

do not show any appreciable potential for accumulation in the adipose tissue.

Analysis of the liver tissue from cows at all dose levels showed very low levels of detectable mefluidide residues; the maximum levels found were <0.01, <0.01, and <0.03 ppm in the 6, 18, and 60 ppm dose groups, respectively.

In the entire study, kidney was the only tissue that contained greater than 0.03 ppm of mefluidide residues. An average of 0.04, 0.19, and 0.26 ppm of mefluidide was found in kidneys of cows administered 6-, 18-, and 60-ppm doses of mefluidide, respectively. The presence of some mefluidide residues in the kidney might be predicted on the basis of findings by Ivie (1980) that an animal excreted the radiocarbon-labeled mefluidide rapidly and almost totally (>93%) via urine. In the radiotracer study, radiocarbon residues in the kidney of the cow corresponded to 0.005 ppm of mefluidide equivalents, with the sample being taken 5 days after the fifth and the final dose of [¹⁴C]mefluidide. In the present 28-day study, however, the animals were sacrificed within 24 h of the final dosing with mefluidide. The results of these two studies suggest that the residues seen in the kidney will fall below GLC detectable levels (0.01 ppm) within a few days following withdrawal of mefluidide-treated feed.

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Flavones of *Scutellaria ovata*

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Seven flavones were isolated and identified from hot methanol extracts of roots, stems, and flowers of *Scutellaria ovata* (skullcap). Five of the compounds, chrysin, spigenin, luteolin, 6-methoxyluteolin, and dinatin, were previously known. Two new flavones, oroxylin 7-O-glucoside and ovatin (5,6-dimethoxyflavone 7-O-glucoside), were identified. Each compound was tested for its effect on radish root and stem growth. Chrysin reduced radish root growth by 20% at 100 ppm as compared with the water control.

The genus *Scutellaria* (skullcap) belongs to the Lamiales family. The chemistry of flavonoids from several species of *Scutellaria*, all of which are common to Europe, has been investigated. Bargellini (1919) found baicalein and baicalin (7-O-glucuronide of baicalein) in *Scutellaria baicalensis*. Bandyakova (1969) isolated the 7-β-D-glucuronoside, scutellarein, from *Scutellaria polyodon*. Denikeena et al. (1970) isolated and identified eight flavonoids from *Scutellaria przewalskii*. Litvinenko et al. (1971) identified baicalin and the glucosides of apigenin and luteolin, wogonin, apigenin, and luteolin from *Scutellaria scordifolia*. The phytotoxicity and structures of flavonoids of *Scutellaria* species indigenous to the United States that have not been investigated are the subject of this report.

MATERIALS AND METHODS

Extraction of *Scutellaria ovata*. Fresh plant material was collected and identified by the Botanical Institute of

Mississippi State University. Roots, leaves, and flowers were separately steeped in hot water (50 °C) for 5 min to stop enzymatic activity. The water was decanted, and the plant material was finely ground in a blender with MeOH and heated at reflux for 2 h. The methanolic extract of flowers (600 g) yielded 5.0 g (0.8% FW) of solids. The methanolic extract of roots (1 kg) also yielded 5.0 g (0.5% FW) of solids.

Fractionation of Methanolic Flower Extract. The scheme for fractionation of the methanolic extract of *Scutellaria ovata* flowers is shown in Figure 1. The flower extract (5 g) was chromatographed on a Sephadex LH-20 column (*l* = 1 m, i.d. = 3 cm; solvent = MeOH) to give two fractions: A, 780 mg (15.5%), and B, 450 mg (9.0%). Fraction A was chromatographed on a silica gel column (*l* = 45 cm, i.d. = 3 cm; solvent = benzene-EtOAc, 8:2 v/v) to give A₁ (300 mg, 38.5%) and A₂ (200 mg, 25.6%). The collection of these two fractions was monitored by TLC plate (silica gel with the same solvent).

A₁ and A₂ were separately introduced into a DCC (Droplet countercurrent apparatus, Tokyo Rikakikai, Toyama-Cho, Kanda Chiyoda-ku, Tokyo, Japan). The upper phase of a solution of CHCl₃-MeOH-water (65:35:20 v/v/v) was the stationary phase, and the lower phase of

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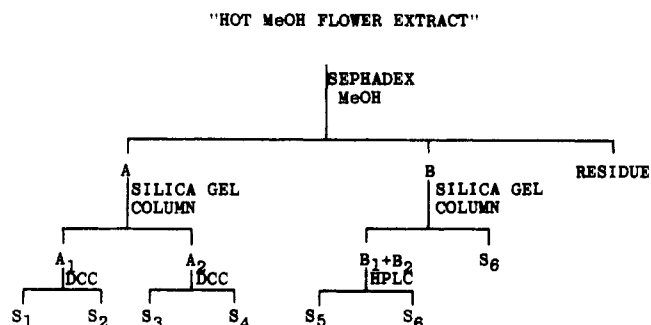


Figure 1. Fractionation scheme of the methanolic extract of *S. ovata* flowers.

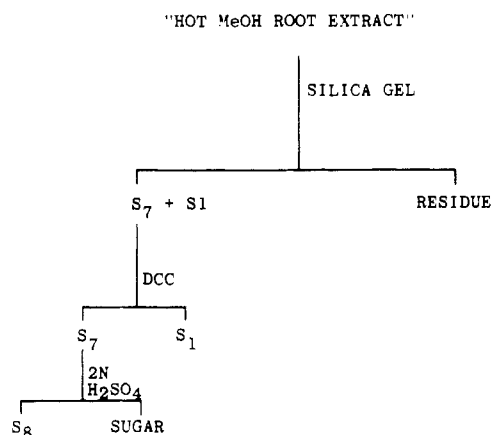


Figure 2. Fractionation scheme of the hot methanolic extract of *S. ovata* roots.

this solvent mixture was the moving phase. In this separation, 72 DCC capillary tubes were used with a flow rate of 16.8 mL/h. The eluates were collected in 10-mL fractions and monitored by TLC on precoated silica gel plates. A₁ gave S₁ (20 mg, 6.7%) and S₂ (220 mg, 73.3%), and A₂ gave S₃ (40 mg, 20.0%) and S₄ (50 mg, 25%). All were flavones (Figure 1).

Fraction B (200 mg) was chromatographed on a silica gel column (*l* = 45 cm, i.d. = 3 cm; solvent = lower phase CHCl₃-MeOH-HOH, 65:35:20 v/v/v) to give B₁ (10 mg) and B₂. Droplet countercurrent separation of B₁ and B₂ was made by using the same solvent system used for A₁ and A₂. Fraction B₁ gave S₅ (10 mg) and B₂ gave S₅ (10 mg) and S₆ (30 mg).

Figure 2 gives the scheme for fractionation of the hot methanolic extract of *S. ovata* roots. The extract (2 g) was chromatographed on a silica gel column (*l* = 45 cm, i.d. = 3 cm; solvent = benzene-EtOAc, 5:5 v/v) to give C₁ and residue. Fraction C₁ was separated by DCC by using the previously described solvent system to give S₁ (5 mg, 0.3%) and S₇ (35 mg, 1.8%).

Bioassay. The radish root growth inhibition bioassay technique was essentially that of Stevens and Merrill (1980) in which purified or partially purified samples were dissolved in 0.5 mL of EtOH or MeOH, mixed with 60 mL of hot 0.35% agar solution, and allowed to heat for 5 min. The agar solution was poured into Petri dishes, and eight radish seeds were placed on each plate of cooled agar. After 4 days at 20 °C the root length was measured to the nearest 0.5 mm and compared with the root length of the control plants on untreated agar plates.

Identifications. Figure 3 gives the structures of the flavonoids isolated and identified from *S. ovata*. Fraction S₁ (mp 162 °C dec) had a bathochromic shift of the λ_{max} with AlCl₃ and AlCl₃ plus HCl characteristic of a 3-

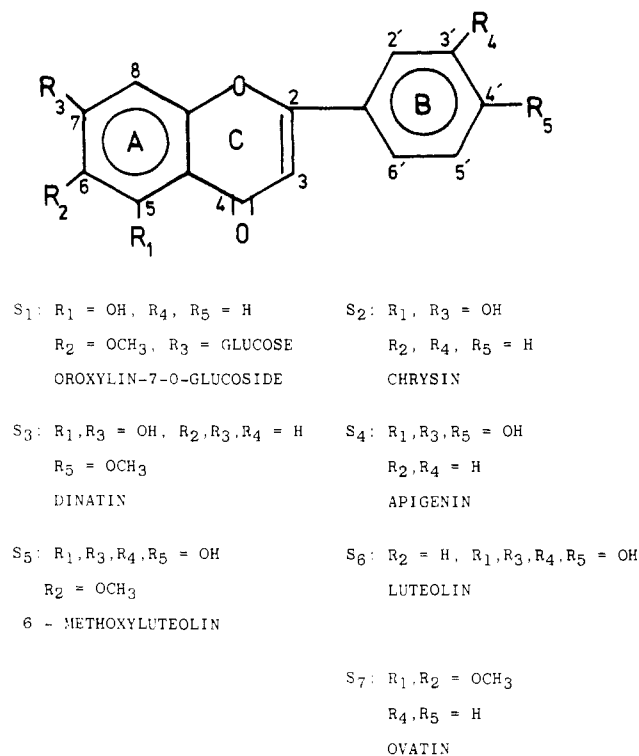


Figure 3. Structures of the flavonoids isolated from *S. ovata*.

oxygenated function on ring A. Hydrolysis of S₁ with 2 N sulfuric acid gave λ_{max} shifts with AlCl₃, AlCl₃ plus HCl, and NaOMe common to oroxylin (Sreevama et al., 1947). The mass spectrum of the hydrolyzed aglycon of S₁ corresponds to the fragmentation of oroxylin (Kingston, 1971). Acid hydrolysis of S₁, followed by neutralization with BaCO₃ and TLC on cellulose [BuOH-pyridine-water (9:5:4 v/v/v)] gave glucose. The ¹H NMR of the aglycon of S₁ was identical with that of oroxylin (Rivaille and Mentzner, 1965): ¹H NMR, S₁-RMS (CDCl₃) δ 7.86-7.79 (2 H, H-2', H-6'), 7.53-7.44 (3 H, m, H-3', H-4', H-5'), 6.65 (1 H, s, H-8) 6.57 (1 H, s, H-3), 5.26 (1 H, s, H-1 glucosyl), 3.75 (3 H, s, 6-OCH₃), 3.5-3.4 (6 H, 6 s, glucosyl). These data indicate S₁ is oroxylin 7-O-glucoside.

Fraction S₂ (mp 279 °C dec). Comparison of UV shifts, ¹H NMR, and mass spectra with those of a pure sample showed it was identical to chrysin.

Fraction S₃ (mp 226 °C dec). The UV shifts of this flavone correspond to those of dinatin (Denikeeva et al., 1970) and the mass spectrum of Kingston (1971); ¹H NMR (CD₃OD) δ 7.86-7.75 (2 H, dd, H-2', H-6', *J* = 8 Hz), 6.96-6.85 (2 H, dd, H-3', H-5', *J* = 8.2 Hz), 6.52 (1 H, s, H-8), 6.48 (1 H, s, H-3), 3.86 (3 H, s, 6-OCH₃).

Fraction S₄ (mp 340 °C). The UV, mass, and ¹H NMR spectra of this component corresponded in detail with those of a pure sample of apigenin.

Fraction S₅ (mp 299 °C). Shifts in the UV spectrum with the shift reagents (AlCl₃; NaOMe) corresponded to those of 6-methoxyluteolin (Chirikdjan and Bleier, 1971); MS *m/e* (rel intensity) 316 (22, M⁺), 301 (14), 298 (15), 273 (100); ¹H NMR (CD₃OD) δ 7.37-7.32 (2 H, m, H-2', H-6'), 6.94-6.83 (1 H, d, H-5', *J* = 9 Hz), 6.51 (1 H, s, H-3), 6.43-6.41 (1 H, d, H-8, *J* = 2 Hz), 6.21-6.18 (1 H, s, H-6, *J* = 2 Hz), 3.88 (3 H, s, 6-OCH₃).

Fraction S₆ (mp 325 °C dec). ¹H NMR of the Me₄Si derivative and UV spectrum were identical with those of luteolin (Mabry et al., 1970); MS *m/e* (rel intensity) 286 (100, M⁺), 270 (14), 258 (27), 153 (28).

Fraction S₇ (mp 149 °C dec). UV (MeOH) 310 nm, 265; UV (AlCl₃) 310 nm, 290 sh, 262; UV (AlCl₃ plus HCl) 310

nm, 290 sh, 263; (NaOMe) 360 nm, 268, 240 sh. The absence of shifts with AlCl_3 is characteristic of flavonoids that do not contain hydroxyl groups at carbons 5, 6, and 7. A bathochromic shift with sodium methoxide is indicative of a sugar or hydroxyl at the 7 position. Hydrolysis of S_7 with H_2SO_4 gave fraction S_8 : $^1\text{H NMR}$ (CD_3OD) δ 7.88-7.56 (2 H, m, H-2', H-6'), 7.53-7.48 (3 H, m, H-3', H-4', H-5'), 6.81 (1 H, s, H-3), 6.63 (1 H, s, H-8), 3.91 (3 H, s, 5- OCH_3), 3.89 (3 H, s, 6- OCH_3) (Massicot and Marthe, 1962). The presence of a methoxyl group at C-5 is shown by proton resonance at 3.95-4.05 ppm (Anker et al., 1969) and with a shift of 0.33 ppm downfield upon the addition of trifluoroacetic acid. The mass spectrum of S_8 was as follows: MS m/e (rel intensity) 298 (9, M^+) 297 (7), 283 (100), 267 (9), 255 (10), 241 (3). The $\text{M} - 1$ fragment confirms the methoxy group (Kingston, 1971). Cellulose TLC (solvent = BuOH-pyridine-water, 9:5:4 v/v/v) of the acid hydrolysate after neutralization with BaCO_3 gave glucose. The conclusion is that S_7 is ovatin (5,6-dimethoxyflavone 7- O -glucoside).

DISCUSSION

The root growth inhibition of individual flavones and aglycons from *S. ovata* was no greater than 20% at 100 ppm. Chrysin had this strongest activity, reducing radish root growth 20% at 100 ppm when compared to the control.

Five of the flavonoids found in *S. ovata* have previously been observed in various species of *Scutellaria* indigenous to Europe. The aglycon of oroxylin 7- O -glucoside (S_1 , Figure 2) was isolated and identified by Naylor and Chaplin (1890) from *Oroxylum indicum* and synthesized by Rivaille and Mentzner (1965). Ovatin (S_7 , Figure 3) has not been found in *Scutellaria* previously but is related to biacalein (5,6-dihydroxyflavone 7- O -glucuronide) found in

the root of *S. biacalensis* (Bargellini, 1919) and *S. scorodifolia* (Popova et al., 1976). Most of the flavones isolated from *Scutellaria* species are characterized by the absence of hydroxyl groups on the B ring, which is rare among flavonoids (Harborne, 1964).

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Caloric Utilization and Disposition of [^{14}C]Polydextrose in the Rat

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Polydextrose is a tasteless, nonsweet, low caloric bulking agent formed by the random polymerization of glucose with lesser amounts of sorbitol and citric acid. It is not absorbed after oral administration, and the major portion (~60%) of polydextrose is excreted in the feces. A fraction of fed polydextrose (~30%) is fermented in the lower gut by the intestinal microflora to products such as volatile fatty acids (VFA) and CO_2 ; the VFA are caloric to the host, but the CO_2 is not. Metabolic studies show that polydextrose has approximately 1 cal/g, or about 25% the value of glucose. Polydextrose can serve as a total or partial replacement for sugar and as a partial replacement for fat and flour in a variety of common processed foods with accompanying caloric reduction of those foods.

The regulation of caloric intake and body weight control for most individuals is desirable but not always readily achievable within the context of modern affluent society. For the most part, caloric control is accomplished by simply limiting the intake of calories so that it is less than or equal to the energy expenditure. Some personal discomfort is commonly experienced while achieving desired goals of weight control.

An attractive approach toward the problem of weight regulation is the normal ingestion of tasteful foods which are devoid of or deficient in calories. It is unreasonable

to expect that an entire palatable, nutritious, and varied diet may be assembled that is essentially free of calories. It is not unrealistic, however, to expect that a moderate portion of a daily diet may be replaced with palatable foods having reduced caloric content.

We report on a substance, polydextrose, which resists enzymatic and microbial attack and thus forms the basis for its utilization as a water-soluble, low caloric bulking agent in food products (Rennhard, 1973, 1981). Applications in a wide variety of foodstuffs show that polydextrose can serve as a total or partial bulk replacement for sugar and as a partial replacement for fat and flour. It may be incorporated into an assorted spectrum of foods such as baked products, candies, ice cream, puddings, salad

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