($CDCl_3$) δ 7 70–7 90 (*m*, 2H), 7 40–7 60 (*m*, 3H), 6 73 (*s*, 1H), 6 64 (*s*, 1H), 6 05 (*s*, 2H), 4 12 (*s*, 3H), ^{13}C NMR ($CDCl_3$): δ 177 5 (*s*, C=O, C-4), 160 8 (*s*, C-2 or C-9), 154 7 (*s*, C-2 or C-9), 152 9 (*s*, C-5), 141 4 (*s*, C-7), 134 9 (*s*, C-6), 131 4 (*s*, C-1'), 131.2 (*d*, C-4'), 128 9 (*d*, C-2' or C-6'), 125 9 (*d*, C-3' or C-5'), 112 9 (*s*, C-10), 108.3 (*d*, C-3), 102 1 (*t*, O–CH_2–O), 93 2 (*d*, C-8), 61 1 (*q*, OMe), EIMS *m/z* (rel Intensity) 296 (M^+ 34 1), 268 (55 5, M-CO), 250 (54 8), 222 (10 6), 237 (10 6), 194 (10 1), 166 (100), 164 (96 7), 136 (33 9), 105 (17 8), 102 (62 1)

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8-HYDROXYTRICETIN 7-GLUCURONIDE, A β-GLUCURONIDASE INHIBITOR FROM *SCOPARIA DULCIS*

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Key Word Index—*Scoparia dulcis*, Scrophulariaceae, flavonoids, 5,7,8,3',4',5'-hexahydroxyflavone 7-*O*-β-D-glucuronide, β-glucuronidase inhibitor

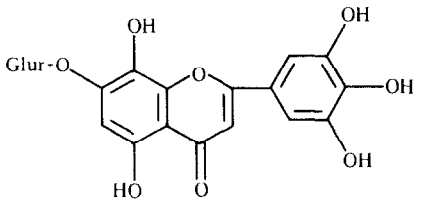
Abstract—A new flavone glycoside has been isolated from *Scoparia dulcis* together with 11 known compounds. The new glycoside was determined as 5,7,8,3',4',5'-hexahydroxyflavone glucuronide by spectral analysis. The new glycoside and isovitexin showed inhibitory activity against β-glucuronidase

INTRODUCTION

Scoparia dulcis L is a perennial herb which has been used for the treatment of stomach disease and hepatosis in Paraguay, as a cure for hypertension in Taiwan [1, 2] and for toothache, blennorrhagia and stomach problems in India [3]. From Indian *S. dulcis*, an antidiabetic compound named amellin was isolated by Nath [4] Earlier phytochemical studies on this medicinal plant resulted in isolation of hexacosanol, D-mannitol, sitosterol [3] and 6-methoxybenzoxazolinone [5] as well as triterpenoids [5, 6] and flavonoids [7] Previously, we reported the isolation and structural elucidation of five new diterpene acids from the 70% ethanolic extract of a plant collected in Paraguay [8–10] In a continuation of this work, we have examined the water-soluble fraction which showed mild inhibitory activity against β-glucuronidase. This paper deals with isolation of flavonoids from this fraction and their inhibitory activity against β-glucuronidase.

RESULTS AND DISCUSSION

Eleven flavonoids (**1**–**11**) and a phenylpropanoid (**12**) were isolated from the water-soluble fraction of a 70% ethanolic extract of *S. dulcis*. The compounds, **1**–**10**, **12**, were identified as apigenin (**1**), scutellarein (**2**), luteolin (**3**), vicenin-2 (**4**), linarin (**5**), vitexin (**6**), isovitexin (**7**), scutellarin (**8**), scutellarin methyl ester (**9**), luteolin 7-glucoside (**10**) and *p*-coumaric acid (**12**) by direct comparison of their physical and spectroscopic properties (mp, IR, UV, ^1H NMR and ^{13}C NMR) with those of authentic samples,



11

respectively Compounds **1**, **2** and **3** are considered to be artifacts from their respective glycosides

Compound **11** gave positive colour reactions with Mg/HCl and $FeCl_3$. The UV spectrum exhibited the characteristic absorptions of a flavonoid skeleton at 348 nm (band I) and 275 nm (Band II) [11]. A large bathochromic shift of band I of 87 nm was observed in the presence of $AlCl_3$ suggesting the presence of free *ortho*-dihydroxyl groups in the B-ring Addition of HCl to this solution caused a hypsochromic shift of Band I of 75 nm, but bathochromic shifts of Band I and Band II still remained relative to the original spectrum indicating the presence of a chelated hydroxyl group at C-5 [11] No bathochromic shift of Band II was observed with sodium acetate indicating the absence of a free hydroxyl group at the C-7 position. The UV spectrum exhibited a bathochromic shift of Band I upon addition of NaOMe suggesting the presence of a free 4′-hydroxyl group. The ^{1}H NMR spectrum revealed five hydroxyl protons (δ12 41, 1H, 9.46, 2H, 9.15, 1H, 8 74, 1H) and four aromatic protons (δ6 62, *s*, 1H, 6 66, *s*, 1H; 7.09, *s*, 2H) indicating oxygenation at C-3′, C-4′, C-5′, C-5, C-7 and C-6 or C-8. The A ring substitution pattern of **11** was determined by comparison of its ^{13}C NMR spectrum with those of scutellarin (**8**) and isoscutellarein 4′-methylether 7-*O*-[2″-*O*-(6‴-acetyl)-β-D-allopyranosyl]-β-D-glucopyranoside (**13**) [12, 13] The chemical shifts of the carbons on the A-ring of **11** were similar to those observed for the A-ring of **13** indicating a 5,7,8-substitution of the A-ring in **11** (See Table 1) The ^{13}C NMR spectrum also showed signals due to a glucuronic acid moiety [14]

Table 1 δ values in ^{13}C NMR spectrum of compounds **11**, a 5,6,7-substituted flavone (**8**) and a 5,7,8-substituted flavone (**13**) in DMSO-d_6

Position	11	13	8
2	164 39 *s*	163 4	164 03 *s*
3	102 66 *d*	103 3	102 44 *d*
4	182 13 *s*	182 2	182 28 *s*
5	150.48 *s*	150 5	146 76 *s*
6	98.00 *d*	99 4	130 35 *s*
7	151 90 *s*	152 0	148 93 *s*
8	126 80 *s*	127 6	93.47 *d*
9	144 47 *s*	143.7	150 88 *s*
10	105 20 *s*	105 5	105 80 *s*

8 = scutellarin, **11** = isoscutellarein 4′-methylether 7-*O*-[2″-*O*-(6‴-acetyl)-β-D-allopyranosyl-β-D-glucuronide [12, 13]

From the coupling constant (6 6 Hz) of the anomeric proton appearing at δ 5.17 in the ^{1}H NMR spectrum of **11**, a β-linkage of glucuronic acid at C-7 was suggested In the EIMS spectrum of **11**, a fragment ion peak at *m/z* 318 (M^+ − glucuronic acid) was observed and its elemental composition determined as $C_{15}H_{10}O_8$ by HRMS. The fragment ion peaks at *m/z* 169 and 150 correspond to the A- and B-rings, respectively Thus, **11** was elucidated as 5,7,8,3′,4′,5′-hexahydroxyflavone 7-*O*-β-D-glucuronide

The inhibitory activities of the isolated flavonoids (**1**–**11**) against β-glucuronidase were measured according to Nobunaga's method with a slight modification [15] The results are shown in Table 2 Of 11 flavonoids, only isovitexin (**7**) and **11** showed mild inhibitory activity against β-glucuronidase from bovine liver The inhibitory activity of **11** was one-tenth of that of the well known β-glucuronidase inhibitor, glucosaccharo-1,4-lactone Kinetic studies were conducted with **11** in order to determine the type of inhibition The Lineweaver-Burk plots of the inhibition by **11** are given in Fig 1, and from these results **11** seems to be a mixed-type inhibitor of β-glucuronidase The inhibitory effect of **11** on β-glucuronidase was also examined in the presence of a large amount of bovine serum albumin (BSA) (100–400 times) In these conditions **11** showed almost the same degree of inhibition in the presence of 400 times of BSA, suggesting that it inhibits the activity of β-glucuronidase even in the presence of other proteins

EXPERIMENTAL

General All mps uncorr The ^{1}H NMR spectra were taken at 270.05 MHz and the ^{13}C NMR spectra were measured at 50 3 MHz in DMSO-d_6 solutions, and chemical shifts are given in δ(ppm) with TMS as int standard The EIMS spectra were obtained at 70 eV

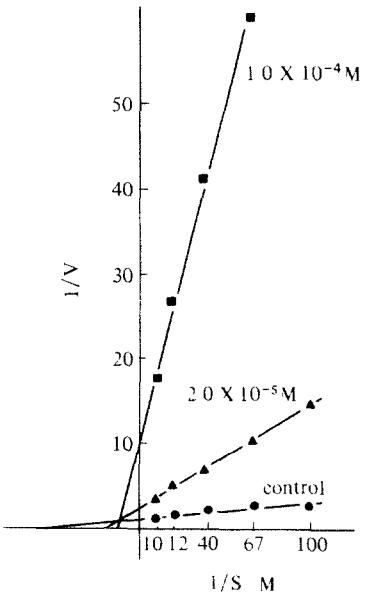


Fig 1. Inhibitory effects of 5,7,8,3′,4′,5′-hexahydroxyflavone 7-*O*-β-D-glucuronide on β-glucuronidase (Lineweaver-Burk plots) 1/V [1/(optical density at 405 nm)] × 1000, 1/S 1/(concentration of *p*-nitrophenyl-β-D-glucuronide)

Plant material *Scoparia dulcis* L. was collected in March 1985, near Asunción, Paraguay Voucher specimens have been deposited in the herbarium of herbal garden, Toyama Medical and Pharmaceutical University

Extraction and isolation of flavonoids. Dried whole plants of *S dulcis* (2 kg) were ground and extracted with hot 70% EtOH × 3. The extract was concd *in vacuo* and the residue still containing water was freeze-dried to give a brown powder (118 g) a 100 g of which was partitioned between H_2O and *n*-hexane and the aqueous layer extracted successively with $CHCl_3$, Et_2O, EtOAc–MeOH (9 1) and EtOAc–MeOH (4·1). The Et_2O extract (1.7 g) was run on a polyamide column followed by separation on a Sephadex LH-20 column to give 3 flavonoids, **1** (7.4 mg), **2** (9.1 mg), **3** (5.1 mg) and **12** (18.3 mg). The EtOAc–MeOH (9 1) extract was fractionated by a combination of polyamide CC, prep. TLC (cellulose) and Sephadex LH-20 CC to afford **4** (trace), **6** (16.3 mg), **9** (20 9 mg) and **10** (6 5 mg) The EtOAc–MeOH (4 1) extract was run on a polyamide column eluted successively with H_2O–MeOH, Me_2CO and DMF The fractions eluted with H_2O, 50–60% MeOH, 60–80% MeOH and DMF were further subjected to cellulose PLC and Sephadex LH-20 CC to afford **4** (541 4 mg), **5** (15.3 mg), **6** (7.1 mg) and **7** (20.8 mg), and **8** (472.6 mg) and **11** (32 2 mg), respectively.

5,7,8,3′,4′,5′-Hexahydroxyflavone 7-O-β-D-glucuronide (**11**). Yellow needles from MeOH–H_2O, mp > 300°. UV λ_{max}^{MeOH} nm 252sh, 275, 315sh, 348; + NaOMe 300sh, 380sh (dec.); + $AlCl_3$ 238sh, 278, 328, 435, + $AlCl_3$ + HCl 280, 330sh, 360, + NaOAc 260sh, 335, 412, + NaOAc + H_3BO_3 260sh, 310sh, 483. EIMS *m/z* 318 (71), 169 (16), 150 (8) HRMS. found, 318.0372 (M^+ − glucuronic acid); calcd. for $C_{15}H_{10}O_8$, 318 0372. ^{1}H NMR (DMSO-d_6). δ 12 41 (1H, *s*, OH), 9 46 (2H, *s*, OH), 9.15 (1H, *s*, OH), 8.74 (1H, *s*, OH), 7.09 (2H, *s*, H-2′,6′), 6.66 (1H, *s*, H-3), 6 62 (1H, *s*, H-6), 5 17 (1H, *d*, *J* = 6 6 Hz, H-1″). ^{13}C NMR (DMSO-d_6): δ 164.39 (*s*, C-2), 102.66 (*d*, C-3), 182 13 (*s*, C-4), 150 48 (*s*, C-5), 98.00 (*d*, C-6), 151.90 (*s*, C-7), 126.80 (*s*, C-8), 144 47 (*s*, C-9), 105.20 (*s*, C-10), 120 40 (*s*, C-1′), 105 73 (*d*, C-2′), 146.04 (*s*, C-3′), 137.82 (*s*, C-4′), 146.04 (*s*, C-5′), 105 73 (*d*, C-6′), 100 33 (*d*, C-1″), 72.69 (*d*, C-2″), 74 80 (*d*, C-3″), 71 14 (*d*, C-4″), 75.22 (*d*, C-5″), 169 91 (*s*, C-6″).

Determination of β-glucuronidase activity β-Glucuronidase activity was determined by measuring the absorbancy at 405 nm of *p*-nitrophenol formed from the substrate by the method of Nobunaga [15] with the following modification. The reaction mixture contained 0 9 ml of 0 1 M acetate buffer (pH 5 0), 0 03 ml of 0.1 M *p*-nitrophenyl-β-D-glucuronide and 0 1 ml of adequately diluted enzyme soln was incubated at 37° for 20 min After addition 0.25 ml of 0 2 M Na_2CO_3 to stop the reaction, the absorbancy at 405 nm was measured. The inhibitory activity was determined as described for the assay of enzyme activity, except that test material dissolved in 0 1 M acetate buffer (pH 5 0) was mixed with substrate and enzyme The inhibitory activity (%) was calculated as follows $(E-S)/E \times 100$, where E is the activity of enzyme without test material and S is the activity of enzyme with test material

Inhibitory activities of flavonoids on β-glucuronidase Compound (IC_{50}). **1** ($>10^{-4}$M), **2** ($>10^{-4}$M), **3** ($>10^{-4}$M), **4** ($>10^{-4}$M), **5** ($>10^{-4}$M), **6** ($>10^{-4}$M), **7** (4.6×10^{-5}M), **8** ($>10^{-4}$M), **9** ($>10^{-4}$M), **10** ($>10^{-4}$M), **11** (1.8×10^{-5}M), glucosaccharo-1 4-lactone (1.8×10^{-6}M, control)

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