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# FLAVONOIDS FROM THE LEAVES OF *CHROMOLAENA SUBSCANDENS*[1]

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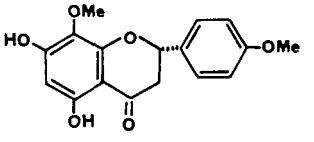
ABSTRACT.—Phytochemical analysis of the leaves from *Chromolaena subscandens* afforded β-sitosterol, umbelliferone, *p*-methoxybenzoic acid, and several known flavonoids, as well as a new one, 5,7-dihydroxy-8,4′-dimethoxyflavanone, which we have named subscandenin [**1**]. The structure of this new flavanone was established by spectral methods.

Several species of the genus *Chromolaena* (Compositae) are used in South American traditional medicine (1), and from this large genus, many potentially active metabolites, mainly terpenoids, flavonoids, and prostaglandin-like acid derivatives, have been isolated (2–5). In a continuation of our phytochemical studies of the Andean flora of Venezuela, we have examined the leaves of *Chromolaena subscandens* (Hieron.) King & Robinson, an evergreen shrub found throughout the temperate Andes from Bolivia to Venezuela, up to an altitude of 2000 m (6).

From $CH_2Cl_2$ and MeOH extracts of the leaves (see Experimental), we have isolated β-sitosterol, *p*-methoxybenzoic acid, umbelliferone, 7-α-(L)-rhamnosyl-kaempferol, kaempferitrin, sakuranetin, 5,4′-dihydroxy-6,7-dimethoxyflavanone (7), and 5,7-dihydroxy-8,4′-dimethoxy-flavanone. This last compound is a new flavanone, which we have named subscandenin [**1**].

Subscandenin [**1**] was isolated from the $CH_2Cl_2$ extract by a combination of Si gel cc and preparative tlc. The compound crystallized as yellow needles, mp 173–175°, $[\alpha]^{25}D$ −43.2°. It showed uv and ir absorption spectra characteristic for flavanones (8), in addition to bands typical of phenolic OH groups. The $^1$H-nmr spectrum displayed a pair of singlets for two phenolic MeO groups (δ 3.82 and 3.93), an aromatic proton singlet (δ 6.10, H-6), a pair of doublets (δ 6.94 and 7.37, $J = 9$ Hz) for 1,4-disubstituted benzene protons, and a characteristic ABX system (δ 5.35, dd, $J_{AX} = 5$ Hz, $J_{BX} = 12.5$ Hz and δ 2.90, m) for H-2 and H-3 signals (9). The hrms of **1** showed the molecular ion at *m/z* 316.09337 $[M]^+$ for $C_{17}H_{16}O_6$, and fragments typical of the cleavage of flavanones at *m/z* 182 $[A_1]^+$, 167 $[A_1 - Me]^+$, 154 $[A_1 - CO]^+$, 134 $[B_3]^+$ and 119 $[B_3 - Me]^+$, which suggested the location of an MeO and two OH groups on the A ring and an MeO group on the B ring (10).




**1**

In the $^{13}$C-nmr spectrum of **1**, the C-4 carbonyl signal appeared at δ 196.7, which is characteristic of a carbonyl associated with a free OH group at C-5 (11). This is in agreement with a dark purple color of the spot corresponding to compound **1** under long wavelength uv light (366 nm) and was further confirmed by the bathochromic shifts of band II in the uv spectra recorded with $AlCl_3$ and $AlCl_3$/HCl, as well as by the $^1$H-nmr spectrum exhibiting a low field singlet (δ 11.76) of a chelated OH group. The localization of the other free OH group could be ascertained because the uv spectra, in the presence of

[1]Part 10 in the series "Phytochemical Studies on the Venezuela Andean Flora." For Part 9, see J.M. Amaro-Luis, *Phytochemistry* (in press).

NaMeO and NaOAc, showed shifts typical of 5,7-dihydroxyflavanones (8).

The 5,7,8 substitution pattern was distinguished from the isomeric 5,7,6 pattern by the chemical shift of the aromatic singlet appearing at 6.10 ppm, which agreed with those reported for the H-6 from other known 5,7,8-substituted flavanones (12,13). On the other hand, the bathochromic shift of 23 nm in band II in the uv spectrum of **1**, after addition of $AlCl_3$ (compared to the spectrum in MeOH), favors the position of the MeO group at C-8 rather than at C-6. The presence of an MeO substituent at C-6 would result in a smaller bathochromic shift due to steric hindrance of complex formation with OH at C-5 (7, 12–15).

Finally, an *S* configuration at C-2 was proposed for **1**, since its specific rotation has a minus sign as in other natural flavanones. All these data are in agreement with a (2*S*)-5,7-dihydroxy-8,4′-dimethoxyflavanone structure for subscandenin [**1**].

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken on Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol III polarimeter. Ir spectra were recorded on a Perkin-Elmer model 377 as KBr pellets. Uv spectra were determined on a Varian Scan 3 spectrophotometer, using 1 cm quartz cells and MeOH as solvent. $^1$H- and $^{13}$C-nmr spectra were measured on a Bruker WP 200 SY and a Varian FT-80A, respectively, in $CDCl_3$ with TMS as internal standard. Hrms were obtained with a VG micomass ZAB-2F at 70 eV and lrms on a Hewlett Packard 5930A spectrometer. Tlc was carried out on 0.25 mm layers of Si gel PF 254 (Merck). Vcc was performated with Si gel 60 (70–230 mesh). Sephadex LH-20 (Pharmacia) was employed for gel filtration.

PLANT MATERIAL.—*C. subscandens* was collected in January 1986 above the small village of El Hato along the road Estánquez-El Molino, Mérida, Venezuela. A voucher specimen (JMAL 1564) was deposited in the MERF Herbarium (Faculty of Pharmacy, ULA).

EXTRACTION.—Air-dried leaves of *C. subscandens* (ca. 1.3 kg) were washed in $CH_2Cl_2$ for 20 min, and the solution was evaporated yielding extract A (27 g). The same leaves, dried and ground, were extracted with MeOH (3 × 10 liters) at room temperature, and the combined extracts were concentrated in vacuo to 3 liters; after adding 600 ml of $H_2O$, the mixture was left standing overnight in a refrigerator at 4°. It was then filtered, obtaining a yellow solid (impure kaempferitrin) and a solution that was successively extracted with $C_6H_{14}$, $CH_2Cl_2$, and EtOAc. The organic solutions were concentrated in vacuo yielding, respectively, extracts B (5 g), C (6 g), and D (21 g).

SEPARATION AND PURIFICATION OF COMPOUNDS.—Extracts A, C, and D were subjected to cc on Si gel. Elution was performed with petroleum ether or hexanes and increasing quantities of EtOAc. Fractions were combined, upon monitoring by tlc, as follows: A-1 [petroleum ether-EtOAc (19:1)] β-sitosterol; A-2 [petroleum ether-EtOAc (9:1)] **1** and 5,4′-dihydroxy-6,7-dimethoxyflavanone; A-3 [petroleum ether-EtOAc (17:3)] sakuranetin; A-4 [petroleum ether-EtOAc (4:1)] and A-5 [petroleum ether-EtOAc (1:1)] noncrystalline products; A-6 [petroleum ether-EtOAc (1:4)] *p*-benzoic acid; C-1 [$C_6H_{14}$-EtOAc (19:1)] umbelliferone; D-9 (EtOAc) 7-α-(L)-rhamnosylkaempferol. The yellow precipitate obtained from the MeOH solution was chromatographed over Sephadex LH-20 (MeOH) yielding pure kaempferitrin (6.3 g). Fraction A-2, subjected to vcc on Si gel, upon elution with $C_6H_{14}$-EtOAc (4:1), gave impure subscandenin [**1**] (120 mg). Final purification was carried out by preparative tlc over Si gel developed with $C_6H_6$-EtOAc (5:1) and filtration on Sephadex LH-20 column eluted with MeOH.

IDENTIFICATION OF COMPOUNDS.—Known compounds were identified by comparison of their physical constants and spectral data with those reported in the literature. Supplementary data are available upon request to the senior author.

*Subscandenin* [**1**].—Yellow needles from hexane/EtOAc: mp 173–175°; $[\alpha]^{25}D$ −43.2° ($c$ = 0.25, $CHCl_3$); ir ν max (KBr) 3050–3680, 2900, 1650, 1590, 920, 850 $cm^{-1}$; uv λ max (MeOH) 293, 331; +NaOMe 251 sh, 329; +NaOAc 253 sh, 329; +$AlCl_3$ 316, 390; +$AlCl_3$/HCl 314, 380; $^1$H nmr (200 MHz) δ ($CDCl_3$) 2.90 (2H, m, H-3), 3.93 (3H, s, OMe), 3.82 (3H, s, OMe), 5.35 (1H, dd, $J$ = 5.0 and 12.5 Hz, H-2), 6.10 (1H, s, H-6), 6.94 (2H, d, $J$ = 9.0 Hz, H-3′ and H-5′), 7.37 (2H, d, $J$ = 9.0 Hz, H-2′ and H-6′); 11.76 (1H, s, OH); $^{13}$C nmr (20 MHz, broad band and APT) δ ($CDCl_3$) 79.1 (C-2), 43.2 (C-3), 196.7 (C-4), 158.7 (C-5)*, 130.6 (C-6), 160.2 (C-7), 94.6 (C-8), 154.1 (C-9), 103.1 (C-10), 129.8 (C-1′), 127.6 (C-2′ and C-6′), 114.3 (C-3′ and C-6′),

157.5 (C-4')*, 55.3 (OMe), 60.8 (OMe) (* asterisk indicates that assignments may be reversed); hreims *m/z* (rel. int.) 316.09337 (15%) ($C_{17}H_{16}O_6$ requires 316.09469), 301 (2), 286 (1), 182 (25), 134 (17); lreims *m/z* (rel. int.) 316 (93%), 301 (9), 286 (6), 183 (10), 182 (90), 167 (71), 154 (20), 149 (28), 139 (10), 134 (40), 121 (27), 119 (19), 91 (22), 83 (55), 69 (100).

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