Biochemical Pharmacology, Vol. 45, No. 2, pp. 509–512, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

SHORT COMMUNICATIONS

Antiplatelet activity of some prenylflavonoids

(*Received* 2 *June* 1992; *accepted* 8 *October* 1992)

Abstract—Eight naturally occurring prenylflavonoids were tested for their antiplatelet activities in rabbit platelet suspension. Cyclomorusin and artomunoxanthone showed strong inhibition of platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) induced platelet aggregation. Cyclomulberrin, dihydroisocycloartomunin, cyclocommunol and cyclocommunin showed strong inhibition of arachidonic acid (AA)- and collagen-induced platelet aggregation. Cyclomorusin also inhibited markedly collagen-induced platelet aggregation. Cyclocommunin, dihydroisocycloartomunin and cyclomulberrin also showed slight but significant antiplatelet effects on the aggregation induced by PAF. Of the compounds tested, cyclocommunin exhibited the most potent inhibition of platelet aggregation induced by collagen (IC_{50} = 14.4 μM) and AA (IC_{50} = 12.5 μM). Thromboxane B_2 formation caused by AA was suppressed by cyclocommunin and artomunoxanthone.

In previous papers [1–4], we reported the isolation of new prenylflavonoids, new pyranodihydrobenzoxanthones and new quinonoid pyranobenzoxanthones from the fresh root bark of *Artocarpus communis*. Prenylflavonoids, isolated from Moraceous plants, especially *Morus alba, Artocarpus communis, Artocarpus heterophyllus* and *Artocarpus rigida* [5], have been investigated as inhibitors of arachidonate 5-lipoxygenase. In this paper, we report the antiplatelet effects and structure–activity relationships of these prenylflavonoids (see Fig. 1 for structures).

Materials and Methods

Materials. Prenylflavonoids were isolated from *Artocarpus communis* as described previously [1–4], and were diluted with dimethyl sulfoxide (DMSO*). In order to eliminate the effects of the solvent on aggregation, the final concentration of DMSO was fixed at 0.5%. Collagen (type 1, bovine achilles tendon), obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.), was homogenized in 25 mM acetic acid and stored at −70° at a concentration of 1 mg/mL. Natural platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) purchased from Sigma was dissolved in chloroform and diluted into 0.1% bovine serum albumin saline solution immediately prior to use. AA, EDTA (disodium salt), bovine serum albumin and aspirin were purchased from Sigma.

Methods. Blood was collected from the rabbit marginal ear vein and was mixed with EDTA to a final concentration of 6 mM. It was centrifuged for 10 min at 90 *g* and room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at 500 *g* for 10 min. The platelet pellets were washed with Tyrode's solution (Ca^{+2}-free) containing 2 mM EDTA, 0.1 mg/mL apyrase and 3.5 mg/mL serum albumin, and centrifuged at 500 *g* for 10 min. Then, the pellets were washed with the above Tyrode's solution without EDTA. After centrifugation under the same conditions, the platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0) and glucose (11.2) containing bovine serum albumin (0.35%).

Platelet aggregation. Aggregation was measured by a turbidimetric method [6]. The aggregation was measured

* Abbreviations: PAF, platelet-activating factor; DMSO, dimethyl sulfoxide; AA, arachidonic acid.

Table 1. Effects of prenylflavonoids on the platelet aggregation induced by arachidonic acid, collagen and PAF in washed rabbit platelets

Treatment	Aggregation (%)		
	AA (100 μM)	Collagen (10 μg/mL)	PAF (2 ng/mL)
Control	87.5 ± 1.7	89.6 ± 0.6	89.3 ± 0.7
Cycloartomunin (**1**) (300 μM)	91.6 ± 3.2	87.5 ± 2.1	92.9 ± 10.0
Cycloartomunin diacetate (**1a**) (75 μM)	87.8 ± 0.3	89.8 ± 0.4	87.5 ± 2.8
Cyclomorusin (**2**) (300 μM)	87.7 ± 2.7	1.5 ± 1.3†	8.0 ± 6.6†
Cyclomorusin diacetate (**2a**) (75 μM)	84.7 ± 1.8	87.2 ± 0.6	86.8 ± 0.6
Didhydrocycloartomunin (**3**) (300 μM)	Induced platelet aggregation without any agonist		
Artomunoxanthone (**4**) (300 μM)	22.0 ± 11.0†	24.6 ± 7.4†	6.5 ± 3.7†
Cyclomulberrin (**5**) (100 μM)	18.6 ± 10.1†	50.6 ± 16.2*	71.7 ± 9.4*
Dihydroisocycloartomunin (**6**) (100 μM)	4.1 ± 1.9†	22.0 ± 7.8†	75.4 ± 5.9*
Cyclocommunol (**7**) (300 μM)	3.9 ± 1.8†	3.7 ± 1.8†	84.6 ± 1.6
Cyclocommunin (**8**) (50 μM)	0.0 ± 0.0†	0.0 ± 0.0†	59.5 ± 14.3*
Aspirin (50 μM)	0.0 ± 0.0†	85.4 ± 3.9	90.5 ± 1.2

Platelets were preincubated with DMSO (0.5%, control) or prenylflavonoid at 37° for 3 min, and the inducer was then added.

Values are presented as means ± SEM (N = 3–5).

* $P < 0.01$, † $P < 0.001$ as compared with the respective control.

in a Lumi-aggregometer (Chrono-Log Co., U.S.A.). All glassware was siliconized. Just 3 min before the addition of the aggregation inducer, the platelet suspension was stirred at 1200 rpm. The percentage of aggregation was calculated as follows (A, absorbance):

Aggregation (%) = (initial A − final A after aggregation)/(initial A–A of suspending medium).

Per cent inhibition was expressed assuming the absorbance of platelet suspension as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation.

Results

The antiplatelet effects of prenylflavonoids were studied on the aggregation of washed rabbit platelets induced by AA (100 μM), collagen (10 μg/mL) and PAF (2 ng/mL). As shown in Tables 1 and 2, cyclomorusin (**2**) and artomunoxanthone (**4**) showed strong inhibition of PAF-induced platelet aggregation, and cyclomulberrin (**5**), dihydroisocycloartomunin (**6**), cyclocommunol (**7**) and cyclocommunin (**8**) showed strong inhibition of both AA- and collagen-induced platelet aggregation. Among these compounds tested, compound **8** exhibited the most potent inhibition of platelet aggregation induced by AA and collagen. Compound **2** also inhibited markedly the platelet aggregation induced by collagen. Compounds **5**, **6** and **8** also showed slight but significant antiplatelet effects on the aggregation induced by PAF. The acetylated product (**2a**,

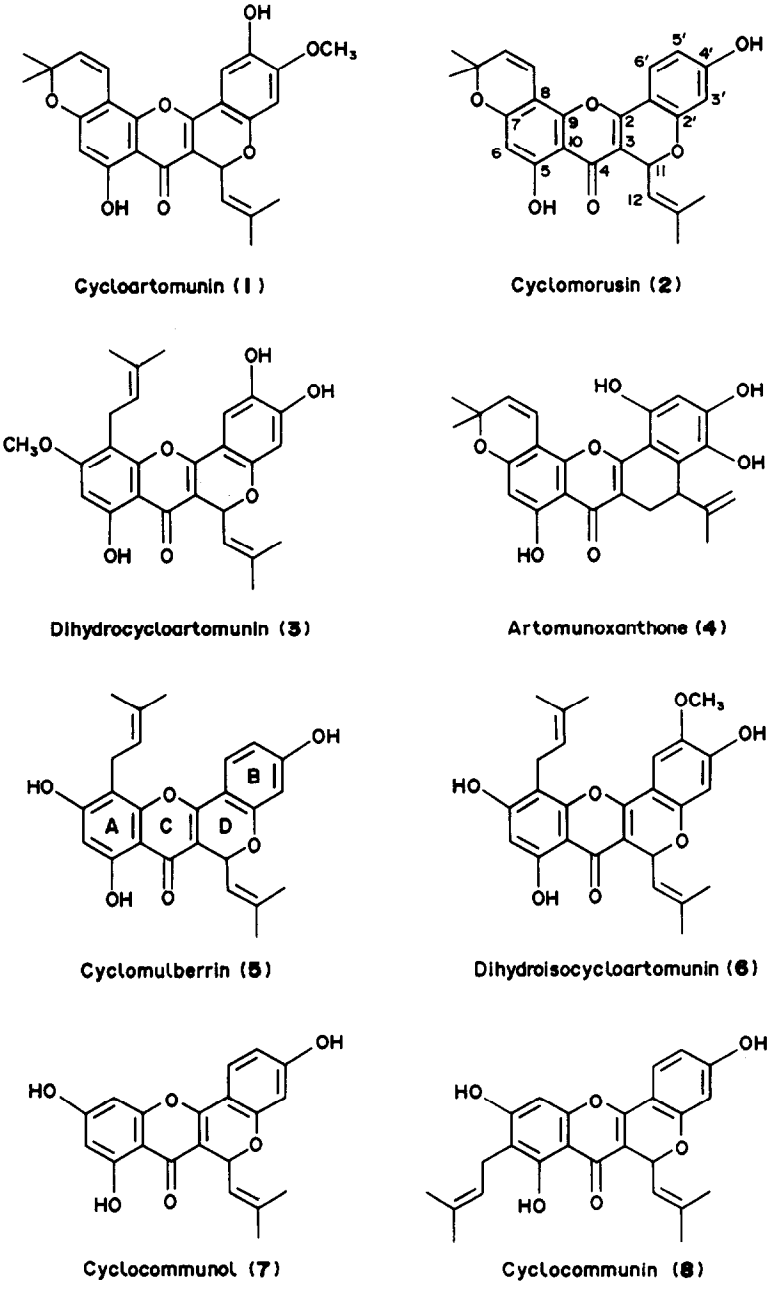

Fig. 1. Structures of prenylflavonoids.

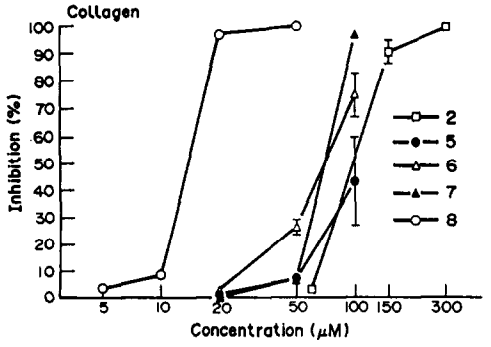

Fig. 2. Concentration-dependent inhibition of cyclomorusin (**2**), cyclomulberrin (**5**), dihydroisocycloartomunin (**6**), cyclocommunol (**7**) and cyclocommunin (**8**) on platelet aggregation induced by collagen of rabbit platelet suspension. Platelets were incubated with different concentrations of prenylflavonoids or DMSO (0.5%) at 37° for 3 min, and collagen (10 μg/mL) was then added to trigger the aggregation. Values are presented as means ± SEM (N = 3–5).

75 μM) of **2** did not show significant antiplatelet effects. Compound **3** induced platelet aggregation without any agonist.

As shown in Tables 1 and 2, a prenyl group substituted at C-6 of **7** (i.e. compound **8**) exhibited much stronger enhancement of antiplatelet effects on AA-, collagen- and PAF-induced aggregation than a prenyl group substituted at C-8 of **7** (i.e. compound **5** or **6**), but a methoxy group substituted at C-3′ of **6** shifted to the C-7 of **6** such as **3** enhanced platelet aggregation. As shown in Tables 1 and 2, and Figs 2 and 3, a chromene ring substituted at C-7 and C-8 of the flavonoid moiety did not enhance the antiplatelet effects of platelet aggregation induced by AA and collagen.

More experiments were performed to study the inhibitory potencies of prenylflavonoids on collagen- and AA-induced platelet aggregation at various concentrations (Figs 2 and 3). Compound **8** was more potent than **2** and **5–7**. Aspirin

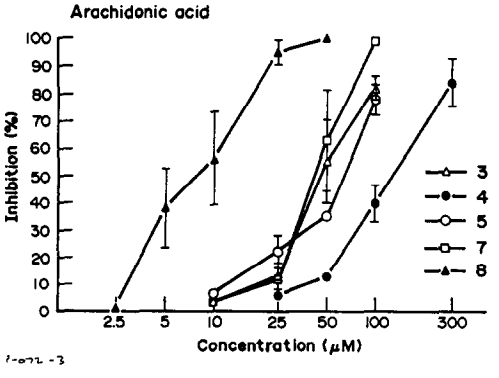

Fig. 3. Concentration-dependent inhibition of dihydrocycloartomunin (**3**), artomunoxanthone (**4**), cyclomulberrin (**5**), cyclocommunol (**7**) and cyclocommunin (**8**) on platelet aggregation induced by AA of rabbit platelet suspension. Platelets were incubated with different concentrations of prenylflavonoids or DMSO (0.5%) at 37° for 3 min, and AA (100 μM) was then added to trigger the aggregation. Values are presented as means ± SEM (N = 3–5).

Table 2. IC_{50} values of prenylflavonoids on the platelet aggregation induced by AA and collagen

Reagent	IC_{50} (μM) AA	Collagen
Cyclomorusin (**2**)	>300	113.4
Artomunoxanthone (**4**)	180.1	—
Cyclomulberrin (**5**)	67.1	128.2
Dihydroisocycloartomunin (**6**)	44.1	72.8
Cyclocommunol (**7**)	57.3	63.5
Cyclocommunin (**8**)	12.5	14.4

Data were calculated from Figs 2 and 3.

was used in this study as a positive control and at 50 μM completely inhibited platelet aggregation induced by AA but not that induced by collagen or PAF.

Discussion

It has been reported that quercetin pentaacetate strongly inhibits the platelet aggregation induced by collagen and AA, but not that induced by PAF. This antiplatelet action is due to inhibition of thromboxane A_2 formation [7]. In this paper, we found that compounds **5–8** showed the same antiplatelet actions on collagen- and AA-induced platelet aggregation as that of quercetin pentaacetate with little or no antiplatelet effects on PAF-induced platelet aggregation. Compound **8** inhibited also the formation of thromboxane B_2 (Table 3), a stable metabolite of thromboxane A_2, induced by AA. Therefore, the antiplatelet mechanism of these compounds is probably due to the inhibition of thromboxane A_2 formation. However, the exact mechanism of action of these prenylflavonoids may be different from that of aspirin, a cyclooxygenase inhibitor, because the latter inhibits AA but not collagen-induced platelet aggregation.

Compounds **5–8**, without a chromene ring, substituted at C-7 and C-8 of 5,7-dihydroxyflavonoid, appear to have the same mechanism of action and the different antiplatelet potency may be due to the position of prenylation. Substitution of the prenyl group at C-6 (compound **8**) is much more potent than at C-8 (compounds **5** and **6**).

As shown in Table 1, compound **3** did not show antiplatelet action, but demethylation at C-7 of **3** (compound

Table 3. Inhibitory effects of cyclocommunin (**8**) and artomunoxanthone (**4**) on AA-induced thromboxane B_2 formation in washed rabbit platelets

Treatment	Thromboxane B_2 (ng/mL)
Resting	0.22 ± 0.04
Control	742.9 ± 88.2
Cyclocommunin (**8**) (50 μM)	71.1 ± 47.1*
Artomunoxanthone (**4**)	
50 μM	351.1 ± 177.0
100 μM	155.4 ± 66.2*
300 μM	90.7 ± 35.0*

Cyclocommunin (**8**), artomunoxanthone (**4**) or DMSO (0.5%, control) was preincubated with platelets at 37° for 3 min, and arachidonic acid (100 μM, except resting) was then added. Aggregation and thromboxane B_2 formation were terminated by EDTA (2 mM) and indomethacin (50 μM) 6 min after addition of the inducer.

Values are presented as means ± SEM (N = 3–5).

* $P < 0.001$ as compared with the control.

6) produced a potent antiplatelet effect. In addition to compound **6**, the active compounds **5**, **7** and **8** all possess a 7-hydroxyl group. This suggests that the 7-hydroxyl group of prenylflavonoids is an important moiety related to antiplatelet effects.

Compounds **2** and **4** possess a chromene ring substituted at C-7 and C-8 of the 5,7-dihydroxyflavonoid moiety, and showed potent inhibition on the platelet aggregation induced by PAF. This indicates that a chromene ring substituted at C-7 and C-8 may modulate antiplatelet effects. However, compound **1** did not possess any antiplatelet action, probably due to methoxyl, not hydroxyl, substitution at C-4′. Thus, the functional group at ring B of prenylflavonoids also modulates the antiplatelet effects.

Acknowledgements—This work was supported, in part, by grants from the National Science Council of the Republic of China (NSC81-0420-B037-15) and the National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

Natural Products Research Center
Kaohsiung Medical College
Kaohsiung 807, and
†Pharmacological Institute
College of Medicine
National Taiwan University
Taipei 100
Taiwan, R.O.C.

CHUN-NAN LIN*
WEN-LIANG SHIEH
FENG-NIEN KO†
CHE-MING TENG

* Corresponding author: Chun-Nan Lin, School of Pharmacy, Kaohsiung Medical College, Kaohsiung 807, Taiwan, R.O.C. Tel. (886) 7 3121101-9 ext 2163; FAX (886) 7 3212062.

REFERENCES

1. Lin CN and Shieh WL, Prenylflavonoids and a pyranodihydrobenzoxanthone from *Artocarpus communis*. *Phytochemistry* **30**: 1669–1671, 1991.
2. Shieh WL and Lin CN, A quinonoid pyranobenzoxanthone and pyranodihydrobenzoxanthone from *Artocarpus communis*. *Phytochemistry* **31**: 364–367, 1992.
3. Lin CN, Shieh WL and Jong TT, A novel pyranodihydrobenzoxanthone epoxide from *Artocarpus communis*. *Phytochemistry* **31**: 2563–2564, 1992.
4. Lin CN and Shieh WL, Pyranoflavonoids from *Artocarpus communis*. *Phytochemistry* **31**: 2922–2924, 1992.
5. Reddy GR, Ueda N, Hada T, Sackeyfio AC, Yamamoto S, Hano Y, Aida M and Nomura T, A prenylflavone, artonin E, as arachidonate 5-lipoxygenase inhibitor. *Biochem Pharmacol* **41**: 115–118, 1991.
6. O'Brien JR, Platelet aggregation. II. Some results from a new method of study. *J Clin Pathol* **15**: 452–455, 1962.
7. Lin HC, Liu HW, Lin CN and Teng CM, Inhibition of thromboxane formation as the antiplatelet mechanism of 3,4-dihydroxyxanthone and quercetin pentaacetate. *Kaohsiung J Med Sci* **7**: 505–512, 1991.