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Anti-platelet aggregation constituents from *Gynura elliptica*

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Abstract

A *p*-hydroxyacetophenone-like derivative, (+)-gynunone, and a chromane, together with six known compounds were isolated from the $CHCl_3$ fraction of the roots of *Gynura elliptica*. Their structures were determined by means of spectral analyses. Among the isolates, 6-acetyl-2,2-dimethylchroman-4-one and vanillin showed anti-platelet aggregation activity induced by arachidonic acid in vitro. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Gynura elliptica*; Compositae; Root; *p*-Hydroxyacetophenone; Chromane; Chroman-4-ol; (+)-Gynunone; Gynunol; Anti-platelet aggregation activity

1. Introduction

Gynura elliptica Yabe & Hayata (Compositae) is a succulent herb endemic to Taiwan, where it grows only along the shores of Lanyu Island (Li, 1978). Phytochemical examination of this species has not yet been conducted. The literature has, however, reported a few components, such as iridoid, terpenyl coumarin, spirostanol steroid, pyrrolizidine, purine, pyrimidine and chromanone constituents from other *Gynura* species (Knaak, 1971; Bohlmann & Zdero, 1977; Takahira, Kondo, Kusano & Nozoe, 1977; Tang, Wu & Fang, 1980; Wiedenfeld, 1982; Wiedenfeld, Kirfel, Roeder & Will, 1983; Hua, Xu, Wei, Tang & Wu, 1983; Liang & Roeder, 1984; Asada, Shiraishi, Takeuchi, Osawa & Furuya, 1985; Liu, Sun & Zhang, 1988; Yuan, Gu & Wei, 1990; Matheson & Robins, 1992; Roeder, Eckert & Wiedenfeld, 1996; Jong & Chou-Hwang, 1997). In particular, the roots of *G. segetum* were used as a folk medicine to promote blood circulation, and these have consequently been extensively studied (Tang et al., 1980; Hua et al., 1983; Liang et al., 1984; Liu et al., 1988; Yuan et al., 1990). Since the methanolic extract of the roots of *G. elliptica* shows anti-platelet aggregation activity, this was used as a rationale for the current study to identify the substances responsible for this effect. Thus, the *p*-hydroxyacetophenone derivative, (+)-gynunone (**1**), as well as the chroman-4-ol, gynunol (**2**), together with six known compounds were isolated from the $CHCl_3$-soluble fraction of the methanolic extract. The latter included two pyrrolizidine alkaloids, (+)-senecionine (**3**) (Hua et al., 1983), and (+)-senkirkine (**4**) (Matheson & Robins, 1992), two chromanones, 6-acetyl-2,2-dimethylchroman-4-one (**5**) (Bohlmann, Zdero & Lonitz, 1977) and 6-hydroxy-2,2-dimethylchroman-4-one (**6**) (Lourenco, Akisue & Roque, 1981), as well as vanillin (**7**) and syringaldehyde (**8**) (Pouchert & Behnke, 1993). These compounds were identified by comparisons of their spectral (UV, IR, ^{1}H-NMR, MS) and/or mp data with corresponding authentic samples or literature data. In this article, we report the isolation and structural elucidation of **1** and **2**, as well as identifying the constituents having anti-platelet aggregation activity.

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2. Results and discussion

(+)-Gynunone (**1**) was isolated as a colorless oil with $[\alpha]_D^{27} + 117.8^\circ$ ($CHCl_3$, *c* 0.15). Its molecular formula was established as $C_{13}H_{14}O_4$ by EIMS ($[M^+]$, *m*/*z* 234) and HR–EIMS. UV absorptions at 218, 237, 277 and 319 nm suggested the presence of an *o*,*p*-dioxygenated acetophenone skeleton (Scott, 1964). An IR absorbance at 1645 cm^{-1}, together with a ^{13}C-NMR spectral resonance at δ 202.4 suggested a conjugated carbonyl group; this was supported by an IR signal for a chelated phenolic hydroxyl (3440 cm^{-1}) as well as a UV bathochromic shift upon addition of KOH. The ^{1}H-NMR spectrum of **1** showed three protons [δ 2.58 (3H, *s*)] assignable to an acetyl unit, one proton [δ 12.99 (1H, *s*, D_2O exchangeable)] assignable to a chelated OH due to the formation of hydrogen-bonding with the neighboring carbonyl group, and two singlets at δ 6.34 and δ 7.79 which were assigned to H-6 and H-3 protons. Furthermore, the saturation index for compound **1** is 7, indicating the presence of another two saturated rings. Two oxymethine protons [δ 5.58 (1H, *d*, *J* = 5.8 Hz, H-7) and 4.94 (1H, *dd*, *J* = 0.8, 5.8 Hz, H-8)], one oxymethylene group [δ 3.63 (2H, *m*, H-10)], one methine proton [δ 2.58 (1H, *m*, H-9), and a methyl group [δ 1.14 (3H, *d*, *J* = 7.2 Hz)] in the ^{1}H-NMR spectrum, together with thirteen signals in ^{13}C-NMR spectrum, indicated the existence of a cyclic 4-methylhexahydro-furo[3,2-*b*]furan ring fused with an *o*,*p*-dioxygenated acetophenone to form a tricyclic compound **1**. Besides, the correlation between an acetyl group [δ 2.58 (3H, *s*)] and H-6 (δ 7.79, *s*) in the NOESY spectrum, and the correlation between a phenolic OH and H-3 (δ 6.34, *s*) in the HMBC spectrum, suggested the acetophenone moiety must be 1-acetyl-2-hydroxyl instead of 2-acetyl-1-hydroxyl. The assignment of H-6 at δ 7.79 was also supported by the HMBC correlation of H-6 with C-7. According to the above data, the structure of gynunone was elucidated as **1**, which was further confirmed by ^{1}H–^{1}H COSY, DEPT, HETCOR and HMBC experiments (see Fig. 1). The relative configuration at H-7 and H-8 was in the *cis*-form, as confirmed by analysis of the NOESY spectrum (see Fig. 2).

Gynunol (**2**) was obtained as colorless oil. Its molecular formula ($C_{11}H_{14}O_3$) was determined by EIMS ($[M]^+$, *m*/*z* 194) and HR–EIMS. The UV spectrum showed maximal absorptions at 230 *sh*, 258 and 290 nm. The IR spectrum showed a hydroxyl absorption at 3400 cm^{-1}. The aromatic region of ^{1}H-NMR spectrum of **2** showed three ABX protons at δ 6.94 (1H, *d*, *J* = 2.8 Hz), 6.70 (1H, *dd*, *J* = 8.0, 2.8 Hz) and δ 6.74 (1H, *d*, *J* = 8.0 Hz), indicating the existence of a 1,2,4-trisubstituted benzene moiety. Furthermore, two methyl groups at δ 1.42, 1.29 (each 3H, *s*), methylene protons at δ 1.84 (1H, *dd*, *J* = 13.6, 9.0 Hz) and δ 2.16 (1H, *dd*, *J* = 13.6, 6.2 Hz), along with a hydroxymethine proton at δ 4.80 (1H, *dd*, *J* = 9.0, 6.2 Hz) indicated the existence of a hydroxy-2,2-dimethylchroman-4-ol moiety in **2** (Bohlmann, Zdero & Lonitz, 1977). Comparison of the ^{1}H-NMR spectrum of **2** with that of 7-hydroxy-2,2-dimethylchroman-4-ol (**9**) (Jennings & Ottridge, 1984) revealed a resonance at δ 6.74 (1H, *d*, *J* = 8.0 Hz, H-8) instead of the resonance at δ 6.26 (1H, *d*, *J* = 3.0 Hz, H-8) observed for **9**. Therefore, the phenolic hydroxyl group of **2** was suggested to be located at C-6 and the structure of gynunol (**2**) was proposed as 6-hydroxy-2,2-dimethylchroman-4-ol.

The $CHCl_3$ and the *n*-BuOH fractions from the root of the *G. elliptica* showed significant anti-platelet activity in vitro using the turbidimetric method (O'Brien, 1962). The clinically-used anti-platelet aggregation agent, aspirin, was used as a reference. Firstly, bioassay-guided fractionation of the $CHCl_3$ fraction led to the isolation of **5** and **7** having anti-platelet aggregation activity (see Table 1). **5** and **7** showed complete inhibitory activity at 100 μg ml^{-1} on platelet aggregation induced by arachidonic acid (AA). Although the anti-platelet aggregation activities of these compounds were less than that of aspirin, both compounds, like aspirin, are highly selective. In addition, *Gynura* species are well-known for possessing pyrrolizidine alkaloids, but the major constituent, **4** in the alkaloidal fraction did not show any anti-platelet aggregation effect (Table 1).

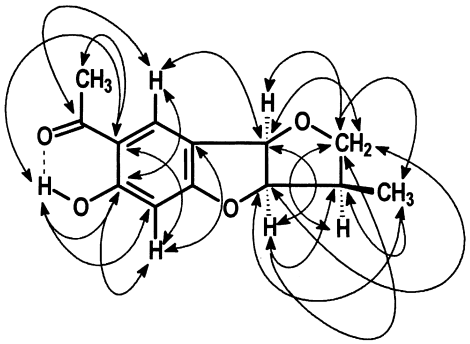


Fig. 1. HMBC correlations of **1**.

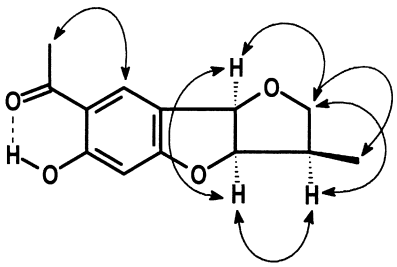
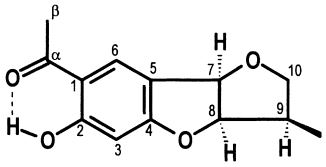
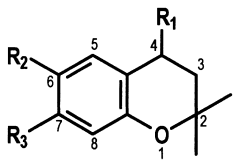


Fig. 2. NOESY correlations of **1**.



1



2 R_1= R_2= OH, R_3= H
9 R_1= R_3= OH, R_2= H

3. Experimental

3.1. General

JASCO DIP-370 Digital polarimeter; MS: direct inlet, 70 eV, VG Biotech Quattro 5022 and Joel JMS-HX 110 mass spectrometer; ^{1}H- and ^{13}C-NMR: Varian Unity 400; 400 and 100 MHz, Varian Gemini 200; 200 and 50 MHz, respectively. Chemical shifts are given in δ with TMS as internal standard. Merck Silica gel 60F (70–230, 230–400 mesh) was used for CC and Merck silica gel Art. 5715 and 5744 for prep. TLC. Silica gel 60 F_{254} was used for TLC.

3.2. Plant material

Root of *G. elliptica* was collected from Lanyu Island, Taitung County, Taiwan, in August 1995. A voucher specimen (Chen 5565) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, ROC.

3.3. Extraction and isolation

The dried root (3.4 kg) was sliced, extracted with MeOH and concd. in vacuo. The MeOH extract (280 g), when partitioned between H_2O and $CHCl_3$ (1:1), afforded $CHCl_3$ and H_2O frs. The $CHCl_3$ fraction was then partitioned between 90% MeOH-*n*-hexane (1:1) to afford an *n*-hexane fr. (fr. A, 24.4 g). The 90% MeOH portion was transferred to $CHCl_3$ and bases were extracted with 2% aq. HCl. The acid-soluble part was made alkaline with NH_4OH and then extracted with $CHCl_3$. The $CHCl_3$ solution was dried ($MgSO_4$) and concentrated in vacuo to afford an alkaloidal fr. (fr. B, 2.10 g). The acid-insoluble part was dried ($MgSO_4$) and concentrated in vacuo to give a neutral $CHCl_3$ fr. (fr. C, 10.04 g). The H_2O fraction was partitioned with *n*-BuOH to yield an *n*-BuOH fr. (fr. D, 26 g) and an H_2O fr. (fr. E, 200 g). Fr. B (2.10 g) was washed with Me_2CO and recrystallized from EtOAc to yield **4** (520 mg). The washings (1.45 g) were chromatographed over silica gel (44 g) and eluting with $CHCl_3$ and $CHCl_3$–MeOH mixtures to give 15 frs. (B-1–B-15). **3** (1.2 mg) was obtained from B-7 (133.6 mg) after washing and recrystallizing from Me_2CO. The washings (B-7-M, 124 mg) were chromatographed over silica gel (5.3 g) and eluted with CH_2Cl_2 and CH_2Cl_2–MeOH mixtures to give 20 frs. (B-7-M-1–B-7-M-20). B-7-M-6 (3.8 mg) was purified by prep. TLC (CH_2Cl_2–MeOH, 10:1) to obtain **2** (0.8 mg). Fr. C (10.04 g) was chromatographed on silica gel (350 g) eluted with CH_2Cl_2 and CH_2Cl_2—MeOH mixtures to give 14 frs. (C-1–C-14). C-6 (185 mg) was rechromatographed on

Table 1
Inhibitory effects of compounds isolated from *Gynura elliptica* on aggregation of washed rabbit platelets induced by thrombin, arachidonic acid, collagen and PAF[a]

Compound	Conc. (μg/ml)	Aggregation (%)			
		Thrombin (0.1 U/ml)	AA (100 μM)	Collagen (10 μg/ml)	PAF (2 ng/ml)
Control		90.4 ± 0.8 (3)	87.6 ± 0.6 (3)	89.3 ± 0.9 (3)	90.2 ± 0.6 (3)
Gynunone (**1**)	100	88.3 ± 0.4 (3)	38.8 ± 15.8 (3)[b]	84.0 ± 0.5 (3)[d]	84.5 ± 1.9 (3)[b]
	50		79.7 ± 0.5 (3)[d]		
	20		84.7 ± 0.1 (3)[c]		
Senkirkine (**4**)	100	91.5 ± 1.8 (3)	85.1 ± 1.1 (3)	86.1 ± 1.0 (3)	91.2 ± 0.7 (3)
6-Acetyl-2, 2-dimethylchroman-4-one (**5**)	100	87.2 ± 1.0 (3)[b]	0.0 ± 0.0 (3)[d]	79.8 ± 2.0 (3)[c]	76.9 ± 4.1 (3)[b]
	50		29.6 ± 16.2 (3)[c]		
	20		81.2 ± 0.9 (3)[d]		
Vanillin (**7**)	100	95.3 ± 0.8 (3)	0.0 ± 0.0 (4)[d]	83.6 ± 5.8 (3)	88.9 ± 1.6 (3)
	50		83.3 ± 4.2 (3)		
Aspirin	50	92.1 ± 1.3 (3)	0.0 ± 0.0 (5)[d]	87.1 ± 2.5 (3)	90.1 ± 1.4 (3)
	20		42.7 ± 15.8 (5)[d]		
	10		90.2 ± 0.9 (5)		

[a] Platelets were preincubated with DMSO (0.5%, control) or each compound at 37°C for 3 min, and then the inducer was added. Aspirin was used as a reference control. Values are presented as means ± s.e. (*n*).
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

silica gel (6.5 g) eluted with CH_2Cl_2 and CH_2Cl_2–MeOH mixtures to give 6 frs. (C-6-1–C-6-6). C-6-3 (199 mg) was washed with Me_2CO and the filtrate (C-6-3-M, 92 mg) was again chromatographed over silica gel (3.8 g), eluting with $CHCl_3$, gradually increasing the polarity with MeOH, to afford 4 frs. (C-6-3-M-1–C-6-3-M-4). C-6-3-M-3 (60 mg) was purified repeatedly with prep. TLC (*n*-hexane–EtOAc, 3:1) to give **1** (3 mg) and **7** (42 mg). C-7 (1.149 g) was chromatographed over silica gel (33 g), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH, and 13 frs. (C-7-1–C-7-13) were collected. C-7-8 (330 mg) was chromatographed on silica gel (1.2 g), eluting with CH_2Cl_2 and CH_2Cl_2–Me_2CO mixtures, to give 7 frs. (C-7-8-1–C-7-8-7). C-7-8-2 (77.8 mg) was chromatographed on silica gel (23.1 g), eluting with CH_2Cl_2 and CH_2Cl_2–Me_2CO mixtures, to give 5 frs. (C-7-8-2-1–C-7-8-2-5). **8** (2.0 mg) was obtained from C-7-8-2-3 (8.0 mg) after purification by prep. TLC (CH_2Cl_2–MeOH, 50:1). **6** (3.0 mg) was obtained from C-7-8-4 (40.6 mg) after purification by prep. TLC (CH_2Cl_2–MeOH, 30:1). C-7-8-6 (20.8 mg) was purified by prep. TLC (*n*-hexane: EtOAc, 3:1) to yield **5**.

3.4. (+)-Gynunone (*1*)

Colorless oil, $[\alpha]_D^{27} + 117.8^\circ$ ($CHCl_3$; *c* 0.15); HR–EIMS *m*/*z* 234.0905 for $C_{13}H_{14}O_4$ (calcd 234.0892); EIMS *m*/*z* (rel. int.): 234 (M^+, 90), 219 (52), 204 (25), 203 (23), 190 (17), 189 (100), 163 (13), 161 (20); IR ν_{max} (Neat) cm^{-1}: 3440 (OH), 1645 (C═O, conjugated); UV λ_{max} (EtOH) nm (log ε): 319 (3.86), 277 (4.10), 237 (4.07), 218 (4.32); + KOH: 356 (3.87), 280 *sh* (0.59), 251 (4.28); ^{1}H-NMR spectral data: δ 1.14 (3H, *d*, *J* = 7.2 Hz, Me-9), 2.58 (1H, *m*, H-9) 2.58 (3H, *s*, Ac-1), 3.63 (2H, *m*, Ha-10 and Hb-10), 4.94 (1H, *dd*, *J* = 5.8, 0.8 Hz, H-8), 5.58 (1H, *d*, *J* = 5.8 Hz, H-7), 6.34 (1H, *s*, H-3), 7.79 (1H, *s*, H-6), 12.99 (1H, *s*, OH-2, D_2O exchangeable); ^{13}C-NMR spectral data: 16.4 (Me-9), 26.3 (C-β), 40.8 (C-9), 71.5 (C-10), 80.7 (C-7), 94.3 (C-8), 98.0 (C-3), 114.7 (C-1), 118.1 (C-5), 129.4 (C-6), 166.8 (C-2), 167.1 (C-4), 202.4 (C-α).

3.5. Gynunol (*2*)

Colorless oil; HR–EIMS *m*/*z* 194.0945 for $C_{11}H_{14}O_3$ (calcd. 194.0943); EIMS *m*/*z* (rel. int.): 194 (M^+, 32), 161 (74), 139 (41), 138 (100), 137 (77), 111 (20); IR ν_{max} (Neat) cm^{-1}: 3400 (OH); UV λ_{max} (EtOH) nm (log ε): 290 (2.99), 258 (3.12), 230 *sh* (3.29); ^{1}H-NMR spectral data: δ 1.29, 1.42 (each 3H, *s*, Me-2), 1.84 (1H, *dd*, *J* = 13.6, 9.0 Hz, H-3a), 2.16 (1H, *dd*, *J* = 13.6, 6.2 Hz, H-3b), 4.80 (1H, *dd*, *J* = 9.0, 6.2 Hz, H-4), 6.70 (1H, *d*, *J* = 2.8 Hz, H-7), 6.74 (1H, *dd*, *J* = 8.0, 2.8 Hz, H-8), 6.94 (1H, *d*, *J* = 8.0 Hz, H-5).

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