Spasmolytic Flavonoids from *Syzygium samarangense* (Blume) Merr. & L.M. Perry

Evangeline C. Amor[a,*], Irene M. Villaseñor[a], M. Nabeel Ghayur[b], Anwar H. Gilani[b], and M. Iqbal Choudhary[c]

[a] Institute of Chemistry, College of Science, University of the Philippines, Diliman 1101, Quezon City, Philippines. Fax: (632)-9205427. E-mail: evangeline.amor@up.edu.ph
[b] Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Stadium Road, Karachi-74800, Pakistan
[c] International Center for the Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c,** 67–71 (2005); received September 10/October 19, 2004

The hexane extract of *Syzygium samarangense* (Ss.Hex) dose-dependently (10–1000 μg/ml) relaxed the spontaneously contracting isolated rabbit jejunum. Four rare C-methylated flavonoids with a chalcone and a flavanone skeleton were isolated from Ss.Hex and were subsequently tested for spasmolytic activity. All flavonoids, identified as 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (**1**), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (**3**), and 7-hydroxy-5-methoxy-6,8-dimethylflavanone (**4**), showed dose-dependent spasmolytic activity in the rabbit jejunum with IC_{50} values of 148.3 ± 69.4, 77.2 ± 43.5, 142.4 ± 58.6 and 178.5 ± 37.5 μg/ml (mean ± SEM), respectively. The dihydrochalcone derivative of compound **1**, 2′-hydroxy-4′,6′-dimethoxy-3′-methyldihydrochalcone (**5**), when tested for spasmolytic activity, did not significantly relax the smooth muscle relative to the other compounds. Verapamil, a standard spasmolytic, has an IC_{50} value of 0.16 ± 0.04 μg/ml. This is the first report of the relaxant activity of chalcones, specifically of compounds **1**–**3**.

Key words: *Syzygium samarangense*, Flavonoids, Spasmolytic Activity

Introduction

Syzygium samarangense (Blume) Merr. & L.M. Perry, commonly known as java apple and locally known in the Philippines as "makopa", is used for the treatment of diarrhea (Morton, 1987). Several flavonoids and triterpenoids that were isolated from the hexane extract of the plant exhibited prolyl endopeptidase activity (Amor *et al*., 2004). Flavonoids are well known for their spasmolytic activity (Williamson *et al*., 1998). However, most spasmolytic flavonoids contain a flavone (Hazekamp *et al*., 2001), flavonol (Bergendorff and Sterner, 1995), isoflavone (Loggia *et al*., 1988) or flavanone (Mata *et al*., 1997; Rojas *et al*., 1996) skeleton. In this investigation, the spasmolytic activity, tested on isolated rabbit jejunum, of the hexane extract of *S. samarangense* and its isolates, which are rare C-methylated chalcones and a flavanone, is reported.

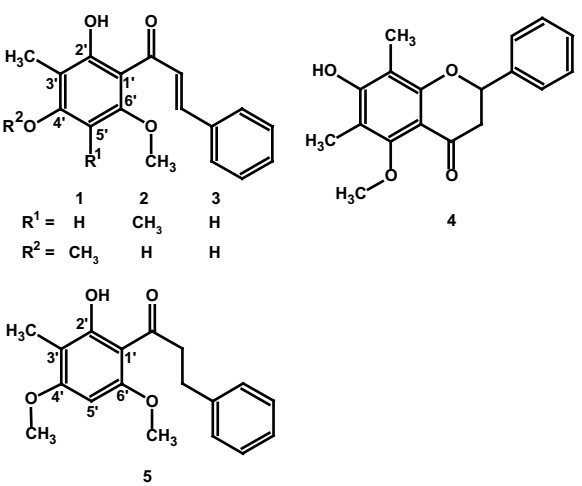

Fig 1. Structures of compounds **1**–**5**: 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (**1**); 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**); 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (**3**); 7-hydroxy-5-methoxy-6,8-dimethylflavanone (**4**); 2′-hydroxy-4′,6′-dimethoxy-3′-methyldihydrochalcone (**5**).

Results and Discussion

The hexane extract of *Syzygium samarangense* (Ss.Hex) dose-dependently (10–1000 μg/ml) relaxed the spontaneously contracting isolated rabbit jejunum with an IC_{50} of 352.0 ± 92.6 μg/ml. Subsequent purification of the hexane extract gave four flavonoids (Fig. 1) that showed dose-dependent (1–1000μg/ml) spasmolytic activity. Compound **2** showed activity at a comparatively small

dose (1 μg/ml), whereas, compounds **1**, **3** and **4** exhibited activity starting at a dose of 3 μg/ml and with more or less the same dose range and potency. Table I summarizes the IC_{50} values of Ss.Hex and compounds **1**–**4**. The spasmolytic activity of the extract and the flavonoids was comparable to the effect seen with verapamil, a standard spasmolytic (Samueli *et al.*, 1984).

The active flavonoids are structurally related. Their spasmolytic activity can be arranged in the following manner: **2** > **3** ≅ **1** > **4**. Among the chalcones **1**, **2** and **3**, the presence of C-5′–CH_3 in compound **2** enhanced the spasmolytic activity. Whereas, a –OCH_3 or a –OH at C-4′ has no effect on the spasmolytic activity of the chalcones because **3** and **1** have comparable activities. Compound **1** was hydrogenated in order to compare the relative spasmolytic activity of a chalcone and a dihydrochalcone. Hydrogenation of **1** to yield **5** (Fig. 1) resulted in a drastic decrease in spasmolytic activity because the isolated rabbit jejunum started to relax at a high dose of 500 μg/ml. This suggests that the C_α,C_β double bond is essential for the activity. The rigidness of the flavanone structure of **4** (Fig. 1), an isomer of **2**, appears to be detrimental to its spasmolytic activity.

The isolated flavanone **4** (IC_{50} of 178.5 ± 37.5 μg/ml) is less active than the known spasmolytic flavanone pinostrobin (Fig. 2), 7-hydroxy-5-methoxyflavanone (IC_{50} of 6.91 μg/ml) (Mata *et al.*, 1997). Compound **4** is more sterically hindered than pinostrobin because of its additional –CH_3 groups at C-6 and C-8, which could explain the decrease in potency of **4**. An additional –OH group in the C ring as in sakuranetin (Fig. 2), 4′,7-dihydroxy-5-methoxyflavanone (IC_{50} of 15.98 μg/ml) (Rojas *et al.*, 1996), decreased the activity in comparison with pinostrobin.

Table I. Comparative IC_{50} values of the test samples.

Test sample	IC_{50} [μg/ml][a]
Ss.Hex[b]	352.0 ± 92.6
1	148.3 ± 69.4
	(0.50 ± 0.23 mM)
2	77.2 ± 43.5
	(0.26 ± 0.15 mM)
3	142.4 ± 58.6
	(0.50 ± 0.21 mM)
4	178.5 ± 37.5
	(0.60 ± 0.13 mM)
Verapamil	0.16 ± 0.04
	(0.33 ± 0.08 μM)

[a] Values shown are means ± SEM.
[b] Ss.Hex = *Syzygium samarangense* hexane extract.

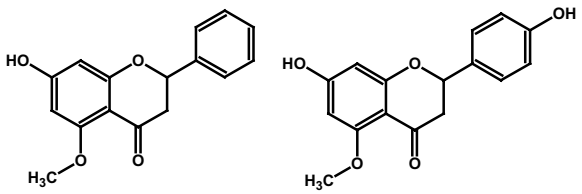

Fig. 2. Structures of spasmolytic flavanones: pinostrobin (7-hydroxy-5-methoxyflavanone); sakuranetin (4′,7-dihydroxy-5-methoxyflavanone).

Hence, among the C-methylated chalcones, dihydrochalcone and flavanone, the chalcones are the most active with an increased activity attributed to the presence of a C-5′–CH_3 and a C_α,C_β double bond.

In conclusion, the relaxant activity of the hexane extract of "makopa" may be attributed to the chalcones **1**–**3** and the flavanone **4** isolated from it. As yet, there are no spasmolytics found in the literature, which have a chalcone skeleton. In addition, among the rare C-methylated isolates, a chalcone with a C-5′–CH_3 is the most active. This observed relaxant activity might also explain the use of *S. samarangense* for the treatment of diarrhea (Morton, 1987).

Experimental

General

All the solvents used for extraction, isolation and purification were technical grade and were distilled before use. Normal phase column chromatography (NPCC) with gradient elution was generally employed for the isolation of the compounds using silica gel, type-60 (70–230 mesh; Merck). Thin layer chromatography (TLC) with GF-254 aluminum plates (Merck) was used to monitor the separation. TLC plates were visualized under an UV lamp at 254 and 365 nm. The spray reagent used was 65% $CeSO_4 \cdot H_2SO_4$.

Plant material

Leaves of the plant were sampled from Parañaque, Metro Manila. They were authenticated and a voucher specimen with accession No. 14258 was submitted to the Dr. Jose Vera Santos Herbarium,

Institute of Biology, University of the Philippines in Diliman.

Extraction and isolation

The ground air-dried leaves of *S. samarangense* (2.9 kg) were extracted at room temperature with methanol and subsequently partitioned between H_2O/hexane (1:6 v/v), from which the hexane extract was obtained and concentrated (Ss.Hex; 140 g oily residue). It was subjected to fractionation on silica gel (1:10 m/m) employing gradient elution (10% increments) with hexane, dichloromethane/hexane, dichloromethane, methanol/dichloromethane and finally with methanol, giving fractions 1–4.

Compound **1** (20 mg) crystallized out of fraction 1, eluted with hexane and 10% dichloromethane/hexane. Compound **1** was purified by recrystallization in acetone.

Sequential NPCC of fraction 2, eluted with 20% to 50% dichloromethane/hexane, on silica gel employing gradient elution (10% increments) with hexane and dichloromethane/hexane yielded compound **2** (3 g).

Sequential NPCC of fraction 3, eluted with 60% dichloromethane/hexane to 30% methanol/dichloromethane, on silica gel employing gradient elution (10% increments) with hexane, dichloromethane/hexane, dichloromethane, methanol/dichloromethane yielded compounds **3** (100 mg) and **4** (50 mg).

2′-Hydroxy-4′,6′-dimethoxy-3′-methylchalcone (**1**)

M.p. 145 °C. – UV: λ_{max} (MeOH) = 344 nm; λ_{max} ($AlCl_3$, HCl) = 374.6 nm; λ_{max} (NaOMe) = 339.6 nm; λ_{max} (NaOAc, H_3BO_3) = 349.8 nm. – FT-IR (KBr): ν_{max} = 3130, 2941, 2860, 1625, 1563, 1428, 1332, 1223, 1142, 980, 872, 791, 749, 706 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$) [NOE, enhanced signal]: δ = 2.04 (3H, s) [14.08], 3.90 (3H, s) [5.99], 3.95 (3H, s) [5.99], 5.99 (1H, s) [3.95], 7.40 (3H, br m), 7.60 (2H, br m), 7.76 (1H, d, J = 15.6 Hz), 7.89 (1H, d, J = 15.6 Hz), 14.08 (1H, s). The intensity of the signal at 14.08 decreased upon D_2O shake. – EIMS (70 eV): *m/z* (rel. int. %) = 298.1 (75.31) ($C_{18}H_{18}O_4$), 281.0 (9.39), 270.0 (13.49), 221.1 (100), 195.1 (42.69), 179.1 (15.47), 165.0 (15.87), 136.0 (35.29), 103.1 (47.11), 91.0 (18.46), 77.0 (60.09), 51.0 (48.51).

2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**)

M.p. 125–126 °C. – UV: λ_{max} (MeOH) = 335.8 nm; λ_{max} ($AlCl_3$, HCl) = 368.8 nm; λ_{max} (NaOMe) = 409.8 nm; λ_{max} (NaOAc, H_3BO_3) = 421.4 nm. – FT-IR (KBr): ν_{max} = 3335, 2945, 2860, 1629, 1548, 1424, 1359, 1312, 1231, 1169, 1115, 985, 911, 818, 760, 691 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$) [NOE, enhanced signal]: δ = 2.15 (3H, s), 2.17 (3H, s), 3.66 (3H, s) [2.17, 8.00], 5.88 (1H, s), 7.41 (3H, m), 7.63 (2H, m), 7.84 (1H, d, J = 15.7 Hz), 8.00 (1H, d, J = 15.7 Hz), 13.69 (1H, s). The intensity of the signals at 5.88 and 13.69 decreased upon D_2O shake. – EIMS (70 eV): *m/z* (rel. int. %) = 298.1 (100) ($C_{18}H_{18}O_4$), 221.0 (93.12), 194.0 (80.67), 166.1 (24.11), 136.0 (20.47), 103.1 (33.04), 83.0 (49.84), 77.0 (21.92), 69.1 (14.18).

2′,4′-Dihydroxy-6′-methoxy-3′-methylchalcone (**3**)

M.p. 198–203 °C. – UV: λ_{max} (MeOH) = 346.0 nm; λ_{max} ($AlCl_3$, HCl) = 372.2 nm; λ_{max} (NaOMe) = 389.8 nm; λ_{max} (NaOAc, H_3BO_3) = 370.2 nm. – FT-IR (KBr): ν_{max} = 3142, 2933, 2725, 1625, 1536, 1447, 1339, 1231, 1150, 1119, 976, 864, 795, 760, 699 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$) [NOE, enhanced signal]: δ = 2.00 (3H, s), 3.92 (3H, s) [6.15], 6.15 (1H, s) [3.92], 7.44 (3H, m), 7.71 (2H, m), 7.74 (1H, d, J = 15.6 Hz), 8.02 (1H, d, J = 15.6 Hz), 14.51 (1H, s) [–]. The intensity of the signal at 14.51 decreased upon D_2O shake. – EIMS (70 eV): *m/z* (rel. int. %) = 284.1 (100) ($C_{17}H_{16}O_4$), 267.1 (41.15), 256.1 (37.33), 207.1 (99.1), 181.1 (62.20), 165.0 (42.87), 151.1 (37.39), 122.0 (55.53), 103.1 (51.14), 77.0 (47.71).

7-Hydroxy-5-methoxy-6,8-dimethylflavanone (**4**)

M.p. 210–211 °C. – UV: λ_{max} (MeOH) = 284.4 nm; λ_{max} ($AlCl_3$, HCl) = 278.8 nm; λ_{max} (NaOMe) = 334.0 nm; λ_{max} (NaOAc, H_3BO_3) = 278.4 nm. – FT-IR (KBr): ν_{max} = 3281, 3010, 2933, 2837, 1648, 1582, 1467, 1428, 1305, 1223, 1208, 1111, 996, 934, 764, 699 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$) [NOE, enhanced signal]: δ = 2.14 (3H, s) [3.81, 5.37], 2.14 (3H, s) [3.81, 5.37], 2.83 (1H, dd, J = 16.7, 3.2 Hz), 2.97 (1H, dd, J = 16.7, 12.8 Hz), 3.81 (3H, s) [2.14], 5.35 (1H, br s) [2.14, 2.14], 5.41 (1H, dd, J = 12.8, 3.2 Hz), 7.35–7.50 (5H, br m). – EIMS (70 eV): *m/z* (rel. int. %) = 298.0 (67.82) ($C_{18}H_{18}O_4$), 221.0 (14.94), 194.0 (100), 166.0 (20.13), 136.0 (27.87), 104.1 (11.98), 83.0 (35.30), 77.0 (21.71).

Hydrogenation of compound **1**

Hydrogenation (Lasswell and Hufford, 1977) was done on a Parr hydrogenation apparatus model 3911 (shaker type hydrogenator) with a 66CA 50 ml reaction bottle. Analytical grade diethyl ether was used as solvent. The sample, weighing 30 mg, was placed in the reaction vessel together with 30 ml of the solvent and 20 mg of the catalyst, 10% palladium in carbon. The pressure was maintained at 0.21 MPa and the reaction time was 2 h. The mixture in the reaction bottle was filtered and the filtrate concentrated *in vacuo*. The crude product was chromatographed on silica gel and purified through a dropper column using isocratic elution with dichloromethane. Hydrogenation of compound **1** gave compound **5** (9 mg).

2'-Hydroxy-4',6'-dimethoxy-3'-methyldihydrochalcone (**5**)

M.p. 143–145 °C. – UV: λ_{max} (MeOH) = 283.4 nm; λ_{max} ($AlCl_3$, HCl) = 339.8 nm; λ_{max} (NaOMe) = 283.2 nm; λ_{max} (NaOAc, H_3BO_3) = 282.8 nm. – FT-IR (KBr): ν_{max} = 3026, 2945, 1613, 1599, 1413, 1281, 1212, 1139, 884, 791, 737, 699 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 2.02 (3H, s), 3.00 (2H, t, J = 7.8 Hz), 3.33 (2H, t, J = 7.8 Hz), 3.88 (3H, s), 3.89 (3H, s), 5.96 (1H, s), 7.20–7.33 (5H, br m). – EIMS (70 eV): *m/z* (rel. int. %) = 300.0 (59.99) ($C_{18}H_{20}O_4$), 283.1 (12.77), 269.0 (11.52), 195.0 (100), 180.0 (15.97), 168.0 (57.85), 151.9 (13.66), 137.0 (9.76), 122.0 (10.39), 109.0 (14.54), 91.0 (40.62), 77.0 (18.70).

Spasmolytic activity

Ss.Hex as well as all the flavonoids were dissolved in 10% DMSO and then the respective dilutions were made in distilled water just before the start of the experiment. 10% DMSO (negative control) was inert and devoid of any activity on the contractility of the isolated rabbit jejunum (data not shown).

Isolated tissue experiments were carried out as described previously (Gilani *et al.*, 1994). Segments of rabbit jejunum tissue, 2 cm long, were suspended in a 10 ml tissue bath containing Tyrode's solution, bubbled with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37 °C. The composition of the Tyrode's solution was 2.68 mM KCl, 136.9 mM NaCl, 1.05 mM $MgCl_2$, 11.90 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 1.8 mM $CaCl_2$ and 5.55 mM glucose. Intestinal responses were recorded isotonically using Bioscience Transducers and Oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these conditions, the rabbit jejunum exhibits spontaneous rhythmic contractions, allowing to test the relaxant (spasmolytic) activity directly without the use of an agonist. The spasmolytic activity of the test compounds was compared with that of verapamil, a known spasmolytic agent (Samueli *et al.*, 1984).

Acknowledgements

Thanks are due to Ms. Hanshella Magno for the hexane extract, UNESCO for ECA's travel grant to Pakistan, the Department of Science & Technology-Education, Science & Engineering Program (DOST-ESEP) and the Commission on Higher Education (CHED) for the scholarship and research funding of ECA.

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