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A Cytotoxic Flavone from *Scoparia dulcis* L.

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5,7-Dihydroxy-3′,4′,6,8-tetramethoxyflavone (hymenoxin) was isolated from the whole plants of *Scoparia dulcis* L. (Scrophulariaceae) and found to be cytotoxic to cultured human cells.

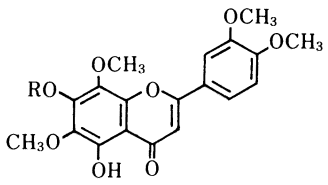
Keywords——flavone; hymenoxin; 5,7-dihydroxy-3′,4′,6,8-tetramethoxyflavone; *Scoparia dulcis*; Scrophulariaceae; cytotoxicity

In a continuation of our studies on biologically active substances of the Paraguayan crude drug, "Typychá kuratũ" (*Scoparia dulcis* L., Scrophulariaceae),[1] we isolated a polyoxygenated flavone (**1**), which was found to exhibit cytotoxicity against human cultured cells such as HeLa 229,[2] HeLa S3,[3] HEp-2,[4] FL,[5] Chang liver[6] and intestine 407[7] cells. Some flavonoids have been reported to be cytotoxic to KB,[8] P-388[9] or HeLa S3[10] cells. However, no comparative data on different cell lines have been reported so far. In this paper we report the identification of this flavone and its cytotoxicity to various cultured cells.

Results and Discussion

Dried, ground whole plants of *Scoparia dulcis* L. were extracted with 70% ethanol, and the extract was partitioned between chloroform ($CHCl_3$) and water. The $CHCl_3$-soluble fraction was repeatedly separated by column chromatograpy on silica gel to yield a polyoxygenated flavone (**1**) along with a diterpenoid (**2**) and an alkaloid (**3**). The compounds **2** and **3** were identified as scopadulcic acid B[1a] and 6-methoxybenzoxazolinone,[11] respectively, both of which were previously isolated from the same plant.

Compound **1** was obtained as yellow prisms, mp 221.5—223 °C. It was positive to the magnesium–hydrochloric acid (Mg–HCl) and the ferric chloride ($FeCl_3$) tests, results which are indicative of a flavonoid. The high-resolution mass spectrum (MS) of **1** exhibited a molecular ion $(M)^+$ at *m/z* 374.0985 for $C_{19}H_{18}O_8$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the presence of four methoxyls, two hydroxyls and four aromatic protons. The splitting pattern of aromatic proton signals is very similar to that of a 3′,4′,5,6,7,8-hexasubstituted flavone such as 5,6-dihydroxy-3′,4′,7,8-tetramethoxyflavone,[12] 4′,5-dihydroxy-3′,6,7,8-tetramethoxyflavone,[13] and 3′,4′,5-trihydroxy-6,7,8-trimethoxyflavone.[14] The mass spectral peaks at *m/z* 359 $(M-Me)^+$, 197 $(A_1-Me)^+$ and 169 $(A_1-Me-CO)^+$ indicated the A-ring to be substituted by two methoxyl and two hydroxyl groups,[15] suggesting the other two methoxyl groups to be at C-3′ and C-4′. The presence of a hydroxyl proton signal at δ 12.75 indicated that one of the hydroxyl groups must be at C-5. This was confirmed by the fact that methylation of **1** with diazomethane gave 5-hydroxy-3′,4′,6,7,8-pentamethoxyflavone (**4**), which was identified by direct comparison with an authentic sample isolated previously from *Citrus* species by Iinuma *et al.*[16] The ultraviolet (UV)



1 : R=H
4 : R=CH_3

Chart 1

TABLE I. ID_{50} Values of Hymenoxin for Cell Growth

Cell line	ID_{50}[a]
HeLa 229	0.097
HeLa S3	0.140
HEp-2	0.148
FL	0.283
Chang liver	0.510
Intestine 407	0.264

a) Dose necessary to inhibit 50% of the cell growth. Values are expressed as μg/ml.

spectra of **1** in methanol and methanol with shift reagent were very similar to those of hymenoxin isolated from *Hymenoxys scaposa* by Thomas and Mabry.[17] The direct comparison of the spectral data of **1** with those of hymenoxin synthesized by Horie *et al.*[18] revealed both compounds to be identical. Thus, compound **1** was characterized as 5,7-dihydroxy-3′,4′,6,8-tetramethoxyflavone. This is the first report of isolation from *Scoparia dulcis*.

Next, the cytotoxic effects of **1** were examined in six cell lines derived from human cancer tissues (HeLa 229, HeLa S3 and HEp-2) and human normal tissues (FL, Chang liver and intestine 407). As shown in Table I, **1** exhibited significant inhibitory effects on the growth of all cell lines. There was a difference of susceptibility to **1** between the cell lines from cancer tissues and those from normal tissues, the former being relatively sensitive and the latter resistant. These results, however, do not always imply that compound **1** is a selective inhibitor of cancer cells. For the evaluation of this compound as an anti-cancer agent, further detailed *in vivo* tests would be necessary.

Experimental

Apparatus——Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. UV spectra were recorded on a Hitachi 220 S spectrophotometer and IR spectra were taken on a Hitachi 260-10 infrared spectrophotometer. ^{1}H-NMR spectra were measured with a JEOL GX-270 (270 MHz) spectrometer. Chemical shifts are given in δ-values (ppm) with tetramethylsilane as an internal standard. Mass spectra were measured with a JEOL JMS-D 200 mass spectrometer at an ionization voltage of 70 eV.

Plant Materials——Plants of *Scoparia dulcis* L. were collected in Paraguay and the voucher specimens have been deposited in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, and Sección Botanica, Facultad de Ciencias Quimicas, Universidad Nacional de Asunción.

Extraction and Isolation——Dried, ground whole plants (3 kg) of *Scoparia dulcis*, collected in March, 1987, were extracted with 70% EtOH three times. The combined extracts were concentrated under reduced pressure and the aqueous residue was further lyophilized to give a brown powder (439 g). The extract was partitioned between $CHCl_3$ and water. The $CHCl_3$-soluble fraction was concentrated under reduced pressure to afford a dark brown residue (110 g). The $CHCl_3$-soluble material (46 g) were chromatographed on a silica gel column with an MeOH–$CHCl_3$ stepwise gradient and the fractions showing yellow spots on a thin layer chromatography (TLC) plate after spraying 10% H_2SO_4 followed by heating were further chromatographed on a silica gel column using $CHCl_3$–MeOH to give a mixture of compounds **1**—**3**. The mixture was purified to give **1** (100 mg), **2** (5.8 g) and **3** (67 mg) by stepwise recrystallization. Compound **2** was identified as scopadulcic acid B by direct comparison with an authentic sample (mp, ^{1}H-NMR, IR), and the spectral data (IR, UV, ^{1}H-NMR) of **3** agreed with those reported for 6-methoxybenzoxazolinone.[11]

5,7-Dihydroxy-3′,4′,6,8-tetramethoxyflavone (1)——Yellow prisms ($CHCl_3$–MeOH), mp 221.5—223 C. High-resolution MS *m/z*: 374.0985 (Calcd for $C_{19}H_{18}O_8$: 374.1000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1660, 1620, 1590. UV λ_{max}^{MeOH} nm (log ε): 242 sh, 250 sh, 281 (4.24), 340 (4.30); $\lambda^{MeOH+NaOMe}$: 231 sh, 283, 310 sh, 378; $\lambda^{MeOH+AlCl_3}$: 232 sh, 254 sh, 297, 360; $\lambda^{MeOH+AlCl_3+HCl}$: 232 sh, 254 sh, 297, 358; $\lambda^{MeOH+NaOAc}$: 238 sh, 284, 314 sh, 374; $\lambda^{MeOH+NaOAc+H_3BO_3}$: 283, 335. ^{1}H-NMR ($CDCl_3$) δ: 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 6.42 (1H, s, 7-OH), 6.60 (1H, s, 3-H), 7.01 (1H, d, J=8.8 Hz, 5′-H), 7.41 (1H, d, J=2.2 Hz, 2′-H), 7.59 (1H, dd, J=8.8, 2.2 Hz, 6′-

H), 12.75 (1H, s, 5-OH). MS *m/z*: 374 $(M)^+$ (93), 359 (100), 356 (3), 197 (17), 169 (23). These spectral data agreed with those of authentic hymenoxin.

Methylation of 1——Compound **1** (10 mg) was treated with diazomethane as usual to afford a crystalline material (12 mg), which was purified by recrystallization from $CHCl_3$–MeOH to give yellow needles, mp 149—151 °C. High-resolution MS *m/z*: 388.1148 (Calcd for $C_{20}H_{20}O_8$: 388.1157). ^{1}H-NMR ($CDCl_3$) δ: 3.96 (3H, s, OCH_3), 3.98 (9H, s, $3 \times OCH_3$), 4.12 (3H, s, OCH_3), 6.62 (1H, s, 3-H), 7.01 (1H, d, J=8.8 Hz, 5′-H), 7.43 (1H, d, J=2.2 Hz, 2′-H), 7.59 (1H, dd, J=8.8, 2.2 Hz, 6′-H), 12.55 (1H, s, OH). These spectral data agreed with those of authentic 5-hydroxy-3′,4′,6,7,8-pentamethoxyflavone.

Cytotoxicity Assay——HeLa 229, HeLa S3, HEp-2, FL, Chang liver and intestine 407 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS). For growth inhibition studies, 2×10^4 cells in 0.4 ml of DMEM supplemented with 10% FCS were seeded into each well of 24-well plates, and cultured for 72 h at 37 °C in the presence of increasing amounts of the compound. After the medium was removed, cell monolayers were trypsinized and the cell number was determined with a conventional hemocytometer using the trypan blue-exclusion method. The inhibition data were plotted as dose–effect curves from which the 50% inhibitory doses (ID_{50}) were obtained. The ID_{50} values are the average of values obtained in three assays with four concentrations within the inhibitory range of the compound.

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