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Flavonoids from the Stem of *Eriophorum scheuchzeri*

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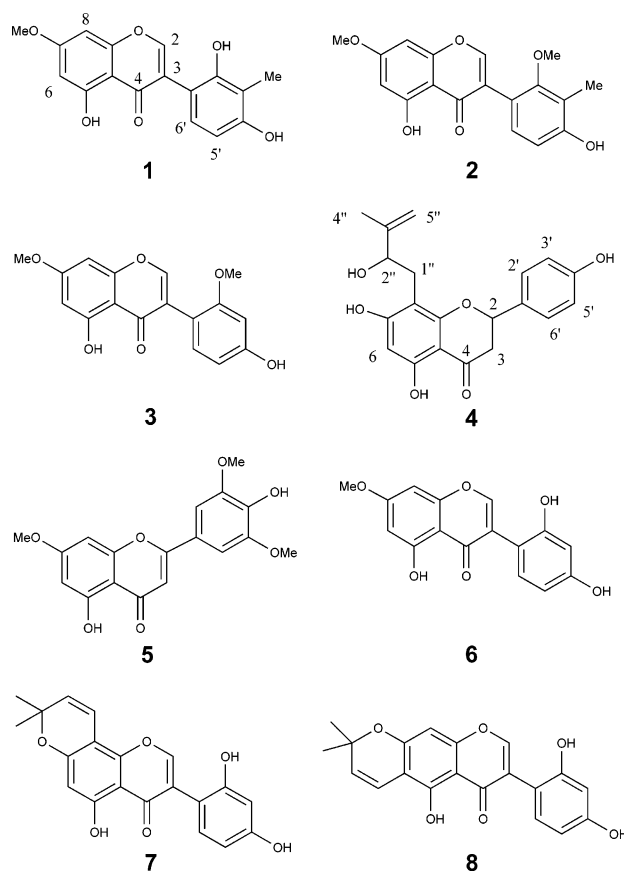
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Activity-guided isolation of antifungal, antibacterial, and antioxidant compounds from the dichloromethane extract of the aerial parts of *Eriophorum scheuchzeri* led to the isolation of eight flavonoids. Three isoflavones and one flavanone are new natural compounds. The isolation of the bioactive compounds was performed by centrifugal partition chromatography. Fractions and pure compounds were monitored by a direct TLC bioautographic assay. Their structures were elucidated by spectroscopic and chemical methods.

As a part of our ongoing investigations of alpine plants from the Swiss flora, the aerial parts of *Eriophorum scheuchzeri* Hoppe (Cyperaceae)¹ have been studied. *Scheuchzer's* cottongrass is a summer-green, strongly rhizomatous or stoloniferous species growing at an altitude between 1500 and 2600 m in wet, peaty soils that are poor in nutrients.^{1,2} The extreme habitat of this alpine plant suggested a strong adaptation to UV-B irradiation, which may partly consist of an increase in the amount of UV-absorbing phenolic compounds.³ Therefore, the antioxidant potential of the CH₂Cl₂ extract was evaluated by TLC autography with DPPH (2,2-diphenyl-1-picrylhydrazyl) radical as spray reagent.⁴ Its antifungal activities were also measured, and the screening of the extract against *Cladosporium cucumerinum* and *Candida albicans*^{5,6} revealed the presence of several lipophilic antifungal agents.

In addition to the biological screening, a LC-UV-MS analysis using an electrospray ionization interface⁷ gave a first indication of the type of constituents present in the crude extracts (Figure 1). The LC-UV/DAD analysis of the CH₂Cl₂ extract displayed UV spectra characteristic for isoflavone-type compounds, which consist of an intense band II (250–275 nm) of low intensity and band I as a shoulder (310–330 nm). Compound **6** showed a major absorption peak (band II) at 277 nm, which is characteristic for flavanones or dihydroflavonols.⁸ The associated protonated molecules recorded by ESI-MS displayed molecular weights ranging from 301 to 329 (compounds **1**, **4**, **5**, **7**, **8**), suggesting the presence of aglycones substituted by a combination of OH, OMe, and Me groups. The ESIMS spectra also showed molecular ions of 353 and 357 (compounds **2**, **3**, **6**), indicating other types of substituents. Only one peak was associated with a flavone (MW 330), which dereplicated as tricin (**5**), a widespread flavone aglycone in the Cyperaceae family.⁹ On the basis of these screening results, a targeted isolation of the CH₂Cl₂ extract constituents was performed since no isoflavones have been characterized previously in the Cyperaceae family.

The activity of the CH₂Cl₂ extract was strongly enhanced by the removal of a large amount of pigments and fatty acids. The enrichment step was performed by a liquid–liquid partition with hexane and 80% aqueous MeOH, followed by dilution of the 80% aqueous MeOH fraction to 60% aqueous MeOH with H₂O and extraction of the resulting aqueous phase with CHCl₃. Both resulting fractions were tested against *Cladosporium cucumerinum*,



Candida albicans, and DPPH using TLC bioautographic assays, and only the CHCl₃ fraction was shown to be bioactive. To avoid irreversible adsorption that can occur on silica gel columns, the latter was fractionated by centrifugal partition chromatography (CPC)¹⁰ using the quaternary system of heptane/EtOAc/MeOH/H₂O (6:5:6:5, v/v/v/v), yielding eight enriched fractions (1–8) (Figure 2). By this means, a good resolution of the bioactive compounds was obtained, leading to the isolation of four known compounds and four new compounds active as radical scavengers and/or antifungals against *Cladosporium cucumerinum* and *Candida albicans*.^{4–6}

Compound **1** was isolated as an amorphous greenish powder. The UV spectra in MeOH and after addition of UV–vis shift reagents suggested an isoflavanone or isoflavone-type structure.¹¹ The high-resolution ESIMS displayed a pseudomolecular ion at *m/z* 337.0683 [M + Na]⁺,

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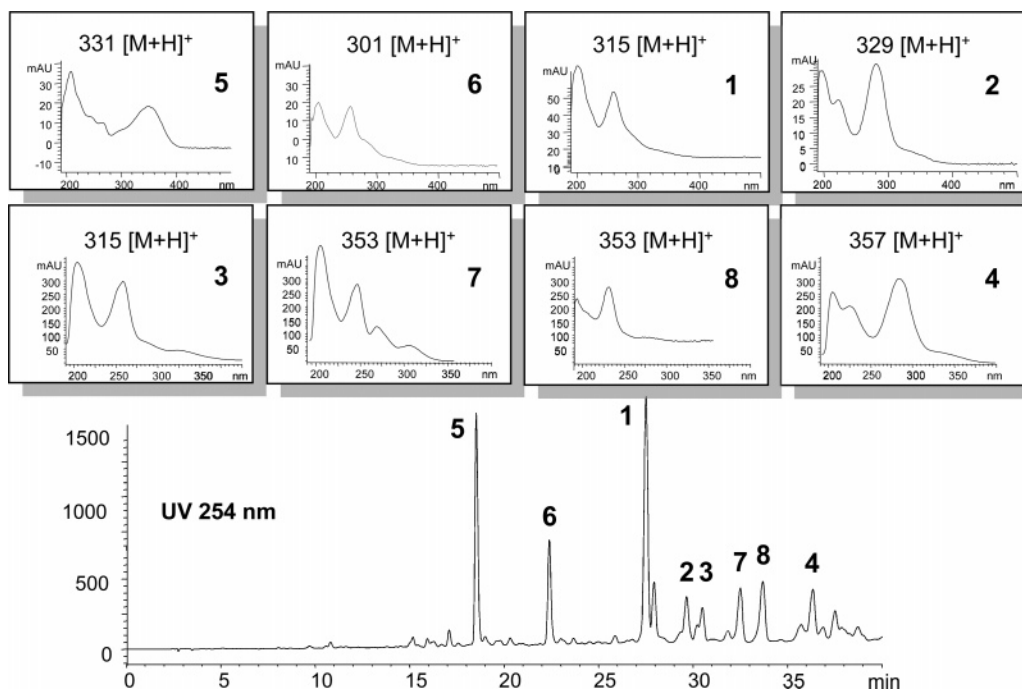


Figure 1. LC/UV/ESIMS analysis of the CH_2Cl_2 extract of *E. scheuchzeri*.

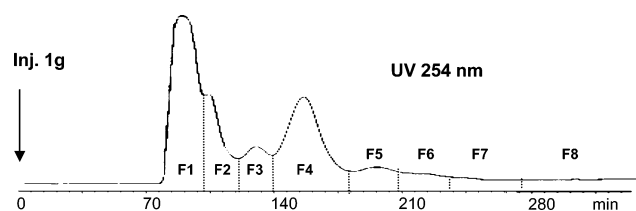


Figure 2. CPC purification of the CH_2Cl_2 extract of *E. scheuchzeri*.

in agreement with the molecular formula $\text{C}_{17}\text{H}_{14}\text{O}_6\text{Na}$, corresponding to an isoflavone skeleton substituted by three hydroxyl, one methoxy, and one methyl group. The EIMS showed a molecular ion at m/z 314.3 $[\text{M}]^+$ and fragment ions at m/z 166.9 $[\text{A}_1]^+$ resulting from a retro-Diels–Alder fragmentation (RDA),¹² indicating the presence of one hydroxyl and one methoxyl group in ring A. The fragment at m/z 147.7 $[\text{B}_1]^+$ suggested the presence of two hydroxyl and one methyl group in ring B.

The ^1H NMR spectrum showed a signal at δ_{H} 8.08 characteristic for a proton at H-2 in isoflavones.⁸ The presence of a doublet at δ_{H} 6.39 and at 6.58 (both with $J = 1.8$ Hz) was attributed to the protons at H-6 and H-8 in ring A. On the other hand, doublets at δ_{H} 6.44 and 6.86 (both with $J = 8$ Hz) were attributed to the protons H-5' and H-6' in ring B. Two other signals were observed at δ_{H} 2.11 (3H) and 3.86 (3H) corresponding to a methyl and a methoxyl group, respectively. The correlations observed in the HMBC spectrum between δ_{H} 2.11 and δ_{C} 157.1 (C-4') and 154.0 (C-2') suggested the presence of a methyl group in position C-3'. The position of the methoxyl group was obtained by a NOESY experiment. The correlations between δ_{H} 3.86 and the aromatic protons at δ_{H} 6.39 and 6.58 indicated the position of a methoxyl group at C-7. Compound **1** was therefore identified as 5,2',4'-trihydroxy-7-methoxy-3'-methylisoflavone and named eriophorin A.

The UV spectrum of **2** showed typical absorption bands of an isoflavanone skeleton.⁸ The high-resolution ESIMS displayed a pseudomolecular ion at m/z 351.0741 $[\text{M} + \text{Na}]^+$, in agreement with the molecular formula $\text{C}_{18}\text{H}_{16}\text{O}_6\text{Na}$, corresponding to an isoflavone skeleton substituted by two hydroxyl, two methoxyl, and one methyl group. The

fragments at m/z 178 $[\text{B}_3 - 2]^+$ and 162 $[\text{B}_1]^+$ from the RDA pattern observed in the EIMS spectrum suggested that ring B was substituted by one methoxyl, one methyl, and one hydroxyl group. The fragment at m/z 167 $[\text{A}_1]^+$ indicated the presence of one methoxyl and one hydroxyl group in ring A. The substitution of ring A was confirmed by the presence in the ^1H NMR spectrum of the signals at δ_{H} 6.39 and 6.42 attributable to protons at C-6 and C-8. Doublets at δ_{H} 7.08 and 6.60 ($J = 8.3$ Hz) were attributed to the aromatic protons H-5' and H-6', respectively. Two signals at δ_{H} 3.57 and 3.80 were attributed to the presence of two methoxyl groups. The correlations between δ_{H} 3.80 and the two aromatic protons at δ_{H} 6.39 and 6.42 observed in the NOE spectrum suggested the presence of a methoxyl group at C-7. In the same way, the second methoxyl group was placed at C-2'. The ^1H NMR spectrum showed another signal at δ_{H} 2.22 attributed to the aromatic methyl group. The position of this group was obtained by the NOE experiment (Figure 3). On this basis, this new compound was characterized as 5,4'-dihydroxy-7,2'-dimethoxy-3'-methylisoflavone and named eriophorin B.

Compound **3** was isolated as an amorphous white powder. The UV spectra in MeOH and after addition of UV–vis shift reagents suggested an isoflavone-type structure.⁸ The EIMS showed a molecular ion at m/z 314 $[\text{M}]^+$ and fragment ions at m/z 148 $[\text{B}_1]^+$ and 167 $[\text{A}_1]^+$ resulting from a retro-Diels–Alder fragmentation,¹¹ indicating the presence of one hydroxyl and one methoxyl group on ring A and a second methoxyl group with a hydroxyl on ring B.

The substitution of ring A was confirmed by analysis of the HMBC spectrum. The signals of two aromatic protons at δ_{H} 6.46 and 6.50 were attributed to the aromatic protons at H-6 and H-8, respectively. The correlations observed in the NOE experiment between the methoxyl signal at δ_{H} 3.91 and the two aromatic protons at δ_{H} 6.46 and 6.50 were indicative of the presence of a methoxyl group at position C-7. The ^1H NMR spectrum showed an ABX system at δ_{H} 7.09, 6.57, and 6.65 attributed to the aromatic protons at H-6', H-5', and H-3', respectively. This hypothesis was confirmed by the COSY and HMBC data. The NOE experiment showed correlations between the second meth-

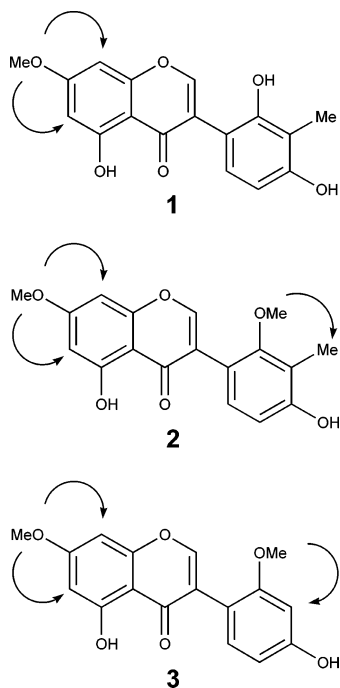


Figure 3. Correlations observed in the selective NOE spectra of compounds **1**, **2**, and **3**.

oxyl group (δ_{H} 3.8) and H-3' (δ_{H} 6.65), confirming the presence of the methoxyl at C-2'. Compound **3**, identified as 5,4'-dihydroxy-7,2'-dimethoxyisoflavone, was named eriophorin C.

The UV spectra of eriophorin C (**4**) showed bathochromic shifts with NaOMe and AlCl_3 , typical for a 5,7,4'-trihydroxyflavanone.⁸ The EIMS displayed a pseudomolecular ion at m/z 356.0 $[\text{M}]^+$, in agreement with the molecular formula $\text{C}_{20}\text{H}_{20}\text{O}_6$. The signals of a three-proton spin system δ_{H} 2.7 (H-3), 3.12 (H-3), and 5.34 (H-2) in the ^1H NMR spectrum were characteristic of a C-3 unsubstituted flavanone nucleus.¹² The substitution of ring A was confirmed by analysis of the HMBC spectrum. The aromatic proton signal at δ_{H} 5.69 was attributed to the isolated proton H-6.

The ^1H NMR spectrum showed the presence of a 2-hydroxy-3-methylbutenyl side chain: one methyl group at δ_{H} 1.77 (s, 3H), an alcoholic OH (δ_{H} 5.03, br s), a methylene at δ_{H} 2.85–3.25 (2H, m, H-1''), and two olefinic protons at δ_{H} 4.92 and 5.07.¹² The correlations observed in the HMBC experiment between the allylic methylene at δ_{H} 2.85–3.25 and the two oxygenated carbons at δ_{C} 166.1 (C-8a) and 170.2 (C-7) showed the presence of the 2-hydroxy-3-methylbutenyl side chain moiety at position C-8. The ^1H NMR spectrum confirmed the hypothesis of the monosubstitution for ring B by the presence of two doublets at δ_{H} 7.33 and 6.83, each one integrating for two protons, corresponding to the four aromatic protons in ring B. Thus, compound **4** was identified as a 5,7,4'-trihydroxy-8-(2-hydroxy-3-methylbutenyl)flavanone.

In addition, compounds **6**, **7**, and **8** were identified as cajanin¹³ and parvisoflavones-A¹⁴ and -B,¹⁴ respectively, from their spectroscopic data (UV, EI-, and D/CI-MS, ^1H and ^{13}C NMR).

The antifungal activity of the isolated compounds was investigated against *C. cucumerinum* and *C. albicans*. The compounds were also tested in routine screening for radical-scavenging activity in a DPPH assay.⁴ Radical-scavenging properties of compounds **1**–**8** were evaluated against the DPPH radical. Quercetin and BHT [2,6-

Table 1. Bioactivities of Isolated Compounds from *E. scheuchzeri* in TLC-Based Assays

compound	<i>C. cucumerinum</i> ^a			<i>C. albicans</i> ^a		DPPH assay ^a		
	10 μg	5 μg	1 μg	10 μg	5 μg	10 μg	5 μg	1 μg
1	+	–	–	+	+	+	+	+
2	+	+	+	–	–	–	–	–
3	–	–	–	–	–	+	+	–
4	+	+	–	+	+	+	+	+
5	–	–	–	–	–	+	+	+
6	+	+	+	–	–	–	–	–
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
reference compound	miconazole 1 μg			miconazole 0.1 μg		quercetin 1 μg		

^a Amounts given are the quantities applied on the TLC plates.

di(*tert*-butyl)-4-methylphenol] were used as reference compounds.^{15,16} The bioactivities obtained using the direct TLC bioautographic assays^{4–6} are presented in Table 1.

Despite the small number of compounds isolated, it is possible to make a correlation between the structures and the biological activities of some of them. Compounds **7** and **8** showed good activity against *C. cucumerinum* and *C. albicans*. The position of the dimethylpyran ring does not seem to influence this activity. The comparison between **1** and **2** showed the importance of the presence of the phenolic group in C-2': compound **1** with a free hydroxyl group in this position is active against *C. albicans*, while compound **2** with a methoxyl group is completely inactive.

The Cyperaceae family is well known for elaborating flavonoids, benzoquinones, and essential oils, but no publications have reported yet the presence of isoflavones in this family.¹⁷ The mixture of different types of radical scavengers (isoflavones and flavanone derivatives) in *E. scheuchzeri* provides good protection against strong UV irradiation and other extreme conditions. Studies of variation of the antioxidant composition of this plant in relation to altitude and light exposure are planned.

Experimental Section

General Experimental Procedures. Optical rotations were determined using a Perkin-Elmer 241 polarimeter (MeOH, c in g/100 mL). UV spectra were measured on a Perkin-Elmer Lambda 20 spectrophotometer. ^1H and ^{13}C NMR were recorded on a Varian Inova 500 spectrometer (500 and 125 MHz, respectively) in CDCl_3 or CD_3OD ; chemical shifts in ppm as δ relative to Me_4Si (internal standard). HRESIMS were recorded on a Bruker FTMS 4.7 T mass spectrometer. EIMS and D/CIMS spectra were obtained on a Finnigan-MAT/TSQ-700 triple stage quadrupole instrument (EIMS: 70 eV; D/CI-MS: NH_3 , positive-ion mode). Mass spectra were obtained on a Finnigan-MAT/TSQ-700 triple stage quadrupole instrument. LC/MS analyses were performed on a Finnigan MAT (Finnigan MAT, San Jose, CA) ion trap mass spectrometer equipped with a Finnigan electrospray interface with the following conditions: capillary voltage 30 V, capillary temperature 200 $^\circ\text{C}$, source voltage 4.5 kV, source current 80 μA , nitrogen as sheath gas flow, and positive-ion mode. Spectra (180–1200 m/z) were recorded every 3 s. TLC: silica gel 60 F₂₅₄ Al sheets (Merck), detection at 254 nm and with vanillin-sulfuric acid reagent.¹⁸ Analytical HPLC was carried out on a HP 1090 system equipped with a photodiode array detector (Agilent Technologies). Extracts and fractions were analyzed on a Nova-Pak C₁₈ column (5 μm , 4.6 \times 250 mm; Waters). Centrifugal partition chromatography (CPC) was performed on a Pharmatech instrument (capacity 350 mL; 2.6 mm i.d. coil) equipped with two Waters 600A pumps (with a 20 mL sample loop), Knauer UV detector, and LKB Bromma 2210 recorder. Semipreparative HPLC was carried out with a Shimadzu LC-8A pump

equipped with a Knauer UV detector using a μ Bondapak C₁₈ prepacked column (10 μ m, 25 \times 100 mm; Waters), flow rate 10 mL/min, detection UV 254 nm.

Plant Material. The aerial parts of *Eriophorum scheuchzeri* Hoppe (Cyperaceae) were collected in Valais, Switzerland, in September 2001. A voucher specimen was deposited at the Laboratory de Pharmacognosy of Phytochemistry, Geneva, Switzerland (no. 2001073).

Extraction and Isolation. The dried stems (3 kg) were ground in liquid nitrogen, successively extracted by solvents of increasing polarity (CH₂Cl₂ and MeOH), and concentrated under vacuum to give 35 g of CH₂Cl₂ extract and 300 g of MeOH extract. The CH₂Cl₂ and MeOH extracts were tested for their antifungal activities. The CH₂Cl₂ extract was weakly active against *C. cucumerinum*. Thus, fractionation of the extract was undertaken to remove fatty acids, pigments, and chlorophyll. The CH₂Cl₂ extract was first fractionated by liquid-liquid partition with hexane and 80% aqueous MeOH, followed by dilution of the 80% aqueous MeOH fraction to 60% aqueous MeOH with H₂O, and extraction of the resulting aqueous phase with CHCl₃. The fractions were tested against *C. cucumerinum* using the TLC bioautographic assay, and only the CHCl₃ fraction (3 g) proved to be bioactive. The active CHCl₃ fraction (3 g) was purified by CPC using the quaternary system of heptane/EtOAc/MeOH/H₂O (6:5:6:5) (v/v/v/v). The coil was first filled with the two phases (1/1), and rotation was set to the desired speed (800 rpm). The lower phase was then pumped into the column at a flow rate of 3 mL/min using the mode head to tail (mobile phase = lower phase; stationary phase = upper phase). After equilibrium between the two phases, the sample solution (1 g in 7 mL of upper phase and 7 mL of lower phase) was injected. After fraction no. 8, the rotation was inverted to the mode tail to head (mobile phase = upper phase; stationary phase = lower phase). Afterward the rotation was stopped and the contents of the column were pushed out by MeOH. The fractions were analyzed by HPLC and grouped in eight fractions (0–7), most of them bioactive against *C. cucumerinum* and *C. albicans*. The first fraction (500 mg) appeared to be a pure compound, the known methoxyflavone tricin (8). Fraction 2 (100 mg) was purified by semipreparative HPLC using a μ Bondapak C₁₈ prepacked column (10 μ m, 25 \times 100 mm, Waters, MeCN/H₂O, 37:63, flow rate 10 mL/min, UV 254 nm), yielding **7** (7 mg, *t*_R = 15 min). Fraction 3 (150 mg) was purified by semipreparative HPLC using MeCN/H₂O, 68:32, yielding **1** (3 mg, *t*_R = 9 min), **2** (4 mg, *t*_R = 15 min), and **3** (5 mg, *t*_R = 18.5 min). Fraction 4 (30 mg) was purified by semipreparative HPLC using MeCN/H₂O, 42:58, yielding **4** (2 mg, *t*_R = 9 min) and **5** (3 mg, *t*_R = 9 min). Fraction 7 (130 mg) was purified by semipreparative HPLC using MeCN/H₂O 68:32, yielding **6** (5 mg, *t*_R = 7 min).

5,2',4'-Trihydroxy-7-methoxy-3'-methylisoflavone (1): UV (MeOH) λ_{\max} (log ϵ) 264, 312 (sh), 346 nm; UV (MeOH+NaOMe) λ_{\max} 277, 334 (sh), 374 nm; UV (MeOH+NaOAc) λ_{\max} 266, 309 (sh), 350 nm; UV (MeOH+AlCl₃) λ_{\max} 273, 312 (sh), 374 nm; ¹H NMR (CDCl₃, 500 MHz) δ 2.11 (3H, s, CH₃), 3.86 (3H, s, OCH₃), 6.39 (1H, d, *J* = 1.8 Hz, H-6), 6.44 (1H, d, *J* = 8 Hz, H-5'), 6.58 (1H, d, *J* = 1.8 Hz, H-8), 6.86 (1H, d, *J* = 8 Hz, H-6'), 8.08 (1H, s, H-2); ¹³C NMR (CDCl₃, 125 MHz) δ 7.52 (CH₃), 56.6 (OCH₃), 92.1 (C-8), 98.1 (C-6), 105.6 (C-4a), 107.3 (C-5'), 110.7 (C-1'), 112.6 (C-3'), 122.8 (C-3), 127.1 (C-6'), 154.0 (C-2'), 157.0 (C-2), 157.1 (C-4'), 158.7 (C-8a), 162.2 (C-5), 166.2 (C-7), 182.1 (C-4); EIMS *m/z* (rel int) 314 [M]⁺ (58), 296 [M – 18]⁺ (24), 177 (23), 166.9 [A]⁺ (100), 147.7 [B]⁺ (42), 114 (12), 94 (15); HRESIMS *m/z* 337.0683 (calcd for C₁₇H₁₄O₆Na, 337.0682).

5,4'-Dihydroxy-7,2'-dimethoxy-3'-methylisoflavone (2): UV (MeOH) λ_{\max} (log ϵ) 260, 300 (sh), 338 nm; UV (MeOH+NaOMe) λ_{\max} 275, 320 (sh), 374 nm; UV (MeOH+NaOAc) λ_{\max} 266, 309 (sh), 350 nm; UV (MeOH+AlCl₃) λ_{\max} 270, 308 (sh), 370 nm; ¹H NMR (CDCl₃, 500 MHz) δ 2.22 (3H, s, CH₃), 3.57 (3H, s, OCH₃), 3.80 (3H, s, OCH₃), 6.39 (1H, d, *J* = 1.8 Hz, H-6), 6.42 (1H, d, *J* = 1.8 Hz, H-8), 6.60 (1H, d, *J* = 8.4 Hz, H-5'), 7.08 (1H, d, *J* = 8.4 Hz, H-6'), 7.96 (1H, s, H-2); ¹³C NMR (CDCl₃, 125 MHz) δ 9.21 (CH₃), 55.6 (OCH₃), 60.8 (OCH₃), 92.6

(C-8), 98.0 (C-6), 106.8 (C-4a), 111.1 (C-5'), 115.2 (C-1'), 117.1 (C-3'), 121.5 (C-3), 132.0 (C-6'), 155.2 (C-2), 157.3 (C-2'), 157.5 (C-5), 157.9 (C-4'), 158.2 (C-8a), 165.8 (C-7), 181.4 (C-4); EIMS *m/z* (rel int) 328 [M]⁺ (67), 297 [M – 31]⁺ (100), 178 [B₃ – 2]⁺ (17), 167 [A]⁺ (77), 162 [B]⁺ (43), 148 (57), 134 (25); HRESIMS *m/z* 351.0741 (calcd for C₁₈H₁₆O₆Na, 351.0654).

5,4'-Dihydroxy-7,2'-dimethoxyisoflavone (3): UV (MeOH) λ_{\max} (log ϵ) 260, 330 (sh) nm; UV (MeOH+NaOMe) λ_{\max} 268, 360 (sh) nm; UV (MeOH+NaOAc) λ_{\max} 260, 330 (sh) nm; UV (MeOH+AlCl₃) λ_{\max} 270, 311 (sh), 373 nm; ¹H NMR (CDCl₃, 500 MHz) δ 3.8 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 6.46 (1H, br s, H-6), 6.50 (1H, br s, H-8), 6.57 (1H, d, *J* = 8.3 Hz, H-5'), 6.65 (1H, d, *J* = 1.6 Hz, H-3'), 7.09 (1H, d, *J* = 8.3 Hz, H-6'), 8.00 (1H, s, H-2), 12.2 (1H, s, OH-5); ¹³C NMR (CDCl₃, 125 MHz) δ 55.5 (OCH₃), 56.2 (OCH₃), 93.2 (H-8), 99.6 (H-6), 104.2 (C-3'), 106.1 (C-4a), 108.3 (C-5'), 112.1 (C-1'), 123.8 (C-3), 130.1 (C-6'), 157.0 (C-2'), 157.2 (C-2), 157.9 (C-8a), 162.1 (C-4'), 162.4 (C-5), 166.4 (C-7), 182.6 (C-4); EIMS *m/z* (rel int) 314 [M]⁺ (83), 297 [M – 15]⁺ (25), 271 (10), 167 [A]⁺ (17), 148 [B]⁺ (42), 133 [B₁ – 15]⁺ (17); HRESIMS *m/z* 337.0658 (calcd for C₁₇H₁₄O₆Na, 337.0661).

5,7,4'-Trihydroxy-8-(2-hydroxy-3-methylbutenyl)flavanone (4): UV (MeOH) λ_{\max} (log ϵ) 295 (4.07), 277 (sh), 343 (sh) nm; UV (MeOH+NaOMe) λ_{\max} 237, 331, 382 (sh) nm; UV (MeOH+NaOAc) λ_{\max} 295, 277, 340 (sh) nm; UV (MeOH+AlCl₃) λ_{\max} 315, 286, 385 (sh) nm; ¹H NMR (CDCl₃, 500 MHz) δ 1.77 (3H, s, H-4''), 2.7 (1H, m, H-3), 2.85 (1H, m, H-1''), 3.12 (1H, m, H-3), 3.25 (1H, m, H-1'), 5.34 (1H, m, H-2), 5.03 (1H, br s, H-2''), 5.07 (1H, m, H-5''), 4.92 (1H, m, H-5'), 5.69 (1H, s, H-6), 6.83 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 7.30 (2H, d, *J* = 8.5 Hz, H-2', H-6'); ¹³C NMR (CDCl₃, 125 MHz) δ 16.2 (C-4''), 30.2 (C-1''), 42.8 (C-3), 81.2 (C-2), 84.6 (C-2''), 90.1 (C-6), 104.4 (C-4a), 106.7 (C-8), 112.8 (C-5''), 115.3 (C-3'), 116.5 (C-5'), 128.1 (C-2'), 129.5 (C-6'), 131.2 (C-1'), 145.5 (C-3''), 159.4 (C-4'), 160.0 (C-5), 166.1 (C-8a), 170.2 (C-7), 198.6 (C-4); EIMS *m/z* (rel int) 356.04 [M]⁺ (23), 338 [M – 18]⁺ (42), 325 [M – 30]⁺ (100), 297 (15), 218 (22), 203 (24), 175 (14), 147 (21), 120 [B₃]⁺ (18), 90 (22); HRESIMS *m/z* 379.2325 (calcd for C₁₉H₂₀O₆Na, 379.2327).

Antifungal Assay. Pure compounds, fractions, and extracts were tested for antifungal activity by bioautography on thin-layer chromatograms⁵ and by the agar overlay method.⁶ Each pure compound (10–0.0001 μ g), fraction (100–20 μ g), or extract (100–20 μ g) was applied on aluminum-backed silica gel plates (*C. cucumerinum*) or glass-backed silica gel plates (*C. albicans*). The plates were developed in CH₂Cl₂/MeOH (95:5) thoroughly dried for complete removal of solvent and sprayed with a conidial suspension of *C. cucumerinum* in a nutrient medium. Clear inhibition zones were observed against a dark gray background after 3 days incubation at room temperature in a humid atmosphere. For *C. albicans*, an inoculum of the yeast (approximately 10⁷ cells/mL) in malt agar was prepared and spread over the TLC plate. The plates were incubated overnight at 30 °C and then sprayed with methylthiazolyltetrazolium bromide (MTT). Active compounds appeared as clear spots against a purple-colored background. Miconazole (Sigma) was used as reference compound.

DPPH Assay.⁴ After developing and drying, TLC plates were sprayed with a 0.2% diphenylpicrylhydrazyl (DPPH) solution in MeOH. The plates were examined 30 min after spraying. Active compounds appeared as yellow spots against a purple background.

Acknowledgment. The Swiss National Science Foundation (grant number 2000-063670.00 to K.H.) is gratefully acknowledged for supporting this work.

Supporting Information Available: Spectral data for the known compounds **5–8** are available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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