Antiplatelet Effects and Vasorelaxing Action of Some Constituents of Formosan Plants

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ANTIPLATELET EFFECTS AND VASORELAXING ACTION OF SOME CONSTITUENTS OF FORMOSAN PLANTS

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ABSTRACT.—Various xanthones as well as quercetin have been shown to exhibit antiplatelet activity. A series of anthraquinones analogues structurally related to xanthones and a series of quercetin-related compounds were tested for their antiplatelet effects. Emodin, frangulin B, kaempferol tetraacetate, quercetin-3-*O*-galactoside octaacetate, rhamnazin triacetate, and rhamnetin tetraacetate were found to be potent antiplatelet agents, and 1,8-dihydroxy-6-methoxy-3-methylanthraquinone 8-*O*-rhamnosyl-(1→2)-glucoside, rhamnustrioside undecaacetate, rutin decaacetate, and quercetin-3-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-galactopyranoside decaacetate showed significant antiplatelet effects. Quercetin showed vasorelaxing action in rat thoracic aorta.

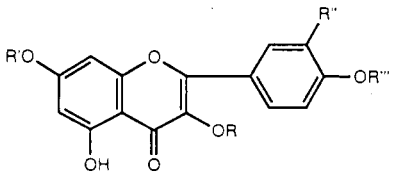
Thrombosis plays an especially important role in the causes of cerebral stroke and cardiac diseases. However, effective antithrombotic drugs still require research and development. Thrombolytic vasoactive Chinese herbs have been used as a treatment in traditional medicine. We are trying to isolate those biologically active substances from natural products and carry out screening work for the thrombolytic vasoactive Chinese herbs, especially those having antiplatelet and vasodilating effects.

Recently, we demonstrated that some xanthone derivatives and quercetin pentaacetate inhibited the aggregation and release reaction of rabbit platelets caused by various inducers, and norathyriol relaxed the rat thoracic aorta (1–3). In our search for bioactive compounds from Formosan Gentianaceous and *Rhamnus* plants (4–9), we studied antiplatelet effects of 11 flavonol derivatives. Since some xanthones possess novel antiplatelet effects (1), the antiplatelet activity of anthraquinones, which possess closely related chemical structures to xanthones, was also examined. The vasorelaxing effects of 4 flavonol derivatives were also studied.

RESULTS AND DISCUSSION

The antiplatelet effects of kaempferol [**1**] tetraacetate, rhamnustrioside [**2**] undecaacetate, rhamnetin [**4**] tetraacetate, rhamnazin [**5**] triacetate, quercetin-3-galactoside [**6**], **6** octaacetate, rutin [**7**], **7** decaacetate, quercetin-3-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-galactopyranoside [**8**] decaacetate, rhamnazin 3-isorhamninoside [**8**] decaacetate, chrysophanol [**10**], physcion [**11**], **11** diacetate, emodin [**12**], **12** triacetate, frangulin B [**13**], **13** hexaacetate, and physcion 8-*O*-rhamnosyl-(1→2)-glucoside [**14**] were studied on the aggregation of washed rabbit platelets induced by ADP (20 μM), arachidonic acid (AA) (100 μM), collagen (10 μg/ml), and PAF (2 ng/ml), and the results are shown in Tables 1–3. Compounds **1** tetraacetate and **4** tetraacetate strongly inhibited platelet aggregation induced by arachidonic acid, collagen, and PAF. Compound **5** triacetate and **6** octaacetate strongly inhibited arachidonic acid- and collagen-induced platelet aggregation. Compound **7** decaacetate and **8** decaacetate significantly inhibited the platelet aggregation induced by arachidonic acid and collagen. The esterified quercetin-related compounds show enhancement of their antiplatelet effects. The glycosylate of quercetin-related compound at 3-OH with one molecule of sugar showed enhancement

TABLE 1. Effects of Various Quercetin-related Compounds on the Platelet Aggregation Induced by Arachidonic Acid (AA), Collagen, and Platelet-activating Factor (PAF).[a]



1 R=R′=R″=R‴=H
2 R=R′=R″=H, R‴=rha(1→2)-*O*-[rha-(1→6)]galactosyl
3 R=R′=R‴=H, R″=OH
4 R=R‴=H, R′=Me, R″=OH
5 R=R‴=H, R′=Me, R″=OMe
6 R=galactosyl, R′=R‴=H, R″=OH
7 R=rutinoyl, R′=R‴=H, R″=OH
8 R=gal(6→1)ara, R′=R‴=H, R″=OH
9 R=glc(6→1)rha(4→1)rha, R′=Me, R″=OMe, R‴=H

Inducer	Control	**1** tetraacetate	**2** undecaacetate	**3** pentaacetate[b]	**4** tetraacetate
ADP	84.8 ± 4.4 (4)				
AA	90.7 ± 0.9 (4)	17.4 ± 0.9 (3)[c]	88.5 ± 0.3 (3)	2.4 ± 2.0 (3)[c]	2.6 ± 2.2 (3)[c]
Collagen	93.1 ± 2.1 (4)	15.9 ± 1.7 (3)[c]	86.5 ± 1.1 (3)[c]	17.4 ± 2.9 (3)[c]	0.0 ± 0.0 (3)[c]
PAF	92.9 ± 1.2 (3)	14.5 ± 4.7 (3)[c]	89.9 ± 0.6 (3)	89.2 ± 1.5 (3)	0.0 ± 0.0 (3)[c]

Inducer	**5** triacetate	**6**	**6** octaacetate	**7**	**7** decaacetate
ADP		71.4 ± 1.3 (2)		75.0 ± 2.5 (2)	72.4 ± 2.8 (3)
AA	10.0 ± 4.4 (3)[c]	92.2 ± 2.3 (2)	0.0 ± 0.0 (3)[c]	93.5 ± 0.9 (2)	25.9 ± 21.1 (3)[c]
Collagen	14.3 ± 1.7 (3)[c]	87.3 ± 4.6 (2)	0.0 ± 0.0 (3)[c]	87.0 ± 3.7 (2)	27.3 ± 17.1 (3)[c]
PAF	84.5 ± 1.1 (3)	93.8 ± 0.4 (2)	78.5 ± 2.9 (3)[c]	91.1 ± 0.8 (2)	88.7 ± 0.9 (3)

Inducer	**8** decaacetate	**9** decaacetate	Indomethacin		
ADP			69.5 ± 7.2 (3)		
AA	78.3 ± 2.9 (3)[d]	88.8 ± 1.4 (3)	0.0 ± 0.0 (3)[c]		
Collagen	38.5 ± 15.4 (4)[d]	87.8 ± 0.8 (3)	72.3 ± 6.0 (3)		
PAF	87.7 ± 1.9 (3)	90.8 ± 1.6 (3)	89.4 ± 0.4 (3)		

[a]Platelets were preincubated with various agents (100 μg/ml) or DMSO (0.5%, control) at 37° for 3 min, then ADP (20 μM), arachidonic acid (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. Percentages of aggregation are presented as means ± SEM. Number of samples is shown in parentheses.

[b]Data in this column are from Lin *et al.* (3).

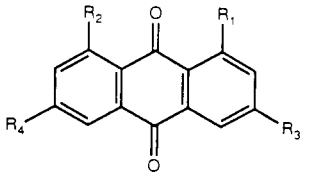
[c]$p<0.05$ as compared with control values.

[d]$p<0.01$ as compared with control values.

[e]$P<0.001$ as compared with control values.

of antiplatelet effects, but the antiplatelet effects decreased as the number of molecules of sugar increased. The antiplatelet actions of **1** tetraacetate, **4** tetraacetate, **5** triacetate, and **6** octaacetate inhibited collagen-induced platelet aggregation in a dose-dependent manner, and all showed greater antiplatelet effects than **3** pentaacetate (3). Figure 1 summarizes the biological activities of the quercetin-related compounds. Compound **6** octaacetate was the most potent inhibitor with a minimal effective concentration of 5 μg/ml and a maximal effective concentration of 100 μg/ml. Compound **6** octaacetate was much more potent than norathyriol [**15**] tetraacetate (1) in inhibiting platelet aggregation induced by collagen. Compounds **12** and **13** strongly inhibited platelet

TABLE 2. Effects of **10**, **11**, **11** Diacetate, **12**, **12** Triacetate, **13**, **13** Hexaacetate, **14**, and Indomethacin on the Platelet Aggregation Induced by ADP, Arachidonic Acid, PAF, and Collagen.[a]



10 $R_1=R_2=OH$, $R_3=Me$, $R_4=H$
11 $R_1=R_2=OH$, $R_3=Me$, $R_4=OMe$
12 $R_1=R_2=R_4=OH$, $R_3=Me$
13 $R_1=R_2=OH$, $R_3=Me$, $R_4=O$-rhamnosyl
14 $R_1=OH$, $R_2=O$-rhamnosyl-(1→2)-glucoside, $R_3=Me$, $R_4=OMe$

Inducer	Control	**10**	**11**	**11** diacetate	**12**
ADP	89.4 ± 0.4 (25)	82.6 ± 2.1 (4)	78.9 ± 6.0 (3)	76.5 ± 8.5 (3)	65.1 ± 19.8 (3)
AA	93.3 ± 0.2 (4)	90.6 ± 1.9 (3)	89.6 ± 0.8 (3)	90.3 ± 2.8 (3)	24.3 ± 7.4 (3)[b]
Collagen	93.8 ± 1.6 (3)	85.4 ± 3.9 (3)	86.8 ± 0.8 (3)	89.4 ± 0.3 (3)	75.7 ± 13.5 (3)
PAF	94.5 ± 0.6 (3)	88.3 ± 2.8 (3)	80.7 ± 7.3 (3)	93.9 ± 0.6 (3)	76.4 ± 14.8 (3)
Inducer	**12** triacetate	**13**	**13** hexaacetate	**14**	Indomethacin
ADP	72.2 ± 7.9 (3)	60.7 ± 7.3 (8)[b]	80.1 ± 2.1 (3)	78.2 ± 7.9 (3)	69.5 ± 7.2 (3)
AA	82.5 ± 10.6 (3)[b]	91.7 ± 2.4 (3)	85.6 ± 3.0 (3)	75.9 ± 2.7 (3)	0.0 ± 0.0 (3)[b]
Collagen	89.6 ± 6.2 (3)	20.6 ± 2.3 (3)[b]	82.5 ± 3.1 (3)	76.7 ± 0.8 (3)[b]	72.3 ± 6.0 (3)
PAF	84.5 ± 2.7 (3)	73.0 ± 9.4 (3)	82.8 ± 2.7 (3)	86.6 ± 3.0 (3)	89.4 ± 0.4 (3)

[a]Platelets were preincubated with **10** (50 μg/ml), **11** (25 μg/ml), **11** diacetate (25 μg/ml), **12** (100 μg/ml), **12** triacetate (100 μg/ml), **13** (100 μg/ml), **13** hexaacetate (100 μg/ml), **14** (100 μg/ml) or DMSO (0.5%) (control) at 37° for 3 min; then ADP (20 μM), arachidonic acid (AA, 100 μM), PAF (2 ng/ml) or collagen (10 μg/ml) was added. Values are presented as means ± SEM. Number of samples is shown in parentheses.

[b]$p<0.001$ as compared with the respective control.

TABLE 3. Effects of Various Concentrations of **12** and **13** on the Platelet Aggregation Induced by Arachidonic Acid and Collagen.[a]

Treatment	Aggregation (%)	
	Arachidonic acid (100 μM)	Collagen (10 μg/ml)
Control	88.3 ± 1.3 (10)	89.3 ± 0.6 (11)
12 (25 μg/ml)	90.3 ± 0.8 (6)	
12 (50 μg/ml)	64.5 ± 13.3 (6)[b]	83.3 ± 3.8 (6)[b]
12 (100 μg/ml)	33.6 ± 6.8 (10)[d]	61.3 ± 6.4 (11)[d]
Control	90.2 ± 0.8 (9)	89.2 ± 0.8 (9)
13 (10 μg/ml)		86.5 ± 3.4 (6)
13 (25 μg/ml)		36.7 ± 10.5 (6)[d]
13 (50 μg/ml)		19.4 ± 10.1 (6)[d]
13 (100 μg/ml)	94.9 ± 1.4 (8)[c]	10.3 ± 5.2 (9)[d]

[a]Percentages of aggregation are presented as means ± SEM. Number of replicates is shown in parentheses.

[b]$p<0.05$.

[c]$p<0.01$.

[d]$p<0.001$.

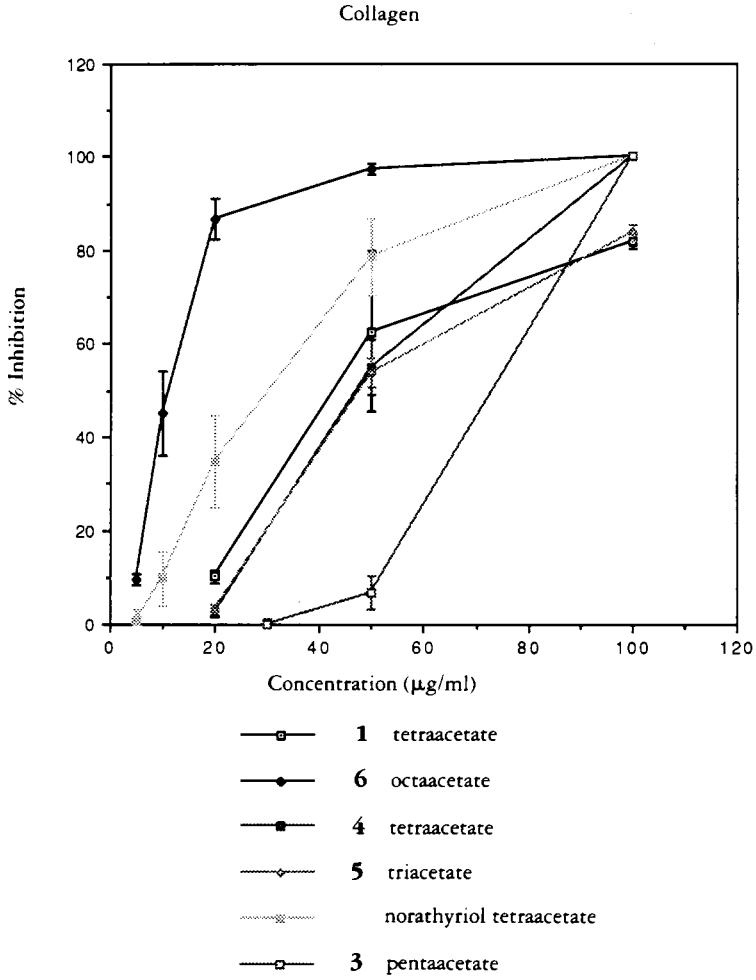


FIGURE 1. The effects of quercetin-related compounds **1** tetraacetate, **3** pentaacetate, **4** tetraacetate, **5** triacetate, **6** octaacetate, norathyriol tetraacetate on the platelet aggregation induced by collagen. Washed rabbit platelets were incubated with various concentrations of agents and collagen (10 μg/ml) was added. Data of **3** pentaacetate are from Lin *et al.* (3).

aggregation induced by arachidonic acid and collagen, respectively. Compound **13** significantly inhibited the platelet aggregation induced by ADP. The esterified anthraquinones did not show enhancement of their antiplatelet effects, and the **12**-related anthraquinones showed greater antiplatelet effects than did the **11**-related anthraquinones. The antiplatelet actions of **12** and **13** were further examined as shown in Tables 2 and 3. Compound **13** inhibited both arachidonic-acid- and collagen-induced platelet aggregation; it did not inhibit that caused by arachidonic acid even at 100 μg/ml. This implies different mechanisms of action are possibly involved in the antiplatelet actions of **12** and **13**. Further experiments are needed to elucidate their actions. In the rat thoracic aorta, **3** depressed markedly the contractions induced by Ca^{2+} (1.9 mM) in high-K^+ (80 mM) medium and norepinephrine (3 μM) (Table 4). Norathyriol and apigenin, a xanthone and a flavonoid analogue, respectively,

TABLE 4. Effects of Various Quercetin-related Compounds on High K^+- and Ca^{++}-induced and Norepinephrine-induced Contraction of Rat Thoracic Aorta.[a]

	K^+ (80 mM) + Ca^{++} (1.9 mM)	NE (3 μM)-phasic	NE (3 μM)-tonic
Control	100.0 ± 9.3	100.0 ± 3.4	100.0 ± 10.5
3 (100 μg/ml)	13.9 ± 2.0[b]	27.0 ± 11.2[b]	0.0 ± 0.0[b]
3 (50 μg/ml)		15.0 ± 3.5[b]	0.0 ± 0.0[b]
3 (20 μg/ml)		44.9 ± 1.1[b]	29.1 ± 1.7[b]
3 (10 μg/ml)		105.0 ± 3.5	101.9 ± 1.3
6 (100 μg/ml)	120.9 ± 0.6	100.0 ± 0.0	98.8 ± 6.3
7 (100 μg/ml)	112.9 ± 1.0	103.8 ± 2.7	120.6 ± 1.4
8 (100 μg/ml)	100.0 ± 0.0	100.0 ± 0.0	108.4 ± 3.3

[a]Rat aorta were preincubated with various compounds or DMSO (0.1%, control) at 37° for 15 min; then high K^+ (80 mM) and Ca^{++} (1.9 mM) or norepinephrine (NE, 3 μM) was added. Percentages of the control contraction were calculated and presented as means ± SE ($n = 3$).

[b]$p<0.01$ as compared with respective control.

markedly inhibited AA- and collagen-induced aggregation by the inhibition of thromboxane A_2 formation in washed rabbit platelets (1,10). In rat thoracic aorta, they also inhibited K^+- and NE-induced contractions by suppression of Ca^{2+} influx (2,11). Thus, **3** may possess antiplatelet and vasorelaxing actions similar to those of norathyriol and apigenin.

EXPERIMENTAL

MATERIALS.—Compounds **1**, **2**, **4**, and **6**–**14** were isolated and identified as previously reported (4–9). Compounds **2** undecaacetate, **4** tetraacetate, **5** triacetate, **6** octaacetate, **7** decaacetate, **8** decaacetate, **9** decaacetate, **11** acetate, **12** triacetate, and **13** hexaacetate were prepared by usual methods and identified from physical and spectral data.

PLATELET AGGREGATION.—Washed rabbit platelets were obtained from EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously (12). Platelets were counted by a Coulter Counter (Model ZM), adjusted to 4.5×10^8 platelets/ml, and suspended in Tyrode's solution containing (mM) NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CuCl_2$ (1.0), and glucose (11.2) with bovine serum albumin (0.35%). Aggregation was measured by the turbidimetric method (13), designed with the absorbance of platelets in suspension at 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation. The aggregation was measured by a Lumi-aggregometer (Chrono-Log Co.) connected to dual channel recorders. The platelet suspension was stirred at 1200 rpm. To eliminate the effect of the solvent on the aggregation, the final concentration of DMSO was fixed at 0.5%.

AORTIC CONTRACTION.—Wistar rats of either sex weighing 250 to 300 g were killed by a blow to the head. The thoracic aorta was isolated and excess fat and connective tissue were removed. Vessels were cut into rings of about 5 mm in length, mounted in organ baths containing 5 ml of Krebs solution, maintained at 37°, and bubbled with a 95% O_2 and 5% CO_2 mixture. Two stainless steel hooks were inserted into the aortic lumen; one was fixed while the other was connected to a transducer. Aorta were equilibrated in the medium for 90 min with three changes of Krebs solution and maintained under an optimal tension of 1 g before specific experimental protocols were initiated; contractions were recorded isometrically via a force displacement transducer connected to a Gould polygraph (Model 2400). The final concentration of DMSO was fixed at 0.1%.

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