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ANTIPLATELET CONSTITUENTS OF FORMOSAN *RUBIA AKANE*

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ABSTRACT.—A known steroid, in addition to triterpenoids, anthraquinones, naphthalenes and a new anthraquinone glycoside, xanthopurpurin 3-*O*-β-D-glucoside, were isolated from the roots of *Rubia akane* grown in Taiwan. Mollugin, a naphthohydroquinone, showed strong inhibition of arachidonic acid (AA)-induced and collagen-induced platelet aggregation. In contrast, 2-methyl-1,3,6-trihydroxyl-9,10-anthraquinone, xanthopurpurin 3-*O*-β-D-glucoside, and xanthopurpurin showed mainly strong inhibition of collagen-induced platelet aggregation.

In the course of continued screening work on antithrombotic Chinese herbs, especially those having antiplatelet and vasodilating effects, mollugin [**1**], 1-hydroxyl-2-methyl-9,10-anthraquinone [**2**], rubiprasin A, rubiprasin B, 2-methyl-1,3,6-trihydroxyl-9,10-anthraquinone [**3**], rubiarbonol D, 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 1,4-di-*O*-β-glucoside [**4**], 2-methyl-1,3,6-trihydroxyl-9,10-anthraquinone 3-*O*-(3′-*O*-acetyl)-α-rhamnosyl(1→2)glucoside [**5**], 2-methyl-1,3,6-trihydroxyl-9,10-anthraquinone 3-*O*-α-rhamnosyl (1→2)glucoside [**6**], xanthopurpurin 3-*O*-β-D-glucoside [**7**], lucidin primeveroside [**9**], and β-sitosterol β-D-glucoside, have been isolated from the roots of *Rubia akane* (Rubiaceae), collected in Taiwan. Compound **7** is a new glycoside isolated from a natural source, and rubiprasin A, rubiprasin B, rubiarbonol D, and β-sitosterol β-D-glucoside are known compounds (1–4) isolated for the first time from this plant. In this paper, we report the isolation and characterization of **7**. Because the anthraquinones showed antiplatelet effects (5-6), we also report the antiplatelet effects of compounds **2**, **3**, **5**, **6**, **7**, **8** (aglycone of **7**), **9**, and the related constituents **1** and **4**.

Compound **7**, $C_{20}H_{18}O_9$, mp 244–245°, showed uv absorption maxima characteristic of anthraquinone (7). The ir spectrum showed a strong absorption band at 3400 cm^{-1} due to a free hydroxyl group and a chelated carbonyl group absorption at 1630 cm^{-1}. On acid hydrolysis, it afforded glucose, detected by tlc, and xanthopurpurin [**8**], identified by uv, ir, ms and nmr (8). In the negative-ion fab mass spectrum, the peak of highest mass number was observed at *m/z* 401 $[M-1]^-$ and confirmed the $[M]^+$ of **7** to be *m/z* 402.

The ^1H-nmr spectrum of **7** indicated the presence of a glucosyl anomeric proton signal at δ 5.18 (d, J=7.5 Hz), a pair of 1H doublet signals (meta coupled) at δ 6.97 (d, J=2.4 Hz, H-2) and 7.26 (d, J=2.4 Hz, H-4), a pair of 2H multiplet signals at 7.93 (m, H-6 and H-7) and 8.19 (m, H-5 and H-8), and a chelated phenolic hydroxyl signal at δ 12.69. In the ^{13}C-nmr spectrum of **7** (Table 1), the chemical shift values for the carbon atoms (except for C-3 and C-13) corresponded very well with those of **8** (Table 1) and, with the exception of C-3, also corresponded closely with those of xanthopurpurin 3-methyl ether [**10**] (Table 1) (8). In addition to the above observations, the resonance of C-9 in **7** had shifted 5.1 ppm downfield with respect to the carbonyl signal of anthraquinone. The above evidence clearly indicated that a sugar moiety was located at C_3-OH (9), and **7** was characterized as

TABLE 1. ^{13}C-Nmr Chemical Shift Values of **7**, **8**[a], and **10**.[b]

Carbon atom	Compound		
	7[c]	**8**[c]	**10**[d]
C-1	164.3	164.8	164.6
C-2	108.6	107.7	107.6
C-3	163.7	165.3	165.5
C-4	108.6	108.3	106.5
C-5	126.9	126.7	127.3
C-6	134.7	134.6	134.0
C-7	134.7	134.6	134.0
C-8	126.5	126.3	126.1
C-9	186.5	185.9	185.7
C-10	181.4	181.8	181.4
C-11	132.9	133.0	133.4
C-12	132.9	133.0	133.4
C-13	111.1	108.4	110.8
C-14	134.9	134.9	134.9
1′	99.9		
2′	73.1		
3′	76.2		
4′	69.5		
5′	77.3		
6′	60.5		
Me			55.9

[a]The number of protons directly attached to each carbon was verified with the DEPT pulse sequence.

[b]Data are from ref. 8.

[c]Spectra recorded in DMSO-d_6.

[d]Spectrum recorded in $CDCl_3$.

1,3-dihydroxyl-9,10-anthraquinone 3-*O*-β-D-glucoside [7].

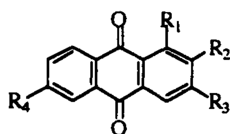
Platelets play a very important role in the hemostatic process and their aggregation induced by thrombin, arachidonic acid, adenosine diphosphate (ADP), collagen, and platelet-activating factor (PAF) will cause arterial thrombosis. Therefore, antiplatelet agents require investigation. Because some anthraquinones have exhibited antiplatelet activity (6), the antiplatelet activities of compounds **1–9** were studied by their effects on the aggregation of washed rabbit platelets induced by thrombin (0.1 u/ml), arachidonic acid (AA) (100 μM), collagen (10 μg/ml) and PAF (2 ng/ml), and the results are shown in Table 2. Compound **1** strongly inhibited platelet aggregation induced by AA and collagen and also showed slight but significant inhibition on the aggregation induced by PAF. Compound **4** did not possess any antiplatelet effects on the aggregation induced by thrombin, AA, collagen, or PAF. Thus, it is clear that opening the chromene ring and glycosylating the two phenolic hydroxyl groups of **1** eliminate its antiplatelet effects.

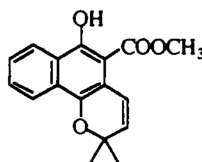
Compound **3** strongly inhibited platelet aggregation induced by collagen and also showed slight but significant inhibition of the aggregation induced by thrombin, AA, and PAF. Compounds **5** and **6** did not significantly inhibit platelet aggregation induced by thrombin, AA, collagen and PAF, although **6** did slightly inhibit platelet aggregation induced by AA. These results indicate that the glycosylation of C_3-OH of **3** with two moles of sugar reduced the inhibitory effects on the aggregation induced by collagen. Compounds **7** and **8** also showed strong inhibitory effects on aggregation induced by collagen. This implies that **5**, **7**, and **8** may be selective inhibitors of collagen-induced platelet aggregation. The inhibition of **1** on the platelet aggregation induced by AA (100 μM) was concentration-dependent and the IC_{50} on aggregation of washed rabbit platelets was about 86.6 μM, with minimal effect at 50 μM and maximal effect at 100 μM. The inhibition of **3** and **7** on the platelet aggregation induced by collagen (10 μg/ml) was also concentration-dependent and their IC_{50} values on aggregation of washed rabbit platelets were about 260.8 and 215.7 μM, respectively, with the same minimal effect at 100 μM and maximal effect at 300 μM. Further experiments are needed to elucidate their mechanism(s) of action.

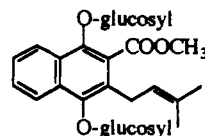
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps are uncorrected. Ft nmr spectra were performed on a Varian VXR-300/51 superconducting high-resolution Ft-nmr system; the ir spectra were recorded with a Hitachi model 260–30; the uv spectra were run on a Shimadzu UV-Visible

TABLE 2. Effects of Compounds **1–9** and Aspirin on the Platelet Aggregation Induced by Thrombin, Arachidonic Acid (AA), Collagen and PAF.[a]







2 R_1=OH, R_2=CH_3, R_3=R_4=H

3 R_1=R_3=R_4=OH, R_2=CH_3

5 R_1=R_4=OH, R_2=CH_3, R_3=*O*-(3′-*O*-acetyl)-rhamnosyl-(1→2)-glucoside

6 R_1=R_4=OH, R_2=CH_3 R_3=*O*-rhamnosyl-(1→2)-glucoside

7 R_1=OH, R_2=R_4=H, R_3=*O*-glucoside

8 R_1=R_3=OH, R_2=R_4=H

9 R_1=OH, R_2=CH_2OH, R_3=*O*-xylosyl-(1→6)-glucoside, R_4=H

1 **7**

Inducer	Control	1	2	3	4	5
Thrombin	90.4±0.7 (4)	90.9±1.9 (3)	88.7±0.9 (3)	85.5±1.2 (3)[c]	91.3±0.4 (3)	82.4±4.8 (3)
AA	90.2±1.2 (4)	0.0±0.0 (3)[b]	81.6±1.2 (3)	83.0±3.2 (3)[d]	86.9±2.1 (3)	90.6±1.7 (4)
Collagen	85.7±0.7 (4)	34.1±10.5 (3)[b]	85.7±0.9 (3)	8.2±4.2 (4)[b]	81.2±4.7 (3)	82.4±1.7 (4)
PAF	92.1±2.3 (4)	62.5±9.8 (3)[c]	82.3±1.1 (3)	77.8±4.4 (4)[d]	90.3±1.7 (4)	81.5±4.4 (4)

Inducer	6	7	8	9	Aspirin
Thrombin	87.0±1.3(3)	85.2±1.1 (3)[b]	86.9±1.7 (3)	92.2±1.6 (3)	91.9±2.5 (3)
AA	83.4±1.1 (3)[d]	71.9±7.1 (4)[d]	85.0±3.8 (3)	88.5±1.8 (3)	0.0±0.0 (5)[b]
Collagen	84.0±1.5 (3)	0.0±0.0 (4)[b]	3.8±3.3 (4)[b]	83.5±1.3 (4)	85.4±3.9 (3)
PAF	89.4±1.8 (3)	65.5±5.0 (4)[b]	82.6±3.0 (4)[d]	89.1±1.3 (4)	90.5±1.2 (3)

[a]Platelets were preincubated with various agents (300 μM), aspirin (50 μM) or DMSO (0.5%, control) at 37° for 3 min, then thrombin (0.1 u/ml), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. Percentages of aggregation are presented as means±SEM (n).

[b]p<0.001 as compared with control values.

[c]p<0.01 as compared with control values.

[d]p<0.05 as compared with control values.

recording spectrophotometer; and ms were obtained on a JMX-HX 110 mass spectrometer.

EXTRACTION AND ISOLATION.—The fresh roots (1.08 kg) of *R. akane* were collected at Kaohsiung Hsien, Taiwan, during July 1991, and were chipped and extracted with hot MeOH. A voucher specimen is deposited in our laboratory. The MeOH extract was chromatographed over a Si gel column. Elution with cyclohexane-C_6H_6 (19:1) yielded **1**. Elution with cyclohexane-C_6H_6 (9:1) yielded **2** in the first fractions and rubiprasin B and rubiprasin A in the later fractions, respectively. Elution with cyclohexane-EtOAc (3:2) yielded **3** in the first fractions and rubiarbonol D in subsequent fractions, respectively. Elution with $CHCl_3$-MeOH-H_2O (8:3:0.5) yielded **4**. Elution with EtOAc-MeOH-H_2O (7:1:0.5) yielded **5** and β-sitosterol β-D-glucoside in the first fractions and **6** in the later fractions, respectively. Elution with EtOAc-MeOH-H_2O (4:1:0.25) yielded **7**, while elution with EtOAc-MeOH-H_2O (4:1:0.5) yielded **9**. β-Sitosterol β-D-glucoside was identified by ir, nmr, ms, and comparison of mmp and spectral data with those of an authentic sample. The other known compounds [**1–6**, **9**] were identified by uv, ir, nmr, and ms and chemical reactions (1–4).

*1,3-Dihydroxyl-9,10-anthraquinone 3-*O*-β-D-glucoside (xanthopurpurin 3-*O*-β-D-glucoside)* [**7**].—Yellow powder (MeOH): mp 244–245°; ir ν max (KBr) 3400, 1670, 1630 cm^{-1}; uv λ max (MeOH) (log ε) 239 (4.39), 244 (4.39), 262 (4.35), 275 (sh) (4.29), 330 (3.54), 400 (3.81) nm; ^1H nmr, see text; ^{13}C nmr, see Table 1; negative-ion fabms m/z $[M-1]^-$ 401 (23), $[401-162]^-$ 239 (24), $[401-162-H]^-$ 238 (36), $[401-162-2H]^-$ 237 (100). Acid hydrolysis (7% HCl-MeOH) of **7** yielded **8**, as orange needles, mp 264–266° (MeOH), which was identified by uv, ir, nmr,

and ms (8). The sugar portion was examined by tlc in $CHCl_3$-MeOH-Me_2CO-H_2O (3:3:3:1) on Si gel [methyl glucopyranoside (R_f 0.59)].

PLATELET AGGREGATION.—Washed rabbit platelets were obtained from EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously (10). 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), $CaCl_2$ (1.0) and glucose (11.2) with bovine serum albumin (0.35%). Aggregation was measured by the turbidimetric method (11), designed with the absorbance of platelets in suspension as 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%.

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LITERATURE CITED

1. H. Itokawa, K. Mihar, and K. Takeya, *Chem. Pharm. Bull.*, **31**, 2353 (1983).
2. H. Itokawa, Y.F. Qiao, and K. Takeya, *Phytochemistry*, **28**, 3465 (1989).
3. H. Itokawa, Y.F. Qiao, K. Takeya, and Y. Iitaka, *Chem. Pharm. Bull.*, **37**, 1670 (1989).
4. H. Itokawa, Y.F. Qiao, and K. Takeya, *Chem. Pharm. Bull.*, **38**, 1435 (1990).
5. H.S. Yun-Choi, J.H. Kim, and M. Takido, *J. Nat. Prod.*, **53**, 630 (1990).
6. M.I. Chung, K.H. Gan, and C.N. Lin, *J. Nat. Prod.*, **56**, 929 (1993).
7. A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon, London, 1964, p. 286.
8. R. Wijnsma and R. Verpoorte, "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1986, pp. 129–130.
9. Y. Berger and A. Castonguay, *Org. Magn. Reson.*, **11**, 375 (1978).
10. C.M. Teng, W.Y. Chen, C.W. Ko, and C. Ouyang, *Biochem. Biophys. Acta*, **294**, 375 (1987).
11. J.R. O'Brien, *J. Clin. Pathol.*, **15**, 452 (1962).

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