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New Flavones from *Bauhinia championii* BENTH.

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Three new flavones, 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavone (I), 5,6,7,3′,4′,5′-hexamethoxyflavone (II) and 5,7,5′-trimethoxy-3′,4′-methylenedioxyflavone (IV), together with known flavones, 5,6,7,3′,4′-pentamethoxyflavone (III), 5,7,3′,4′,5′-pentamethoxyflavone (V), and 5,7,3′,4′-tetramethoxyflavone (VI), have been isolated and identified from the root of *Bauhinia championii* BENTH.

Keywords——*Bauhinia championii*; Leguminosae; 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavone; 5,6,7,3′,4′,5′-hexamethoxyflavone; 5,7,5′-trimethoxy-3′,4′-methylenedioxyflavone; coronary dilator; cardiac muscle inotropy; platelet aggregation

Bauhinia championii BENTH. (Leguminosae) grows in the mountainous areas and dense forests of Taiwan. The root of this plant has been reported to possess piscicidal activity.[1] The purpose of this study was to determine whether the root of this plant contains flavonoids, and if so, to examine the biological activities of the individual flavonoids. Three new natural flavones, 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavone (I), 5,6,7,3′,4′,5′-hexamethoxyflavone (II), and 5,7,5′-trimethoxy-3′,4′-methylenedioxyflavone (IV), together with 5,6,7,3′,4′-pentamethoxyflavone (III), 5,7,3′,4′,5′-pentamethoxyflavone (V) and 5,7,3′,4′-tetramethoxyflavone (VI), were isolated from the ethanolic extract of the root of this plant. This paper deals with the elucidation of the structures and the biological activities of these flavones.

Compound I was obtained as colorless prisms, M^+ 386, mp 200—201 °C. The magnesium–hydrochloric acid (Mg–HCl) test gave an orange color and the Labat test was positive. The infrared (IR) and ultraviolet (UV) spectra suggested the presence of a flavone skeleton. The nuclear magnetic resonance (NMR) spectrum showed signals due to four methoxyl groups at δ 3.91 (3H, s) and 3.98 (9H, s) and a methylenedioxy group at 6.02 (2H, s). The alkaline degradation of I gave 5-methoxy-3,4-methylenedioxybenzoic acid, showing the position of the methylenedioxy group at C-3′ and C-4′ and a methoxyl group at C-5′ on ring B of I. The singlet at δ 7.03 (2H, s) was assigned to the protons at C-2′ and C-6′. The mass spectra (MS) fragment *m/z* 176 (20) further supported the proposed substitution pattern on ring B. The C-5 must have been substituted by a methoxyl group, since there is no signal in the region of δ 7.9 to 8.2.[2,3] The MS fragments of I at *m/z* 210, 195 and 167 indicated that another three methoxyl groups were located in ring A. The base peak at *m/z* 371 ($M^+ - 15$) showed that three methoxyl groups must be located at C-5, C-6, and C-7.[4] Hence the two singlets at δ 6.53 (1H) and 6.77 (1H) were attributed to the protons at the C-3 and C-8 positions, respectively. In order to obtain further information about the structure of I, Eu(fod)$_3$, a NMR shift reagent, was equilibrated with I at increasing concentrations up to 1.0 molar ratio. The "Δ_{Eu} values" are listed in Table I. The structure of I was further supported by comparing the "Δ_{Eu} values" with the data reported by Okigawa *et al.*[5,6] According to the above evidence, the structure of I could be assigned as 5,6,7,5′-tetramethoxy-3′,4′-methylenedioxyflavone. This was confirmed by mixed mp, Co-TLC and NMR spectral comparison with a synthetic sample.[7)]

Compound II was obtained as colorless prisms, M^+ 402, mp 116—117 °C. The Mg–HCl test gave an orange-red color. The IR and UV spectra suggested that II also has a flavone skeleton. The NMR spectrum showed six methoxyl groups at δ 3.92 (6H, s), 3.95 (6H, s) and 4.00 (6H, s). The aromatic region of the spectrum, which was very similar to that of I, also showed three singlets at δ 6.60 (1H, s), 6.80 (1H, s) and 7.07 (2H, s). The MS base peak at m/z 387 ($M^+ - 15$) indicated that the three methoxyl groups should be located at C-5, C-6, and C-7.[4)] The alkaline degradation of II gave 3,4,5-trimethoxybenzoic acid, which confirmed the presence of another three methoxyl groups at the C-3′, C-4′, and C-5′ positions on ring B, and there was an NMR signal at δ 7.07 (2H, s) due to the two symmetrical protons C-2′ and C-6′. Hence, the two singlets at δ 6.60 (1H) and 6.80 (1H) correspond to protons at C-3 and C-8, respectively. The NMR shift reagent $Eu(fod)_3$ was also employed to confirm the structure of II, and the "Δ_{Eu} values" are listed in Table I. The results indicated that the structure of II was 5,6,7,3′,4′,5′-hexamethoxyflavone. This was confirmed by mixed mp, Co-TLC and NMR spectral comparison with a synthetic sample.[7)]

From the spectral data for compound III and its alkaline hydrolysis product, compound III was assigned as 5,6,7,3′,4′-pentamethoxyflavone. It was identified by mixed mp, and Co-TLC comparison with authentic sinensetin,[8)] a compound previously isolated from *Citrus sinensis* (Rutaceae),[2)] *Orthosiphon stamineus* (Labiatae),[8)] and *Eupatorium coelestineum*

TABLE I. δ Values (from TMS) and Europium-induced Shifts (Δ_{Eu} Values)

Compound	Protons	δ	Δ_{Eu} (ppm)
I	MeO-5	3.98	13.02
	MeO-6	3.98	4.92
	MeO-7	3.98	1.22
	MeO-5′	3.91	0.00
	H-3	6.53 (s)	−1.33
	H-8	6.77 (s)	1.53
	H-2′,6′	7.03 (s)	−0.63
	O–CH_2–O-3′,4′	6.02 (s)	0.00
II	MeO-5	4.00	12.24
	MeO-6	3.95	5.59
	MeO-7	4.00	1.04
	MeO-3′	3.92	−0.02
	MeO-4′	3.95	0.00
	MeO-5′	3.92	−0.02
	H-3	6.60 (s)	−1.65
	H-8	6.80 (s)	1.32
	H-2′,6′	7.07 (s)	−0.49
IV	MeO-5	3.98	12.68
	MeO-7	3.95	1.03
	MeO-5′	3.91	−0.02
	H-3	6.55	0.1
	H-6	6.37 (d, $J=2$ Hz)	6.17
	H-8	6.54 (d, $J=2$ Hz)	1.44
	H-2′,6′	7.05	−0.02
	O–CH_2–O-3′,4′	6.06	0.00

Minus signs indicate upfield shifts.
$\Delta_{Eu} = \delta_{Eu}^{n=1} - \delta_{Eu}^{n=0}$ ($CDCl_3$) where n is the molar ratio of shift reagent to solute.

(Compositae).[9]

Compound IV was obtained as colorless prisms, M^+ 356, mp 244—246 °C. The Mg–HCl test gave an orange color. The IR and UV spectra of IV indicated the presence of a flavone skeleton. The NMR spectrum showed a methylenedioxy group at δ 6.02 (2H, s) which was confirmed by the IR bands at 920 and 720 cm^{-1} and the positive response in the Labat test. The alkaline degradation of IV gave 5-methoxy-3,4-methylenedioxybenzoic acid, and thus the ring B of IV has the same substitution pattern as I. The singlet at δ 7.05 (2H, s) was assigned to the protons at C-2′ and C-6′. The NMR spectrum also suggessted a 5,7-disubstituted ring A because of the appearance of two doublets at δ 6.39 and 6.53 showing *meta*-coupling (J= 2 Hz), each integrating for one proton. Hence, the singlet as δ 6.55 (1H) was assigned to the proton at the C-3 position. All these data were consistent with the identity of IV as 5,7,5′-trimethoxy-3′,4′-methylenedioxyflavone. This structure was also confirmed by use of the NMR shift reagent $Eu(fod)_3$. The "Δ_{Eu} values" are listed in Table I. The compound was also identified by mixed mp, Co-TLC, and NMR spectral comparison with a synthetic sample.[7]

5,7,3′,4′,5′-Pentamethoxyflavone (V) and 5,7,3′,4′-tetramethoxyflavone (VI), previously isolated from *Merrillia caloxylone* (Rutaceae),[10] were also obtained from the extract. Their structures were confirmed by the spectral data of the compounds themselves and their alkaline hydrolysis products.

Compounds, I, II and V exhibited a coronary dilating effect in guinea pigs, and also caused a reduction in cardiac muscle inotropy at a concentration of 10 μg/ml. Compounds I, II, IV and V also exhibited an inhibitory activity on adenosine diphosphate (ADP)-induced platelet aggregation at a concentration of 100 μg/ml. Collagen-induced platelet aggregation was inhibited at 50 μg/ml in the cases of compounds I, II and V, and 100 μg/ml in the case of compound IV.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-30 instrument, UV spectra on a Varian Super Scan 3 spectrometer, ^{1}H-NMR spectra on a JEOL FX-100 (100 MHz) instrument with tetramethylsilane as an internal standard (δ value) and MS on a JEOL JMS-D 100 instrument.

Isolation of the Constituents——The dried and chipped roots (2.3 kg) of *Bauhinia championii*, collected at Kuanyinshan, Taipei Prefecture (台北県觀音山), Taiwan, were extracted with boiling *n*-hexane and EtOH successively. The EtOH extract (200 g) was chromatographed on a silica gel column using an *n*-hexane–EtOAc gradient system to give two mixtures, A (12.323 g) and B (9.452 g). Mixture A (1 g) was separated by preparative thin-layer chromatography (PLC) using *n*-hexane–EtOAc (1 : 1) as the solvent system to give three products I (213 mg), II (173 mg) and III (13 mg) as crystalline materials. Mixture B (1 g) was separated by PLC using *n*-hexane–EtOAc (1 : 2) solvent system to give three products IV (32 mg), V (274 mg) and VI (8 mg) as crystalline materials.

5,6,7,5′-Tetramethoxy-3′,4′-methylenedioxyflavone (I)—— Colorless prisms, mp 200—201 °C (MeOH); Mg–HCl test, orange color; Labat test, green color. UV λ_{max}^{MeOH} nm (log ε): 332 (4.37), 270 (sh), 239 (4.39). IR ν_{max}^{KBr} cm^{-1}: 2950, 2830, 1630, 1600, 1510, 1450, 1250, 1120, 1040. MS *m/z* (%): 386 (M^+, 29), 371 (100), 358 (1), 353 (4), 343 (5), 328 (13), 210 (2), 195 (9), 185 (17), 179 (8), 176 (20), 167 (69), 151 (5), 139 (13), 131 (15). NMR ($CDCl_3$) δ: 3.91 (3H, s), 3.98 (9H, s), 6.02 (2H, s), 6.53 (1H, s), 6.77 (1H, s), 7.03 (2H, s).

Alkaline Hydrolysis of I[11]——I (35 mg) in EtOH (5 ml) was refluxed with 50% KOH (5 ml) for 24 h. The reaction mixture was cooled and neutralized with 20% H_2SO_4, then extracted four times with Et_2O. The ethereal extract was washed three times with 5% $NaHCO_3$. The 5% $NaHCO_3$ extract was neutralized with 50% H_2SO_4 and extracted four times with Et_2O. The ethereal solution was dried and evaporated to yield a colorless solid. Recrystallization from MeOH gave 5-methoxy-3,4-methylenedioxybenzoic acid (9.4 mg), mp 211—212 °C [lit.[8] 206—207 °C]. IR ν_{max}^{KBr} cm^{-1}: 3000—2500, 1680, 1630, 1590, 1510, 1270, 1120, 920, 720; MS *m/z* (%): 196 (M^+, 100). NMR ($CDCl_3$) δ: 3.90 (3H, s), 6.02 (2H, s), 7.30 (2H, s).

5,6,7,3′,4′,5′-Hexamethoxyflavone (II)——Colorless prisms, mp 116—117 °C (MeOH); Mg–HCl test, orange-red color. UV λ_{max}^{MeOH} nm (log ε): 317 (4.49), 258 (4.19), 237 (sh). IR ν_{max}^{KBr} cm^{-1}: 2950, 2840, 1640, 1600, 1500, 1450, 1260, 1120, 1060, 1000, 830. MS *m/z* (%): 402 (M^+, 27), 387 (100), 371 (11), 357 (10), 343 (4), 329 (5), 195 (4), 192 (1), 167 (22). NMR ($CDCl_3$) δ: 3.92 (6H, s), 3.95 (6H, s), 4.00 (6H, s), 6.60 (1H, s), 6.80 (1H, s), 7.07 (2H, s).

Alkaline Hydrolysis of II—II (30 mg) was hydrolyzed by the above method to give 3,4,5-trimethoxybenzoic acid

(11.3 mg), mp 165—167 °C [lit.[12] 165—167 °C]. IR ν_{max}^{KBr} cm^{-1}: 3000—2500, 1680, 1590, 1500, 1460, 1270, 1120. MS m/z (%): 212 (M^+, 100). NMR (DMSO) δ: 3.72 (3H, s), 3.82 (6H, s), 7.22 (2H, s).

5,6,7,3′,4′-Pentamethoxyflavone (III)——Colorless prisms, mp 169—171 °C (MeOH); Mg–HCl test, orange color. UV λ_{max}^{MeOH} nm (log ε): 330 (4.45), 266 (4.21), 243 (4.41). IR ν_{max}^{KBr} cm^{-1}: 2910, 2830, 1640, 1600, 1510, 1450, 1320, 1260, 1140, 1120, 1020, 980, 840. MS m/z (%): 372 (M^+, 37), 357 (100), 341 (30), 329 (6), 327 (9), 313 (13), 195 (7), 167 (18), 165 (6), 162 (8), 137 (5). NMR ($CDCl_3$) δ: 3.89 (3H, s), 3.92 (3H, s), 3.95 (6H, s), 3.98 (3H, s), 6.52 (1H, s), 6.74 (1H, s), 6.94 (1H, d, $J=9$ Hz), 7.32 (1H, d, $J=2$ Hz), 7.46 (1H, dd, $J=2$ and 9 Hz).

Alkaline Hydrolysis of III—III (10 mg) was hydrolyzed by the above method to give 3,4-dimethoxybenzoic acid (3.4 mg), mp 178—180 °C [lit.[13] 180—182 °C]. IR ν_{max}^{KBr} cm^{-1}: 3000—2500, 1680, 1600, 1590, 1500, 1270. MS m/z (%): 182 (M^+, 100). NMR ($CDCl_3$) δ: 3.95 (6H, s), 6.92 (1H, d, $J=9$ Hz), 7.58 (1H, d, $J=2$ Hz), 7.76 (1H, dd, $J=2$ and 9 Hz).

5,7,5′-Trimethoxy-3′,4′-methylenedioxyflavone (IV)——Colorless prisms, mp 244—246 °C (MeOH); Mg–HCl test, orange color; Labat test, green color. UV λ_{max}^{MeOH} nm (log ε): 336 (4.17), 268 (sh), 242 (4.22). IR ν_{max}^{KBr} cm^{-1}: 2920, 2820, 1630, 1600, 1500, 1450, 1260, 1100, 920, 720. MS m/z (%): 356 (M^+, 100), 355 (65), 328 (4), 326 (11), 298 (8), 180 (2), 179 (8), 176 (50), 152 (5), 151 (10), 149 (34). NMR ($CDCl_3$) δ: 3.91 (3H, s), 3.95 (3H, s), 3.98 (3H, s), 6.06 (2H, s), 6.39 (1H, d, $J=2$ Hz), 6.53 (1H, d, $J=2$ Hz), 6.55 (1H, s), 7.05 (2H, s).

Alkaline Hydrolysis of IV——Alkaline hydrolysis of IV (10 mg) was performed in the same manner as for I. The product was recrystallized from MeOH to give 3,4-methylenedioxy-5-methoxybenzoic acid (4.2 mg), mp 211—212 °C. It was identified by mixed mp determination and MS, IR and NMR comparison with an authentic sample.

5,7,3′,4′,5′-Pentamethoxyflavone (V)——Colorless prisms, mp 196—197 °C; Mg–HCl test, orange-red color. UV λ_{max}^{MeOH} nm (log ε): 322 (4.37), 268 (4.30), 240 (sh). IR ν_{max}^{KBr} cm^{-1}: 2930, 1640, 1600, 1500, 1260, 1120. MS m/z (%): 372 (M^+, 100), 357 (1), 344 (5), 341 (9), 329 (2), 313 (3), 311 (2), 283 (1), 195 (1), 192 (1), 167 (1), 152 (1), 137 (2). NMR ($CDCl_3$) δ: 3.91 (6H, s), 3.94 (9H, s), 6.38 (1H, d, $J=2$ Hz), 6.56 (1H, d, $J=2$ Hz), 6.61 (1H, s), 7.07 (2H, s).

Alkaline Hydrolysis of V——Alkaline hydrolysis of V (30 mg) was performed in the same manner as for I. The product was recrystallized from MeOH to give 3,4,5-trimethoxybenzoic acid (13.2 mg), mp 165—167 °C. It was identified by mixed mp determination and MS, IR and NMR comparison with the alkaline hydrolysis product of II.

5,7,3′,4′-Tetramethoxyflavone (VI)——Colorless prisms, mp 188—190 °C; Mg–HCl test, orange color. UV λ_{max}^{MeOH} nm (log ε): 332, 266 (sh), 243. IR ν_{max}^{KBr} cm^{-1}: 2930, 2830, 1650, 1610, 1520, 1260, 1120. MS m/z (%): 342 (M^+, 100), 327 (1), 314 (4), 284 (1), 165 (1.5), 162 (9), 152 (1), 137 (3), 122 (1). NMR ($CDCl_3$) δ: 3.98 (3H, s), 3.93 (9H, s), 6.35 (1H, d, $J=2$ Hz), 6.52 (1H, d, $J=2$ Hz), 6.56 (1H, s), 6.90 (1H, d, $J=9$ Hz), 7.28 (1H, d, $J=2$ Hz), 7.46 (1H, dd, $J=2$ and 9 Hz).

Alkaline Hydrolysis of VI——Alkaline hydrolysis of VI (3 mg) was performed in the same manner as for I. The product was identified by comparing its Co-TLC with that of the alkaline hydrolysis product of III.

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