A New Antiplatelet Diarylheptanoid from *Alpinia blepharocalyx*

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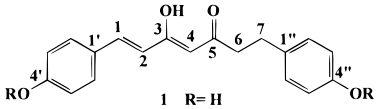
Received June 12, 1997[⊗]

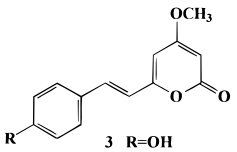
A new diarylheptanoid, 1,7-bis(4-hydroxyphenyl)-3-hydroxy-1,3-heptadien-5-one (**1**), along with seven other known compounds, were isolated from the seeds of *Alpinia blepharocalyx*. Of these, compounds **1** and **3** showed strong inhibition of collagen-induced, arachidonic acid-induced, and adenosine diphosphate-induced platelet aggregation of human whole blood. Compound **3** also strongly inhibited ristocetin-induced platelet aggregation. Structures of these compounds were elucidated by spectroscopic and chemical means.

The seeds of *Alpinia blepharocalyx* K. Schum. (Zingiberaceae) have been used as an aromatic stomachic in China.[1] Previous studies of these seeds have resulted in the isolation of five diarylheptanoids bearing chalcone moieties, namely calyxin A, calyxin B, 3-epicalyxin B, and blepharocalyxins A and B.[2,3] Antiplatelet constituents, 5,6-dehydrokawain, dihydro-5,6-dehydrokawain, and quercetin have also been reported recently from the other species of the same genus, *Alpinia speciose* and *Alpinia ulurensis*.[4,5] As part of our screening of antiplatelet agents from medicinal plants, we further investigated this plant and isolated one new compound, 1,7-bis(4-hydroxyphenyl)-3-hydroxy-1,3-heptadien-5-one (**1**) and seven known compounds, 1,7-bis(4-hydroxyphenyl)-3-hydroxy-1,3,6-heptatrien-5-one (**2**), 4′-hydroxydehydrokawain (**3**), 5,6-dehydrokawain (**4**), 4′,7-dihydroxy-5-methoxyflavanone (**5**), cardamomin (**6**), helichrysetin (**7**), and 4-hydroxybenzaldehyde (**8**), from the seeds of *A. blepharocalyx*. Here, we report the purification and structure elucidation of **1** and the antiplatelet aggregation activity of **1**−**8** on human whole blood.

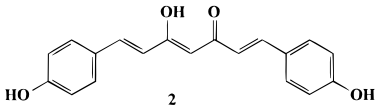
Compound **1** was obtained as yellow needles (Et_2O−C_6H_6), mp 148−149 °C. The molecular formula was determined to be $C_{19}H_{18}O_4$ (M^+ 310.1183) by HRMS. Acetylation of compound **1** gave a diacetate (**1a**), $C_{23}H_{22}O_6$ (*m*/*z* 394, M^+). Both **1** and **1a** were positive to $FeCl_3$ reagent, so they were suggested to be the enol form of a 1,3-diketone.[6] The IR spectrum of **1** indicated the presence of a hydroxyl group (3180 cm^{-1}) and a conjugated ketone (1635 cm^{-1}). ^{13}C NMR, together with DEPT spectrum of **1**, showed 15 carbon signals, including characteristic signals of two methylene carbons (C-6, δ 29.79 and C-7, δ 41.23), a carbonyl group (C-5, δ 199.06), a hydroxylated olefinic carbon (C-3, δ 178.01),



1 R= H
1a R=Ac



3 R=OH
4 R= H



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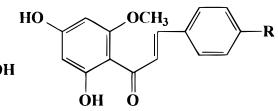
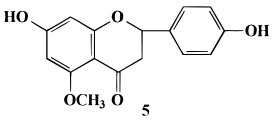


6 R= H
7 R=OH



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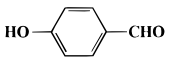

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two phenolic carbons (C-4″, δ 155.49 and C4′, δ 159.63), and an α-carbon of a β-hydroxy-α,β-unsaturated ketone for C-4 (δ 100.09). The ^1H-NMR spectrum of **1** showed the presence of four doublets at 6.66, 6.80, 7.02, and 7.52 ppm (J = 8.5 Hz) due to the two *para*-substituted benzene rings, a pair of trans olefinic doublet protons at 6.54 (H-2, d, J = 16.0 Hz) and 7.48 ppm (H-1, d, J = 16.0 Hz), two methylene protons with chemical shifts 2.64 (t, H-6) and 2.77 ppm (t, H-7), an α-proton of a β-hydroxy-α,β-unsaturated ketone (H-4, 5.84 ppm, s), and a chelated hydroxy proton (15.24 ppm, br s) in the β-hydroxy-α,β-unsaturated ketone moiety. From the above data, the structure of compound **1** was determined to be 1,7-bis(4-hydroxyphenyl)-3-hydroxy-1,3-heptadien-5-one. All the carbons and the protons of compound **1** were assigned with the help of DEPT, ^1H-^1H COSY, ^1H-^{13}C COSY, and COLOC (correlation spectroscopy via long-range coupling) spectral data (see Table 1). 1,7-Bis(4-hydroxyphenyl)-3-hydroxy-1,3,6-heptatrien-5-one (**2**),[7] 4′-hydroxydehydrokawain (**3**),[8] 5,6-dehydrokawain (**4**),[9] 4′,7-dihydroxy-5-methoxyflavanone (**5**),[10] cardamomin (**6**),[6] helichrysetin (**7**),[11] and 4-hydroxybenz-

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[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1997.

S0163-3864(97)00293-0 CCC: $15.00
Published on Web 01/23/1998

Table 1. Chemical Shifts[a] and Correlations of **1** in DMSO-d_6 (400 MHz for ^{1}H, and 100 MHz for ^{13}C-NMR spectrum)

position	^{13}C shift	^{1}H shift	COLOC
1	139.83 (d)	7.46 (1H, d, J = 16.0 Hz)	C2, C3, C2′,C6′
2	119.30 (d)	6.54 (1H,d, J = 16.0 Hz)	C1′, C3, C4
3	178.01 (s)		
4	100.09 (d)	5.84 (1H, s)	C2, C3, C5
5	199.06 (s)		
6	41.23 (t)	2.64 (2H, t, J = 7.0 Hz)	C7
7	29.79 (t)	2.77 (2H, t, J = 7.0 Hz)	C6
1′	126.81 (s)		
2′,6′	130.03 (d)	6.80 (2H, d, J = 8.5 Hz)	C3′,C5′
3′,5′	115.67 (d)	7.52 (2H, d, J = 8.5 Hz)	C2′,C6′, C4′
4′	159.63 (s)		
1″	130.12 (s)		
2″,6″	115.06 (d)	6.66 (2H, d, J = 8.5 Hz)	C1″, C3″,C5″
3″,5″	129.06 (d)	7.02 (2H, d, J = 8.5 Hz)	C2″,C6″, C4″
4′	155.49 (s)		

[a] δ in ppm.

aldehyde (**8**) were identified by comparing their MS, ^{1}H-, and ^{13}C-NMR spectral data with those reported in the literature and with authentic samples.

Platelets play a very important role in the hemostatic process, and their aggregation induced by collagen, arachidonic acid (AA), adenosine diphosphate (ADP), and ristocetin will cause atheromatic plaque formation. In recent years, many antiplatelet agents have been isolated from plants and showed potent activity.[4,5,12] In this work, the effects of compounds **1**−**8** on platelet aggregation of human whole blood were studied, and the results are given in Table 2. Among these compounds, **3** showed strong inhibition on platelet aggregation caused by all four inducers at a concentration of 100 μg/mL. Its IC_{50} values of collagen-, ADP-, AA-, and ristocetin-induced aggregation were 70.9, 20.7, 80.6, and 25.0 μg/mL, respectively. Compound **4**, on the other hand, only strongly inhibited platelet aggregation induced by AA, with a IC_{50} of 25.8 μg/mL. This was in accordance with the result from a previous study in which **4** was reported to inhibit platelet aggregation of platelet rich plasma induced by AA but not by ADP.[4] The structures of compounds **3** and **4** differ only in the presence of a hydroxy group at C-4′ position of the benzene ring in the former. This phenol group may be important with regard to its strong antiplatelet activities. An extra double-bond at the 6 position of compound **2** also changes its antiplatelet effects significantly from compound **1**. Compound **1** strongly inhibited platelet aggregation induced by collagen (IC_{50} = 14.7 μg/mL), AA (IC_{50} = 26.6 μg/mL), and ADP (IC_{50} = 65.7 μg/mL) (Figure 1), whereas compound **2** inhibited only collagen-induced platelet aggregation (IC_{50} = 13.7 μg/mL). Meanwhile, compounds **6** and **7** showed strong inhibition on platelet aggregation induced by AA, with IC_{50} values of 25.4 and 21.7 μg/mL, respectively. Compounds **5** and **8** showed no significant inhibition on platelet aggregation by all four inducers. Thus, the effect of each compound on platelet aggregation should be evaluated individually.

Experimental Section

General Experimental Procedures. All melting points measured were uncorrected. IR spectra were recorded on a Perkin−Elmer 1600 Series FTIR spectrometer. EIMS were obtained on a MACROMASS 7035E mass spectrometer at 70 eV. All spectra (^{1}H, ^{13}C, COSY, COLOC, and HMBC) were recorded on a JEOL GX-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts were reported in parts per million with TMS as an internal standard. TLC was performed on 0.25-mm Si gel (60 F_{254}, Merck) plates. Spots were visualized under UV light (254 nm).

Plant Material. The seeds of *A. blepharocalyx* used in this experiment were collected from the Yunnan Province of China in August 1991. The plant was identified by Professor Wu Te-Lin, South China Institute of Botany, Academia Sinica. A voucher specimen (CPU9008037) was deposited in the herbarium of the China Pharmaceutical University.

Extraction and Isolation. Dried seeds of *A. blepharocalyx* (5 kg) were powdered and percolated with EtOH (20 L) five times at room temperature. The extract was dried under reduced pressure to yield a residue of 750 g. A portion of the residue (400 g) was suspended in 10% aqueous MeOH (1 L) and successively partitioned by hexane (5 × 2 L, 22 g) and Et_2O (5 × 2 L, 225 g). The hexane extract (22 g) was subjected to column chromatography over Si gel 60 and eluted under gradient conditions with increasing amounts of EtOAc in hexane to afford four fractions, fractions A−D. Fraction B was purified again by column chromatography over Si gel using hexane−EtOAc (8:2) as an eluent to afford 10 mg of **8**. Fraction C was subjected to column chromatography in a similar manner using hexane−EtOAc (7:3), yielding 30 mg of **4**. The Et_2O extract (100 g) was also subjected to column chromatography over Si gel, using a linear $CHCl_3$-MeOH gradient system. The fraction eluted with CH_3Cl-MeOH (8:2) was further purified by column chromatography over Si gel and elution with C_6H_6-Et_2O-MeOH (5:4:1) to give 32 mg of **1** and 27 mg of **2**. The fraction eluted with $CHCl_3$-MeOH (7:3) was subjected to column chromatography and elution with $CHCl_3$-MeOH (8:2−6:4) to give **3** (70 mg), **5** (8 mg), **6** (80 mg), and **7** (10 mg).

1,7-Bis(4-hydroxyphenyl)-3-hydroxy-1,3-heptadien-5-one (1): yellow needles (Et_2O-C_6H_6); mp 148−149 °C; HREIMS 310.1183 (calcd for $C_{19}H_{18}O_4$, 310.1205); EIMS m/z 310 (M^+, 36), 295 (96), 208 (37), 206 (43), 189 (100), 161 (80), 147 (23), 120 (16), 107 (52); IR (KBr) ν_{max} 3180 (OH), 1635 (C=O), 1583, 1570, 1550, 1360, 927, 820, 780 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 362 (4.40), 215 (sh), 225 (sh) nm; ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

Acetylation of compound 1. A solution of **1** (10 mg) in Ac_2O (1 mL) and pyridine (1 mL) was stirred at room temperature for 24 h. The acetylated product was purified by preparative TLC (Merck, 0.25 mm; $CHCl_3$, R_f = 0.4) to give diacetate **1a** (9.5 mg), which was positive to $FeCl_3$ reagent: EIMS m/z 394 (M^+, 44), 352 (63), 310 (40), 292 (58), 231 (22), 203 (72), 161 (93), 147 (100), 107 (96), 43 (94); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.29 (3H, s, OCH_3), 2.31 (3H, s, OCH_3), 2.72 (2H, t, J = 7.2 Hz, H-7), 2.98 (2H, t, J = 7.2 Hz, H-6), 5.61 (1H, s, H-4), 6.40 (1H, d, J = 16.0 Hz, H-2), 7.01 (2H, d, J = 8.5 Hz), 7.11 (2H, d, J = 8.5 Hz), 7.21 (2H, d, J = 8.5 Hz), 7.53 (2H, d, J = 8.5 Hz), 7.31 (1H, d, J = 16.0 Hz, H-1), and 15.18 (s, br, 3-OH).

^{13}C-NMR data of compounds **3** and **5**, which have not been published previously, are listed below. Compound **3**: yellow amphous powder; δ (DMSO-d_6, 100 MHz)

Table 2. Percentage Inhibition of Compounds **1**−**8** (100 μg/mL) and Indomethacin on Platelet Aggregation of Human Whole Blood

inducer	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	indomethacin
collagen	77.3 ± 1.3	76.3 ± 1.1	86.9 ± 5.5	48.9 ± 4.4	57.7 ± 7.1	56.0 ± 4.3	32.3 ± 9.2	23.6 ± 4.6	71.1 ± 6.2
ADP	100 ± 0.0[a]	35.4 ± 0.3	100 ± 0.0[a]	43.8 ± 5.3	14.5 ± 1.1	62.9 ± 1.3	48.9 ± 1.0	39.8 ± 1.5	36.7 ± 2.2
AA	100 ± 0.0[a]	46.8 ± 1.4	100 ± 0.0[a]	100 ± 0.0[a]	26.2 ± 0.6	100 ± 0.0[a]	100 ± 0.0[a]	5.4 ± 0.1	100 ± 0.0[a]
Ristocetin	62.8 ± 0.8	38.3 ± 2.1	100 ± 0.0[a]	33.3 ± 3.4	37.2 ± 0.3	0.0 ± 0.0[b]	63.8 ± 0.4	11.0 ± 0.1	52.1 ± 0.1

[a] No aggregation was observed. [b] No inhibition was observed.

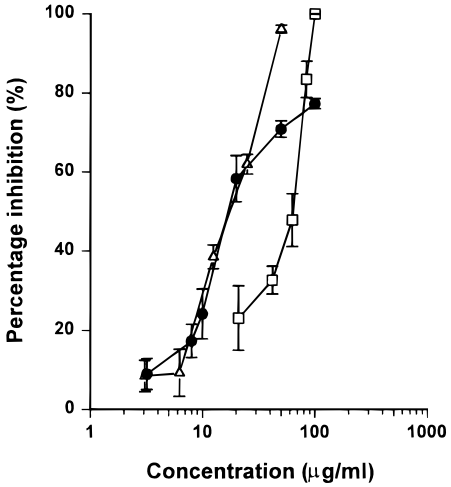

Figure 1. Effects of compound **1** on platelet aggregation induced by collagen (2 μg/mL, ●), AA (0.5 mM, △) and ADP (10 μM, □). The percentage inhibition of aggregation was calculated by taking the value of control (0.5% DMSO) as 100% aggregation.

56.26 (q, OCH_3), 88.01 (d, C-3), 100.03 (d, C-5), 115.76 (d, C-3′,5′), 116.09 (d, C-7), 126.7 (s, C-1′), 129.21 (d, C-2′,6′), 134.40 (d, C-8), 158.93 (s, C-4′,6), 162.72 (s, C-2), 170.95 (s, C-4). Compound **5**: white needles (MeOH−$CHCl_3$), mp 279−281 °C; δ (DMSO-d_6, 100 MHz) 44.78 (t, C-3), 55.59 (q, OCH_3), 78.06 (d, C-2), 93.20 (d, C-6), 95.60 (d, C-8), 104.9 (s, C-10), 115.1 (d, C-3′,5′), 128.1 (d, C-2′,6′), 129.3 (s, C-1′), 157.6 (s, C-4′), 162.5 (s, C-5, 9), 164.3 (s, C-7), 188.5 (s, C-4).

Biological Assay. Whole blood (1 mL) diluted with phosphate-buffered saline (1:1 ratio) was incubated with samples (5 μL, in DMSO) at 37 °C for 2 min, after which collagen (2 μg/mL), ADP (10 μM), AA (0.5 mM), or ristocetin (1 mg/mL) was added to initiate aggregation. The platelet aggregation was measured by a Whole Blood Lumi-Aggregometer (Chrono-Log Corp., Havertown, PA) using an electrical impedance method.[13,14] The mean percentages of aggregation in whole blood was determined at 5 min as the increase in impedance across a pair of electrodes placed in the blood sample by comparison to that of a control group impedance.[15] To eliminate the effect of the solvent on the aggregation, blood with 0.5% DMSO was used as the control. The percentage inhibition of platelet aggregation was calculated as: percentage inhibition (%) = [1 − (aggregation of the sample/aggregation of control)] × 100%. Each sample was measured in triplicate.

Acknowledgment. This work was supported by a grant from the National University of Singapore (RP950389).

References and Notes

(1) Wu, Z. Y.; Zhou, T. Y.; Xiao, P. G. *Xin Hua Ben Cao Gang Yao* **1988**, *1*, 537.
(2) Kadota, S.; Dong, H.; Basnet, P.; Prasain, J. K.; Xu, G. J.; Namba, T. *Chem. Pharm. Bull.* **1994**, *12*, 2647−2649.
(3) Kadota, S.; Prasain, J. K.; Li, J. X.; Basnet, P.; Dong, H.; Tani, T.; Namba, T. *Tetrahedron Lett.* **1996**, *40*, 7283−7286.
(4) Teng, C. M.; Hsu, S. Y.; Lin, C. H.; Yu, S. M.; Wang, K. J.; Lin, M. H.; Chen, C. F. *Chin. J. Physiol.* **1990**, *1*, 41−48.
(5) Tooru, O.; Nobuyuki, S.; Keiichi, N. *Chem. Abstr.* **1996**, *124*, 155972; Japan Patent 07324037A2, 12 Dec 1995.
(6) Kuroyanagi, M.; Noro, T.; Fukushima, S.; Aiyama, R.; Ikuta, A.; Itokawa, H.; Morita, M. *Chem. Pharm. Bull.* **1983**, *5*, 1544−1550.
(7) Kuroyanagi, M.; Natori, S. *Yakugaku Zasshi* **1970**, *11*, 1467−1470.
(8) Talapatra, B.; Pradhan, D. K.; Talapatra, S. K. *Indian J. Chem.* **1976**, *14B*, 300−301.
(9) Itokawa, H.; Morita, M.; Mihashi, S. *Phytochemistry* **1981**, *11*, 2503−2506.
(10) Norbedo, C.; Ferraro, G.; Coussio, J. D. *J. Nat. Prod.* **1982**, *45*, 635−636.
(11) Puyvelde, L. V.; Kimpe, N. *J. Nat. Prod.* **1989**, *3*, 629−633.
(12) Lin, C. N.; Lu, C. M.; Lin, H. C.; Fang, S. C.; Shieh, B. J.; Hsu, M. F.; Wang, J. P.; Ko, F. N.; Teng, C. M. *J. Nat. Prod.* **1996**, *59*, 834−838.
(13) Cardinal, D. C.; Flower, R. J. *J. Pharmcol. Meth.* **1980**, *3*, 135−158.
(14) Ingerman-Wojenski, C. M.; Silver, M. J. *Thromb. Haemostas.* **1984**, *2*, 154−156.
(15) Challen, A.; Branch, W. J.; Cummings, J. H. *J. Pharmcol. Meth.* **1982**, *8*, 115−122.

NP970293I