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# PLANT ANTITUMOR AGENTS, 21. FLAVONES, COUMARINS, AND AN ALKALOID FROM *SARGENTIA GREGGII*

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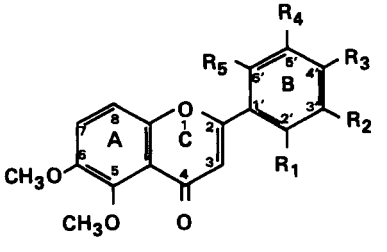
ABSTRACT.—Investigation of the MeOH extracts of *Sargentia greggii* (Rutaceae) led to the isolation of two known flavones, zapotin and 5,6,2′-trimethoxyflavone, and a new flavone, 5,6,2′,3′,4′,6′-hexamethoxyflavone (**1**), whose structure was established by spectral data and confirmed by total synthesis. In addition, two known coumarins, 3-(α,α-dimethylallyl) herniarin and seselin, and a new coumarin, *O*-geranylosthenol (**3**), have been isolated from this plant. The structure of **3** was deduced from spectral data. Although the crude extracts displayed KB activity, none of the crystalline compounds were significantly active.

Crude MeOH extracts of *Sargentia greggii* S. Watts (Rutaceae) exhibited 9 KB activity of the order of $1\times10^{-2}-1\times10^{-3}$. Accordingly, the plant was fractionated in an attempt to isolate compounds with in vivo activity in P-388 mouse leukemia. *S. greggii* has been examined previously, and identified compounds include the furanoquinoline alkaloids, maculine and kokusaginine, and the limonoids, rutaevin and limonin diosphenol (1). Three flavones have also been reported: namely 5,6-dimethoxyflavone, 5,6,3′,5′-tetramethoxyflavone (cerrosillin) (2), and 5,6,3′,4′,5′-pentamethoxyflavone (cerrosillin B) (3). The only other natural source of 5,6-dimethoxyflavones is *Casimiroa edulis* Llave (4).

We wish to report the discovery of two additional known flavones, 5,6,2′,6′-tetramethoxyflavone (zapotin) (5) and 5,6,2′-trimethoxyflavone (6), plus a new compound, 5,6,2′,4′,5′,6′-hexamethoxyflavone (**1**). In addition, two known coumarins, 3-(α,α-dimethylallyl) herniarin (7) and seselin (8), as well as the new *O*-geranylosthenol (**3**), have been isolated from the roots. Because the $^1$H-nmr data for 5,6-dimethoxyflavone was not reported previously (2), it is presented in this paper. Finally, the previously known quinolone, casimiroine (9, 10), has been isolated from this species. Although the crude extracts displayed KB activity, none of the crystalline compounds were significantly active.

The new flavone was shown by high resolution ms to have the molecular formula $C_{21}H_{22}O_8$ and by $^1$H nmr to possess six methoxy groups. A 5,6-dimethoxy substitution of the A-ring was supported by the mass fragmentation (11) (Scheme 1), by similarity of its uv λ max to other 5,6-dimethoxyflavone derivatives, and by similarity of its $^1$H-nmr signal (7.24 ppm, 2H, ABq, $J$=9.2 Hz) for H-7 and H-8. A symmetric




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | $OCH_3$ |
| **2** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | $OCH_3$ |

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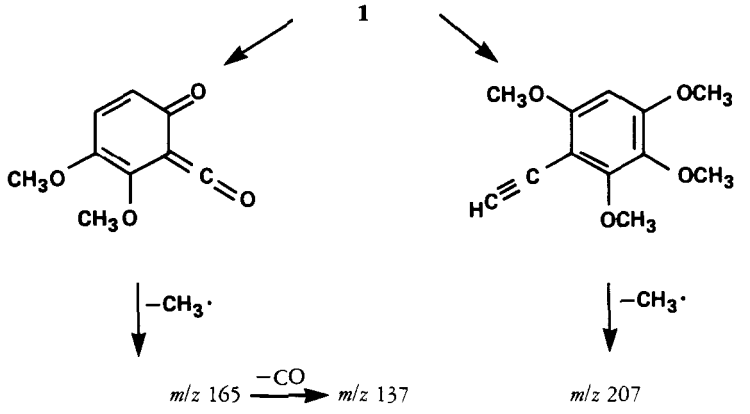
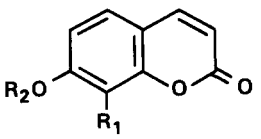
arrangement of four methoxy groups on the B-ring was precluded by the presence of separate signals for each of the methoxy groups in the $^1$H-nmr spectrum. Distinction between structures **1** and **2** was made by use of benzene-induced shifts of methoxy groups (12). Only methoxy groups *ortho* to an aromatic proton are shifted upfield by 0.5 ppm in $C_6D_6$ compared to $CDCl_3$ solution. Three methoxy signals were shifted to such an extent (6,4′ and 6′-$OCH_3$ in **1**). If structure **2** is correct, only two methoxy signals would shift (6 and 3′-$OCH_3$ in **2**). The B-ring substitution of **1** was further supported by comparison of the chemical shift of the lone aromatic proton with recent data for some synthetic flavones with four B-ring methoxy groups (13).

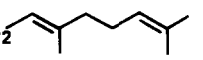


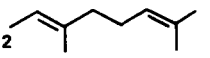

SCHEME 1. Proposed Mass Fragmentation of **1**

There are two precedents for the 2′,3′,4′,6′ pattern of oxygenation in flavones. They are 5,6′-dihydroxy-6,7,2′,3′,4′-pentamethoxyflavone from two species of *Brickellia* (14) and 6,7,2′,3′-tetramethoxy-5,4′,6′-trihydroxyflavone from a fern (15, 16). Structure **1** was confirmed by synthesis from monocyclic precursors. 2,3,4,6-Tetramethoxybenzoyl chloride required for the synthesis of **1** was derived from 6-hydroxy-2,3,4-trimethoxyacetophenone in three steps consisting of methylation, oxidation, and treatment with thionyl chloride. Acylation of 2,3-dimethoxy-6-hydroxyacetophenone (17) with the above acid chloride gave the ester **6**. Baker-Venkatraman rearrangement of **6** with powdered KOH in pyridine (18) gave the β-diketone **7** which was cyclized with sodium acetate in refluxing HOAc to give the flavone **1** identical with the natural product.

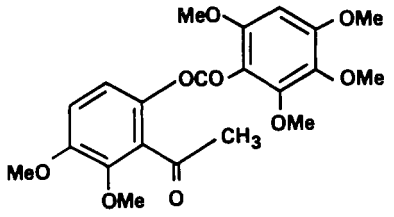
The new coumarin had the molecular formula $C_{24}H_{30}O_3$, as shown by hrms, and was assigned structure **3** by comparison of its $^1$H-nmr signals with literature data for known compounds, i.e., the geranyl side-chain of aurapten (*O*-geranylumbelliferone,




**3** $R_1$=  $R_2$= 

**4** $R_1$=H $R_2$= 

**5** $R_1$=  $R_2$=$CH_3$



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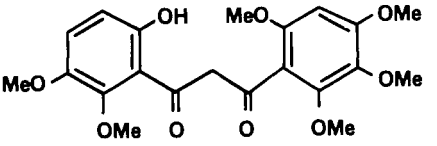

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4) (19) and the coumarin nucleus of osthole (**5**) (20). This is the first report of coumarins in *Sargentia* although their presence was conjectured based on tlc of EtOH extracts by Dominguez (21).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Ir spectra were measured in $CHCl_3$ solution with a Perkin-Elmer 267 spectrophotometer. Uv spectra were measured in absolute EtOH using a Varian 2290 spectrophotometer. $^1$H-nmr spectra were obtained with a Bruker WM 250 spectrometer using TMS as internal standard. Mass spectra were obtained with an Associated Electrical Industries MS-902 instrumnent.

Chromatotron model 7924 (Harrison Research) was employed for preparative radial tlc. Rotors were coated with Kieselgel 60 $PF_{254}$ gipshalting (E. Merck). Rf measurements were made on analytical precoated glass plates, silica gel 60 F-254 (E. Merck). Solvent systems: A, $Et_2O$-hexane-MeOH (10:8:1); B, $Et_2O$-hexane-diethylamine (15:7:3); C, EtOAc-heptane (7:1).

EXTRACTION AND FRACTIONATION.—MeOH extracts of roots of *S. greggii* were supplied by Professor X. Dominguez (Departmento de Quimica, Instituto Technologio Y de Estudios Superiores de Monterrey, Sucursal de Correos "J", Monterrey, N.L., Mexico). Voucher specimen is deposited in the Herbarium of the Botany Department of the Instituto Technologio y de Estudios Superiores de Monterrey. The roots were collected on the slopes of Cerro de La Silla, N.L., Mexico. This extract was partitioned between 10% MeOH in $CH_2Cl_2$ and $H_2O$ (1000 ml each). Solvent was removed from the $CH_2Cl_2$ layer, and the residue (322 g) was partitioned between 90% aqueous MeOH and petroleum ether (800 ml each).

5,6-DIMETHOXYFLAVONE.—A portion (148 g) of a 90% MeOH fraction prepared as described above was applied to a silica gel column (800 g) and eluted with $CHCl_3$ containing 2% (8 liters), 4% (8 liters), 10% (6 liters), 20% (2 liters), and 50% (2 liters) MeOH, while 80-ml fractions were collected. Fractions 17-40 were combined, stripped of solvent (residue 31.0 g), and dissolved in minimal absolute EtOH. After standing 4 days, a crystalline precipitate was removed by filtration and recrystallized twice from absolute EtOH to yield 5,6-dimethoxyflavone (81.6 mg): mp 192-193° [lit. 196° (2)]; $^1$H nmr δ ($CDCl_3$) 7.90 (2H, m, H-3′,5′), 7.51 (3H, m, H-2′,4′,6′), 7.32 (2H, s, H-7,8), 6.69 (1H, s, H-3), 3.99, 3.94 (6H, s, 2 $OCH_3$).

ZAPOTIN.—Solvent was removed from the MeOH layer leaving a syrup (286 g) which stood at 4° for 5 months. A crystalline precipitate was filtered out after first diluting the supernatant slightly with EtOH. The precipitate was recrystallized three times from absolute EtOH and once from $Me_2CO$ to yield 1.02 g of zapotin: mp 144-145° [lit. 147-148° (5)]; $^1$H nmr, ms, and uv matched literature data (5).

5,6,2′-TRIMETHOXYFLAVONE.—Mother liquors (842 mg) from the crystallization of 5,6-dimethoxyflavone were separated by repeated preparative tlc ($Et_2O$-hexane-diethylamine, 12:20:2) to yield 5,6,2′-trimethoxyflavone (Rf 0.29, system B) which was recrystallized from absolute EtOH (24 mg): mp 123-124° [lit. 124-125° (6)]; λ max 235 (log ε 4.23), 267 (4.33), 326 (4.17) (5); ms *m/z* 312.0995 ($C_{18}H_6O_5$=312.0997). $^1$H-nmr data did not match the literature (5) but was identical to the spectrum of an authentic sample: $^1$H nmr δ ($CDCl_3$) 7.86 (1H, dd, $J$=1.7, 7.8 Hz, H-6′), 7.45 (1H, m, H-4′), 7.28 (2H, ABq, $J$=9.2 Hz, H-7, 8), 7.09 (1H, m, H-5′), 7.03 (1H, d, $J$=8.4 Hz, H-3′), 6.99 (1H, s, H-3), 3.98 (3H, s, $OCH_3$), 3.93 (6H, s, 2 $OCH_3$).

5,6,2′,3′,4′,6′-HEXAMETHOXYFLAVONE (**1**).—Fractions 41-56 from column chromatography were combined and stripped of solvent. A portion of the residue (730 mg) was subjected to preparative tlc ($Et_2O$-hexane-diethylamine, 12:20:2). A late-eluting fraction (Rf 0.17, solvent B) yielded 21.4 mg of **1** after recrystallization from $Et_2O$: mp 126-127°; ms *m/z* (rel. int. %) 402.1275 ($C_{21}H_{22}O_8$=402.1313) (78), 387 (100), 373 (8), 371 (8), 357 (21), 349 (7), 329 (7), 207 (10), 165 (8), 164 (5), 137 (8); λ max 229 (log ε 4.48), ca. 255, 326 (4.03); $^1$H nmr δ ($CDCl_3$) 7.24 (2H, ABq, $J$=9.2 Hz, H-7,8), 6.33 (1H,

s, H-5'), 6.28 (1H, s, H-3), 3.98, 3.94, 3.93, 3.90, 3.84, 3.79 (6 $OCH_3$), ($C_6D_6$) 4.03, 3.71, 3.68, 3.29, 3.28, 3.16.

3-(α,α-DIMETHYLALLYL)-HERNIARIN.—Fractions 1-16 from column chromatography were combined and condensed (50.9 g). A portion (1.0 g) was subjected to repeated preparative tlc (hexane-$CHCl_3$, 1:1) to yield 25 mg of the title compound (Rf 0.63, system A) after recrystallization from $Et_2O$-heptane: mp 126-128° [lit. 126-128° (7)]; $^1$H nmr δ ($C_6D_6$) 7.10 (1H, s, H-4), 6.5-6.7 (3H, m, H-5,6,8), 6.19 (1H, dd, $J$=10.7, 17.4 Hz, H-2'), 5.05 (1H, dd, $J$=1.1, 17.6 Hz, H-3'), 5.03 (1H, dd, $J$=1.1, 10.5 Hz, H-3'), 3.08 (3H, s, $OCH_3$), 1.46 [6H, s, $(CH_3)_2$]; ($CDCl_3$) 7.52 (1H, s, H-4), 7.26-7.36, 6.80-6.85 (3H, m, H-5,6,8), 6.18 (1H, dd), 5.10 (1H, d), 5.08 (1H, d), 3.86 (3H, s), 1.47 (6H, s). Uv and ms matched literature data (7).

SESELIN.—Fractions obtained from the repeated preparative tlc of column fractions 1-16 yielded seselin (Rf 0.49, system A) (34 mg), which crystallized from aqueous EtOH upon seeding with authentic material: mp 118-119° [lit. 119-120° (8)]; uv, ms, and $^1$H nmr matched literature data (8).

*O*-GERANYLOSTHENOL (**3**).—Fractions obtained from the repeated preparative tlc of column fractions 1-16 yielded **3** (Rf 0.56, system A) (20 mg) after recrystallization from $Et_2O$-heptane: mp 44-45°; ms *m/z* (rel. int. %) 366.2194 ($C_{24}H_{30}O_3$=366.2195) (8), 351 (4), 323 (6), 310 (7), 298 (5), 283 (2), 255 (2), 231 (78), 230 (100), 215 (24), 187 (46), 175 (73), 137 (19), 136 (24), 71 (85); $^1$H nmr δ ($CDCl_3$) 7.58 (1H, d, $J$=9.4 Hz, H-4), 7.24 (1H, d, $J$=8.7 Hz, H-5), 6.80 (1H, d, $J$=8.7 Hz, H-6), 6.2 (1H, d, $J$=9.4 Hz, H-3), 5.46 (1H, bt, H-2"), 5.22 (1H, bt, H-2'), 5.06 (1H, bt, H-6"), 4.62 (2H, d, $J$=6.5 Hz, $CH_2$-1"), 3.52 (2H, d, $J$=7.3 Hz, $CH_2$-1'), 2.08 (4H, bs, $CH_2CH_2$), 1.81 (3H, s), 1.72 (3H, s), 1.65 (6H, s), 1.59 (3H, s); λ max 248 (log ε 3.70), 257 (3.57), 323 (4.29)

CASIMIROINE.—A portion of the 90% MeOH fraction was applied to a column containing 700 g of silica gel (1.5 in.×40 in.) and eluted with increasing amounts of EtOAc in hexane, then with EtOAc, and then with increasing MeOH in EtOAc. A fraction eluted with 100% EtOAc possessed slight KB activity ($LD_{50}$ 6 μg/ml). This fraction (237 mg) was subjected to preparative tlc, eluting with EtOAc-heptane (1:1, 2:1, 4:1), EtOAc and EtOAc-iPrOH (19:1). A fraction having Rf 0.19 (system C) had KB activity of 8 μg/ml. This fraction (207 mg) was nearly one spot on tlc and was crystallized twice from $Me_2CO$ to yield 105 mg of casimiroine: mp 199-200°; ms *m/z* (rel. int. %) 233.0686 ($C_{12}H_{11}NO_4$=233.0688) (100), 218 (37), 204 (9), 202 (8), 190 (22), 179 (19), 175 (15). Uv (9) and $^1$H nmr (10) matched literature data. Purified casimiroine was not active in the KB assay.

SYNTHESIS OF 5,6,2',3',4',6'-HEXAMETHOXYFLAVONE (**1**).—2,3,4,6-*Tetramethoxyacetophenone*.—Dimethylformamide (20 ml) was added to 1.1 eq of NaH previously washed with heptane, and 6-hydroxy-2,3,4-trimethoxyacetophenone (976 mg) (Pfaltz & Bauer) was added. When bubbling subsided, 744 mg of $Me_2SO_4$ was added and allowed to stir for 30 min. Solvent was removed in vacuo, and the residue was taken up in $CH_2Cl_2$ and filtered. The filtrate was concentrated and the product purified by the Chromatotron ($CH_2Cl_2$-hexane-EtOAc, 65:30:5). The product was a faintly amber oil which crystallized upon standing for 3 days (936 mg, 90%): mp 50-51.5°; $^1$H nmr δ ($CDCl_3$) 2.52 (3H, s, $COCH_3$), 3.90 (6H, s, 2 $OCH_3$), 4.00 (6H, s, 2 $OCH_3$), 6.27 (1H, s, ArH); ir ν max ($CHCl_3$) 1700 $cm^{-1}$.

*Anal.* calcd for $C_{12}H_{16}O_5$: MW, 240.0998. Found: MW, 240.0995 (hrms).

*2,3,4,6-Tetramethoxybenzoic acid.*—The above acetophenone (770 mg) was added to 13.3 ml of 5.25% NaOCl (Clorox®) containing 5% additional NaOH in a cooled flask. The temperature was gradually raised to room temperature and then to 90° for 3 h. $NaHSO_3$ was added to decompose excess NaOCl which was detected in aliquots with the use of acidic KI solution. The mixture was chilled and extracted with three 20-ml portions of $Et_2O$. The aqueous layer was subjected to vacuum to remove $Et_2O$, and acidified with concentrated HCl. The product crystallized with cooling and stirring (554 mg, 65%): mp 151-152°; $^1$H nmr δ ($CDCl_3$) 3.82, 3.90, 3.92, 3.99 (4 $OCH_3$), 6.32 (1H, s, ArH); ir ν max ($CDCl_3$) 1730, 1700 $cm^{-1}$.

*Anal.* calcd for $C_{11}H_{14}O_6$: MW, 242.0790. Found: MW, 242.0792 (hrms).

*2,3-Dimethoxy-6-(2,3,4,6-tetramethoxybenzoyloxy)-acetophenone* (**6**).—The condensation of 2,3-dimethoxy-6-hydroxyacetophenone with the acid chloride of 2,3,4,6-tetramethoxybenzoic acid was low yielding, but the starting materials could be recovered and recycled. Typically, 100 mg of 2,3,4,6-tetramethoxybenzoic acid were dissolved in 3 ml of $SOCl_2$, refluxed under $N_2$ for 30 min, and the $SOCl_2$ was removed in vacuo. The hydroxyacetophenone (100 mg) was dissolved in pyridine with 0.5 mg of 4-dimethylaminopyridine, added to the acid chloride, and left on a steam bath for 15 h. The pyridine was removed in vacuo, and the product was purified by the Chromatotron ($CHCl_3$-EtOAc, 9:1) and recrystallized from heptane-EtOAc as pale yellow needles (47 mg): mp 126-127°; $^1$H nmr δ ($CDCl_3$) 2.54 (3H, s, acetyl), 3.9-4.0 (18H, 6 $OCH_3$), 6.28 (1H, s, H-2'), 6.97 (2H, ABq, $J$=9.2, H-4,5); ir ν max ($CHCl_3$) 1245, 1710 $cm^{-1}$. In all, 545 mg of the acid chloride was processed to yield 293 mg of **6** (26%).

*Anal.* calcd for $C_{21}H_{24}O_9$: MW, 420.1420. Found: MW, 420.1420 (hrms).

*2,3,2',3'4',6'-Hexamethoxy-6-hydroxydibenzoylmethane* (**7**).—The ester **6** (132 mg) was dissolved in 30 ml of pyridine with 300 mg of powdered KOH. The mixture was heated at 55° for 6 h under $N_2$. Solvent was removed in vacuo, and 2 ml of cold $H_2O$ were added. The solution was acidified with HOAc and extracted with five 2-ml portions of $CHCl_3$. The $CHCl_3$ was dried over $Na_2SO_4$ and evaporated, and the residue was purified by the Chromatotron ($CHCl_3$). The diketone **7** (56 mg) was a yellow, noncrystalline solid: $^1$H nmr δ ($CDCl_3$) 3.9-4.0 (18H, 6 $OCH_3$), 4.60 (1H), 6.92 (1H, CO-$CH_2$-CO), 6.31 (1H, s, H-3'), 6.70 (1H, d), 7.06 (1H, d, H-4,5); ir ν max ($CHCl_3$) 1580, 1600 $cm^{-1}$.

*Anal.* calcd for $C_{21}H_{24}O_9$: MW, 420.1420. Found: MW, 420.1420 (hrms).

*5,6,2',3',4',6'-Hexamethoxyflavone* (**1**).—The diketone **7** (49 mg) was dissolved in 3 ml of glacial HOAc with 300 mg of fused sodium acetate and refluxed for 4 h under $N_2$. Then, $H_2O$ (2 ml) was added, followed by 50% NaOH with cooling to neutralize the acid. This mixture was extracted with five 2-ml portions of $CHCl_3$, and the combined washings were dried over $Na_2SO_4$ and evaporated. The product was purified by the Chromatotron using $CHCl_3$ containing 0 to 20% EtOAc. Compound **1** was crystallized from $Et_2O$ to yield 37.4 mg (80%), mp 124-125°. Spectral characteristics were identical to those of the natural product.

## ACKNOWLEDGMENTS

Plant extracts of *S. greggii* were supplied by Professor X. Dominguez. An authentic sample of 5,6,2'-trimethoxyflavone was supplied by Dr. D.L. Dreyer. Authentic seselin was provided by Dr. A.D. Kinghorn. This research was supported by Grant RO1CA29890, Isolation of Antineoplastic Agents from Plants, National Cancer Institute, NIH.

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