11** relative to that of 17β-estradiol (receptor binding affinity, RBA) was determined using purified recombinant ERα and ERβ and a fluorescence polarization approach.[23,26] Table 3 shows data for those of **1**−**11** that displayed RBA values for ERα (RBAα) and/or ERβ (RBAβ) ≥ 0.1. Compounds with RBA ≥ 0.1 at a concentration of 1 μM can

Table 3. ERα- and ERβ-Binding Affinities and Selectivity for ERα[a]

compound	RBAα	RBAβ	RBAα/RBAβ
17β-estadiol	100	100	1
PPT[b]	50.2 ± 2.4	1.43 ± 0,31	36
DPN[b]	0.32 ± 0.04	13.4 ± 2.5	0.02
2	0.15 ± 0.05	0.21 ± 0.05	0.7
3	0.14 ± 0.04	0.06 ± 0.01	2.3
5	0.31 ± 0.01	0.02 ± 0.01	16
6	0.31 ± 0.10	0.49 ± 0.05	0.6
7	1.95 ± 0.17	3.39 ± 0.32	0.6
8	0.06 ± 0.01	0.79 ± 0.10	0.1
9	0.21 ± 0.07	0.30 ± 0.07	0.7
11	2.68 ± 0.39	1.22 ± 0.02	2.2

[a] The RBA values (mean ± SEM of at least three independent experiments) of compounds **1**−**11** for ERα (RBAα) and ERβ (RBAβ) were calculated by [RBA = (IC_{50} 17β-estradiol/IC_{50} compound) × 100], where IC_{50} values are compound (or 17β-estradiol) concentrations that are capable of inhibiting the binding of the fluorescent estrogen ES2 (1 nM) to ERα and ERβ by 50%. IC_{50} values of estradiol for ERα and ERβ were 3.2 ± 0.3 and 2.9 ± 0.5 nM, respectively. The RBAα and RBAβ of 17β-estradiol were set equal to 100. The ERα-binding selectivity was calculated by RBAα/RBAβ. Only those of **1**−**11** that displayed RBAα and/or RBAβ ≥ 0.1 are listed. [b] PPT (propyl-pyrazoletriol) and DPN (diarylpropionitrile) are commercially available synthetic compounds that served as positive controls of high affinity and selectivity for ERα and ERβ, respectively.[46,47]

displace more than 90% of 17β-estradiol bound to ER in the presence of postmenopausal levels of the hormone (ca. 0.1 nM). Considering that isoflavones administered in the form of tablets to healthy volunteers participating in intervention trials can reach circulating concentrations of 2−5 μM,[37] those of **1**−**11** that displayed RBAα and/or RBA$\beta \geq 0.1$ can in principle be considered to be potentially active. The RBAα values of **1**−**11** differed ca. 90-fold, ranging from 0.03 (**1**) to 2.68 (**11**). Those of **1**−**11** that displayed RBA$\alpha \geq 0.1$ can be segregated into two groups, five weak binders ($0.1 \leq$ RBA$\alpha < 1$; **2**, **3**, **5**, **6**, and **9**) and two moderate binders ($1 \leq$ RBA$\alpha < 10$; **7**, **11**). Moderate binders are here considered to be those that at a concentration of 1 μM can displace more than 90% of 17β-estradiol bound to ER in the presence of premenopausal levels of the hormone (ca. 1 nM). The structural determinant underlying this segregation is evidently the free OH at C-7 in conjunction with the presence of an isoprenyl substituent in ring B of **7** and **11**. The presence of a 6-isoprenyl in **6** and an 8- as well as a 3′-isoprenyl in **8** correlated with a drop in RBAα compared to **7** (which possesses only a 3′-isoprenyl) of 84% and 97%, respectively. In addition, the dimethylpyrano group of **9** interfered even more than the 6-isoprenyl of **6** with the role of OH-7 in binding to ERα.

RBAβ values differed ca. 170-fold, ranging from 0.02 (**5**) to 3.39 (**7**). Those of **1**−**11** that displayed RBA$\beta \geq 0.1$ can be similarly segregated into two groups, four weak binders (**2**,**6**, **8**, **9**) and two moderate binders (**7**, **11**). The structural determinant primarily underlying this segregation is again the OH-7 in conjunction with the isoprenyl substituent in ring B of **7** and **11**. The higher RBAβ of **7** compared to **11** can be attributed to the presence of an OH-5 in the former but not the latter. The presence of a 6-isoprenyl in **6** and an 8- as well as a 3′-isoprenyl in **8** is associated with a drop in RBAβ relative to that of **7** of 86% and 77%, respectively, implying that these subtituents obstruct binding to both ERβ and ERα. The obstructive role of the 6- and especially the 8-isoprenyl is more pronounced in ERα, causing **8** to exhibit a 10-fold higher selectivity for ERβ (Table 3). Taken together, these data show that the isoprenyl and dimethylpyrano substituents in ring A cause **6**, **8**, and **9** to bind to ERβ with ca. 100-fold lower affinity than genistein (RBA$\beta = 43.9$[24,27]), whereas the isoprenyl substituent in ring B is better accommodated, allowing **7** to bind with ca. 10-fold lower affinity than genistein.

Interest in plant-derived natural products that display mixed estrogen agonist/antagonist activities as a means to prevent and/or treat endocrine-related diseases has been rising steadily.[38,39] However, many phytoestrogens were found to display ER-binding affinities and estrogenic/antiestrogenic activities that are considerably lower compared to 17β-estradiol.[26,27,40] Interestingly, while the flavanone naringenin exhibits weak estrogenic activity, its isoprenylated derivative, 8-prenylnaringenin, ranks among the most estrogenic natural products discovered to this day, raising interest in the role of the isoprenyl group as a determinant of ER-binding affinity and activity.[41,42] In addition, recent findings show that compounds belonging to different chemical classes and exhibiting pronounced structural diversity can bind ER and differentially activate receptor cross-talk with different signaling pathways of distinct pharmacological importance.[43] Moreover, prenylated phytoestrogens are also known to be cytotoxic toward intensively proliferating cells in a manner that depends on the structure and position of the prenyl substituent.[44,45] While these findings might account for the empirical use of plant extracts containing the isoprenylated isoflavones **1**−**11** for the treatment of amenorrhea, menopausal syndromes, and endocrine-related disorders, it is unlikely that they could also account for the multitude and diversity of health claims ascribed to them, implying that these compounds could target more effectors and modulate other signaling pathways independently of any effects they might exert through the ER.

Experimental Section

General Experimental Procedures. UV−vis spectra were obtained using spectroscopic grade EtOH−MeOH on a Shimadzu-160A spectrophotometer. IR spectra were recorded on a Perkin-Elmer Paragon 500 FT-IR spectrometer. A Thermo Finnigan HPLC system (ThermoFinnigan, San Jose, CA) was employed for the profiling of the extract connected to a Spectral System UV2000 PDA detector. ChromQuest 2.1 software was used for the management of the data. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 400 and 600 MHz spectrometers using $CDCl_3$ (Aldrich) as solvent. The 2D-NMR experiments (COSY, LRCOSY, HMQC, HSQC, and HMBC) were performed using standard Bruker microprograms. ESIMS and APCIMS were run on a Finnigan MSQ mass spectrometer. HREIMS were run on a Thermo Scientific LTQ Orbitrap Discovery mass spectrometer. Column chromatography was carried out using silica gel [Merck, 0.04−0.06 mm (flash) and 0.015−0.04 mm] with an applied pressure of 300 mbar and Sephadex LH-20 (Merck). Precoated TLC silica 60 F_{254} plates (purchased from Aldrich) were used for thin-layer chromatography (0.25 and 2 mm layer thickness for analytical and preparative TLC, respectively). Spots were visualized using UV light and vanillin−sulfuric acid reagent.

Plant Material. The stem bark of *E. poeppigiana* was collected in November 2006 in Sancta Cruz (Bolivia). After being identified by Ing. Mario Saldias Paz, a specimen was deposited in the Museo de Historia Natural, Facultad de Ciencias Agricolas de Santa Cruz-Bolivia, under the voucher number USZ: 71775.

Extraction and Isolation. Well-dried and pulverized bark of the plant (1.86 kg) was extracted successively with CH_2Cl_2, MeOH, and H_2O (2 L of each solvent × 3, 48 h per extraction). After concentration under reduced pressure, 34.0 g (CH_2Cl_2), 90.5 g (MeOH), and 123.1 g (H_2O) residues were obtained, from which 20 g of the CH_2Cl_2-soluble extract was subjected to vacuum-liquid chromatography on silica gel (0.07−0.2 mm) for initial fractionation. Elution with cyclohexene−CH_2Cl_2 and CH_2Cl_2−EtOAc gradients yielded 68 fractions. A qualitative evaluation of the fractions by TLC and HPLC-PDA (using a gradient method with H_2O + 2% acetic acid and CH_3CN + 2% acetic acid) was performed in order to select fractions rich in flavonoids and isoflavonoids. Thus, fractions 35−38 (795 mg) were subjected to a Sephadex LH-20 column (bead size: 25−100 μm). The subfraction 4 (216 mg) was subjected to silica gel, and from fraction 15 compound **5** (1.8 mg) was obtained. Preparative TLC, developed in cyclohexane−EtOAc (50:50), was performed for the purification of the compound. From subfraction 16, compound **9** (2.5 mg) was isolated using preparative TLC developed with CH_2Cl_2−EtOAc (95:5). From the initial fractionation, fraction 40 (840 mg) was also subjected to a silica gel column. Subfraction 420 (147 mg) after silica gel column chromatography followed by a Sephadex gel filtration (fractions 15−48: 74.2 mg) led to compounds **1** (6.7 mg) and **10** (4.4 mg). Subfraction 534 (60 mg) was submitted to a Sephadex LH-20 column, affording compound **4** (1.2 mg), while from subfraction 596, compound **3** (1.7 mg) was obtained after purification with preparative TLC (cyclohexane−CH_2Cl_2−acetone, 17:80:3). From the initial fractionation, fraction 45 (504 mg), after Sephadex gel column chromatography, followed by silica gel column chromatography of fraction 5 (204 mg), compounds **2** (1.5 mg), **6** (2.3 mg), **7** (3.3 mg), and **8** (4.7 mg) were isolated. Finally, fraction 49 (266 mg), after Sephadex gel and silica gel column chromatography of subfractions 5 and 6, afforded compound **11** (2.8 mg).

5,4′-Dihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)isoflavone (1): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (KBr) ν_{max} 3338, 1657, 1620, 1583, 1516 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 600 and 150 MHz, respectively), see Tables 1 and 2; APCIMS(+) *m*/*z* 353 [M + 1] (100), 329 (28), 284 (14); HRESIMS *m*/*z* 353.3867 (calcd for $C_{21}H_{20}O_5$, 353.3864).

5,3′,4′-Trihydroxy-7-methoxy-3′-(3-methylbuten-2-yl)isoflavone (2): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (KBr) ν_{max} 3340, 1658, 1618, 1580, 1513 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 600 and 150 MHz, respectively), see Tables 1 and 2; ESIMS(+) *m*/*z* 369 [M + 1] (100), 337 (28), 301 (14); HRESIMS *m*/*z* 368.3855 (calcd for $C_{21}H_{20}O_6$, 368.3857).

5,4′-Dihydroxy-7-methoxy-3′-(3-methyl-2-hydroxybuten-3-yl)isoflavone (3): amorphous, yellow solid; $[\alpha]^{25}_D$ −8.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (KBr) ν_{max} 3338, 1662, 1631, 1620, 1579, 1515, 1435 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$,

600 and 150 MHz, respectively), see Tables 1 and 2; APCIMS(+) *m/z* 369 [M + 1] (54), 351 (100), 322 (12); HRESIMS *m/z* 368.3852 (calcd for $C_{21}H_{20}O_6$, 368.3851).

3′-Formyl-5,4′-dihydroxy-7-methoxyisoflavone (4): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (KBr) ν_{max} 3392−3200 (OH), 1658 (CO), 1619 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 600 and 150 MHz, respectively), see Tables 1 and 2; APCIMS(−) *m/z* 311 [M − 1] (100), 119 (22); HRESIMS *m/z* 312.2780 (calcd for $C_{17}H_{12}O_6$, 312.2784).

5-Hydroxy-7-methoxy-(3″,4″-dihydro-3″-hydroxy)-2″,2″-dimethylpyrano[5″,6″:3′,4′]isoflavone (5): amorphous, yellow solid; $[\alpha]^{25}_D$ −1.8 (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (KBr) ν_{max} 3366, 1658, 1614, 1577, 1512 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 600 and 150 MHz, respectively), see Tables 1 and 2; APCIMS(−) *m/z* 367 [M − 1] (100), 279 (12); HRESIMS *m/z* 368.3853 (calcd for $C_{21}H_{20}O_6$, 368.3859).

Assessment of Isoflavone Binding to the ERα and ER*β* Receptors. The receptor binding affinities, RBAα and RBA*β*, of **1**−**11** were assessed using a Beacon 2000 fluorescence polarization reader (Invitrogen), as previously described.[23] Briefly, test compound competition of 1 nM ES2 binding to ER obeys the following scheme: Test _ ER-ES2 (high FP) → ER-Test _ ES2 (low FP). The concentrations were determined for 17*β*-estradiol and **1**−**11** that inhibited the binding of the fluorescent estrogen ES2 (Invitrogen) to isolated recombinant human ERα or ER*β* (Invitrogen) by 50% (IC_{50}) and were used to derive the receptor binding affinity values of Table 3, as described in the legend to the table. PPT (propylpyrazoletriol) and DPN (diarylpropionitrile) are synthetic compounds purchased from Tocris Bioscience (UK) that served as positive controls of high affinity and selectivity for ERα and ER*β*, respectively.[46,47]

Supporting Information Available: Table of 2D-HMBC NMR data for **1**−**5** and 1D- and 2D-NMR spectra for **1**−**5**. This material is provided free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Neill, D. *Ann. Missouri Bot. Gard.* **1987**, *74*, 27–41.
(2) Hegde, G. R.; Hegde, S. D.; Kholkute, H. V. *Complementary Ther. Clin. Pract.* **2007**, *13*, 38–45.
(3) Kirandeep, K.; Meenakshi, J.; Tarandeep, K.; Rahul, *J. Bioorg. Med. Chem.* **2009**, *17*, 3229–3256.
(4) More, G.; Tshikalange, T. E.; Lall, N.; Botha, F.; Meyer, J. J. M. *J. Ethnopharmacol.* **2008**, *119*, 473–478.
(5) Maregesi, S. M.; Ngassapa, D. O.; Pieters, L.; Vlietinck, J. A. *J. Ethnopharmacol.* **2007**, *113*, 457–470.
(6) Raupp, I. M.; Sereniki, A.; Virtuoso, S.; Ghislandi, C.; Cavalcanti e Silva, E. L.; Trebien, H. A.; Miguel, O. G.; Andreatini, R. *J. Ethnopharmacol.* **2008**, *118*, 295–299.
(7) Kosalge, S. B.; Fursule, R. A. *J. Ethnopharmacol.* **2009**, *121*, 456–461.
(8) Ji, H.; Dailey, T. L.; Long, V.; Chien, E. K. *Am. J. Obstet. Gynecol.* **2008**, *198*, 536.e1536.e7.
(9) Tanee, F. S. F.; Njamen, D.; Magne Ndé, C. B.; Wanji, J.; Zierau, O.; Fomum, Z. T.; Vollmer, G. *Phytomedicine* **2007**, *14*, 222–226.
(10) Togola, A.; Austarheim, I.; Theïs, A.; Diallo, D.; Paulsen, B. S. *J. Ethnobiol. Ethnomed.* **2008**, *4*, 1–9.
(11) Zhang, Y.; Li, X.-L.; Lai, W. P.; Chen, B.; Chow, H. K.; Wu, C. F.; Wang, N. L.; Yao, X. S.; Wong, M. S. *J. Ethnopharmacol.* **2007**, *109*, 165–169.
(12) Schmidt, B.; Ribnicky, M. D.; Poulev, A.; Logendra, S.; Cefalu, T. W.; Raskin, I. *Metabolism* **2008**, *57*, S3–S9.
(13) Zhang, Y.; Li, X. L.; Yao, X. S.; Wong, M. S. *Arch. Pharm. Res.* **2008**, *31*, 1534–1539.
(14) Krukoff, B. A. *Phytologia* **1982**, *51*, 440–457.
(15) Maraias, J. P.; Mueller-Harvey, I.; Brandt, E. V.; Ferreira, D. *J. Agric. Food Chem.* **2000**, *48*, 3440–3447.
(16) Veitch, C. N. *Nat. Prod. Rep.* **2007**, *24*, 417–464.
(17) Tanaka, H.; Oh-Uchi, T.; Etoh, H.; Shimizu, H.; Tateishi, Y. *Phytochemistry* **2002**, *60*, 789–794.
(18) Tanaka, H.; Oh-Uchia, T.; Etoh, H.; Sako, M.; Sato, M.; Fukai, T.; Tateishi, Y. *Phytochemistry* **2003**, *63*, 597–602.
(19) Wanjala, C. C. W.; Juma, B. F.; Bojase, G.; Gashe, B. A.; Majinda, R. R. T. *Planta Med.* **2002**, *68*, 640–642.
(20) Tanaka, H.; Etoh, H.; Shimizu, H.; Makita, T.; Tateishi, Y. *Planta Med.* **2000**, *66*, 578–579.
(21) Tanaka, H.; Tanaka, T.; Etoh, H. *Phytochemistry* **1997**, *45*, 835–838.
(22) Tanaka, H.; Hirata, M.; Etoh, H.; Sako, M.; Sato, M.; Murata, J.; Murata, H.; Darnaedi, D.; Fukai, T. *Chem. Biodiversity* **2004**, *1*, 1101–1108.
(23) Kuiper, G. G.; Lemmen, J. G.; Carlsson, B.; Corton, J. C.; Safe, S. H.; Van der Saag, P. T.; Van der Burg, B.; Gustafsson, J. A. *Endocrinology* **1998**, *139*, 4252–4263.
(24) Halabalaki, M.; Alexi, X.; Aligiannis, N.; Lambrinidis, G.; Pratsinis, H.; Florentin, I.; Mitakou, S.; Mikros, E.; Skaltsounis, A. L.; Alexis, M. N. *Planta Med.* **2006**, *72*, 488–493.
(25) Katsanou, E. S.; Halabalaki, M.; Aligiannis, N.; Mitakou, S.; Skaltsounis, A. L.; Alexi, X.; Pratsinis, H.; Alexis, M. N. *J. Steroid Biochem. Mol. Biol.* **2007**, *104*, 228–236.
(26) Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engström, O.; Ljunggren, J.; Gustafsson, J. A.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608–4618.
(27) Lambrinidis, G.; Halabalaki, M.; Katsanou, E. S.; Skaltsounis, A. L.; Alexis, M. N.; Mikros, E. *Environ. Chem. Lett.* **2006**, *4*, 159–174.
(28) Henley, D. V.; Korach, K. S. *Endocrinology* **2006**, *147*, S25–S32.
(29) Ito, C.; Itoigawa, M.; Kumagaya, M.; Okamoto, Y.; Ueda, K.; Nishihara, T.; Kojima, N.; Furukawa, H. *J. Nat. Prod.* **2006**, *69*, 138–141.
(30) Ingham, L. J.; Keen, T. N.; Hymowitz, T. *Pytochemistry* **1977**, *16*, 1943–1946.
(31) Dahiya, S. J.; Strange, N. R.; Bilyard, G. K.; Cooksey, J. C.; Garratt, J. P. *Phytochemistry* **1984**, *23*, 871–873.
(32) Tahara, S.; Yasufumi, K.; Ingham, J.; Mizutani, J. C. *Phytochemistry* **1994**, *36*, 1261–1271.
(33) El-Masry, S.; Amer, E. M.; Abdel-Kader, S. M.; Zaatout, H. H. *Phytochemistry* **2002**, *60*, 783–787.
(34) Tanaka, H.; Oh-Uchi, T.; Etoh, H.; Shimizu, H.; Tateishi, Y. *Phytochemistry* **2002**, *60*, 789–794.
(35) Dagne, E.; Gunatilaka, A. A. L.; Kingston, D. C. I. *J. Nat. Prod.* **1993**, *56*, 1831–1834.
(36) Queiroz, F. E.; Atindehou, K. K.; Terreaux, C.; Antus, S.; Hostettmann, K. *J. Nat. Prod.* **2002**, *65*, 403–406.
(37) Gardner, C. D.; Chatterjee, L. M.; Franke, A. A. *J. Nutr. Biochem.* **2009**, *20*, 227–234.
(38) Gerhäuser, C. *Eur. J. Cancer* **2005**, *41*, 1941–1954.
(39) Oseni, T.; Patel, R.; Pyle, J.; Jordan, V. C. *Planta Med.* **2008**, *74*, 1656–1665.
(40) Fokialakis, N.; Lambrinidis, G.; Mitsiou, D. J.; Aligiannis, N.; Mitakou, S.; Skaltsounis, A. L.; Pratsinis, H.; Mikros, E.; Alexis, M. N. *Chem. Biol.* **2004**, *11*, 397–406.
(41) Zierau, O.; Hamann, J.; Tischer, S.; Schwab, P.; Metz, P.; Vollmer, G.; Gutzeit, H. O.; Scholz, S. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 909–916.
(42) Roelens, F.; Heldring, N.; Dhooge, W.; Bengtsson, M.; Comhaire, F.; Gustafsson, J. A.; Treuter, E.; De Keukeleire, D. *J. Med. Chem.* **2006**, *49*, 7357–7365.
(43) Nettles, K. W.; Bruning, J. B.; Gil, G.; Nowak, J.; Sharma, S. K.; Hahm, J. B.; Kulp, K.; Hochberg, R. B.; Zhou, H.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S.; Kim, Y.; Joachmiak, A.; Greene, G. L. *Nat. Chem. Biol.* **2008**, *4*, 241–247.
(44) Daskiewicz, J. B.; Depeint, F.; Viornery, L.; Bayet, C.; Comte-Sarrazin, G.; Comte, G.; Gee, J. M.; Johnson, I. T.; Ndjoko, K.; Hostettmann, K.; Barron, D. *J. Med. Chem.* **2005**, *48*, 2790–2804.
(45) Halabalaki, M.; Alexi, X.; Aligiannis, N.; Alexis, M. N.; Skaltsounis, A. L. *J. Nat. Prod.* **2008**, *71*, 1911–1918.
(46) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Nishiguchi, G.; Carlson, K.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2000**, *43*, 4934–4947.
(47) Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230–4251.

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