Triterpenoids from *Rubia yunnanensis*

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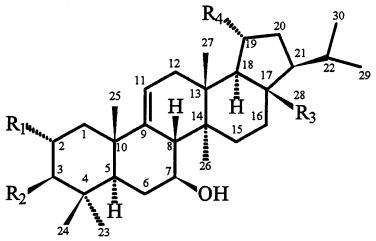
Five new triterpenoids, rubiarbonones D (**1**), E (**5**), and F (**2**), and rubiarbosides F (**3**) and G (**4**), together with nine known compounds, were isolated from the roots of *Rubia yunnanensis*. The structures of **1**−**12** were elucidated by spectroscopic methods. The antiplatelet aggregation activities of rubiarbonone A (**6**) and rubiarbonol A (**8**) and B (**9**) were investigated with a standard protocol.

Rubia yunnanensis Diels (Rubiaceae) is a perennial plant native to Yunnan Province, People's Republic of China, and is utilized as an antitumor drug in Yunnan.[1] It has been used to substitute for the well-known Chinese traditional medicine "Qiancao" (*R. cordifolia*). Several triterpenoids, anthraquinones, and cyclic hexapeptides have been isolated from *R. yunnanensis* previously.[1−12] As part of our research program on bioactive compounds from natural sources, we have further investigated the constituents of *R. yunnanensis*. The present paper reports the isolation and structural elucidation of five new triterpenoids, rubiarbonone D (**1**), rubiarbonone E (**5**), rubiarbonone F (**2**), rubiarboside F (**3**), and rubiarboside G (**4**), together with nine known compounds from the roots of this plant. The anticoagulant activities of the known compounds rubiarbonone A (**6**) and rubiarbonol A (**8**) and B (**9**) were evaluated, and the results are described herein.

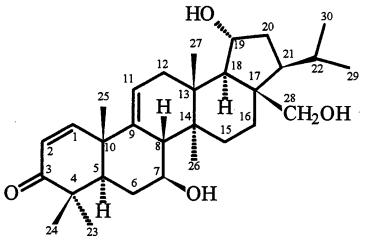
Results and Discussion

The methanol extract of the roots of *R. yunnanensis* was partitioned successively between chloroform and water, then ethyl acetate and water. The chloroform extract yielded rubiarbonone D (**1**), rubiarbonone F (**2**), rubiarbonone E (**5**), rubiarbonone A (**6**),[7] rubiarbonone B (**7**),[9] rubiarbonol A (**8**),[13] rubiarbonol B (**9**),[13] rubiarbonol F (**10**),[13] rubiarbonol G (**11**),[7] stigmasterol, and β-sitosterol by repeated Si gel column chromatography. The ethyl acetate extract on column chromatography over Diaion HP-20 and RP-18 gel afforded rubiarboside F (**3**), rubiarboside G (**4**), and rubiarboside A (**12**).[2]

Rubiarbonone D (**1**) was obtained as colorless needles. The HREIMS of **1** indicated a molecular ion at *m/z* 456.3606, in agreement with the molecular formula $C_{30}H_{48}O_3$. The IR spectrum showed the presence of hydroxyl (3443 cm^{-1}) and carbonyl (1698 cm^{-1}) groups, and an olefinic double bond (1646 cm^{-1}). The ^1H, ^{13}C, $^1H-^1H$ COSY, and HMQC NMR spectra showed the presence of six tertiary methyls at δ 15.5, 16.7, 16.7, 21.0, 22.0, and 25.0, one isopropyl unit at δ 21.9, 22.9, and 30.3, two oxygenated carbons at δ 71.4 and 71.7, and a keto-carbon at δ 216.2. Analysis of this information suggested that compound **1** is an arborane- or a fernane-type triterpenoid. The differences between arborane and fernane triterpenoids are the opposite configurations at C-8, -13, -17, -18, and -21. The relationship between CH_3-10 and H-8 is a *syn*-1,3-diaxial orientation in arborane, but an *anti*-1,3-diaxial orientation in fernane.[13] The NOE correlations observed between H-8 (δ 2.14) and H-25 (δ 1.27), H-27 (δ 0.94), as



	R_1	R_2	R_3	R_4
1	H	=O	CH_3	OH
2	H	=O	CO_2H	OH
3	OH	Oglc	CH_2OH	OH
4	H	Oglc(6→1)glc	CH_2OH	OH
6	H	=O	CH_2OH	OAc
7	H	=O	CH_2OH	OH
8	H	OH	CH_2OH	OH
9	H	OH	CH_3	OH
10	OH	OH	CH_2OH	OH
11	H	OH	CH_2OH	OAc
12	OAc	Oglc	CH_3	OH



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well as H-18 (δ 1.63) and H-26 (δ 0.97), together with the absence of NOE correlations of H-28 (δ 0.81) with H-18 (δ 1.63) and H-21 (δ 1.30) in a ROESY experiment (Table 1), indicated that **1** is an arborane-type triterpenoid. By comparison of the ^1H and ^{13}C NMR spectra of **1** with the arborane-type compounds rubiarbonone A (**6**) and B (**7**), which were also isolated in the present study, two hydroxyl groups substituted at C-7 and C-19 with equatorial β- and α-orientation, respectively, could be concluded, on the basis of the coupling constants of H-7 at δ 3.79 (td, J = 10.4, 5.3 Hz) and H-19 at δ 4.21 (td, J = 9.4, 3.2 Hz). This proposition was further confirmed by $^{13}C-^1H$ long-range correlations of C-7 (δ 71.7) with H-6β (δ 1.73) and H-8 (δ 2.14), and C-19 (δ 71.4) with H-18 (δ 1.63) and H-20β (δ 1.87) in the HMBC experiment (Table 1). A trisubstituted olefinic proton at δ 5.35 (1H, dd, J = 4.4, 1.8 Hz) was assigned to H-11. This result could be further identified

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Table 1. ^{1}H, ^{13}C, HMBC, and ROESY NMR Spectral Data of Compound **1** in $CDCl_3$

	δ_C	δ_H	HMBC	ROESY
1α	37.0 t	1.72 m	C-5	H-2α, 11
1β		2.04 m		H-2α, 2β, 11, 25
2α	34.8 t	2.38 ddd (15.1, 4.6, 3.1)	C-8	H-1α, 1β, 2β
2β		2.74 td (15.1, 5.8)	C-1, 3	H-1β, 2α, 24, 25
3	216.2 s			
4	47.2 s			
5	49.1 d	1.34 dd (12.8, 2.5)	C-4, 6, 23, 24, 25	H-6α, 7, 23
6α	33.7 t	1.86 m		H-5, 7, 23
6β		1.73 m	C-5, 7	H-24, 25
7	71.7 d	3.79 td (10.4, 5.5)		H-5, 6α, 26
8	48.9 d	2.14 br d (10.4)	C-7, 9, 11, 14, 26	H-25, 27
9	144.9 s			
10	39.0 s			
11	118.2 d	5.35 dd (4.4, 1.8)	C-8, 10, 13	H-1α, 1β, 12α, 12β
12α	36.9 t	1.98 m	C-9, 11, 27	H-11, 26
12β		1.84 m	C-9, 11, 14, 27	H-11
13	37.6 s			
14	39.4 s			
15α	31.9 t	2.01 m	C-17	H-16β
15β		1.64 m		H-27, 28
16α	36.4 t	1.53 td (13.8, 3.9)		H-26
16β		1.62 m	C-15, 18	H-15α, 29
17	43.8 s			
18	59.3 d	1.63 d (9.4)	C-13, 17, 19, 21, 27	H-26
19	71.4 d	4.21 td (9.4, 3.2)		H-20β, 27, 28
20α	40.9 t	1.69 m	C-17	H-30
20β		1.87 m	C-19, 22	H-19, 22, 28, 30
21	57.2 d	1.30 br t (9.5)	C-17, 20, 22, 28	H-29, 30
22	30.3 d	1.43 m	C-21	H-20β, 28, 29, 30
23	25.0 q	1.06 s	C-3, 4, 5, 24	H-5, 6α
24	22.0 q	1.07 s	C-3, 4, 5, 23	H-2β, 6β
25	21.0 q	1.27 s	C-1, 5, 9, 10	H-1β, 2β, 6β, 8
26	16.7 q	0.97 s	C-8, 13, 14, 15	H-12α, 16α, 18
27	16.7 q	0.94 s	C-12, 14, 18	H-8, 15β, 19
28	15.5 q	0.81 s	C-16, 17, 18, 21	H-15β, 19, 20β, 22
29	21.9 q	0.88 d (6.5)	C-21, 22, 30	H-16β, 21, 22
30	22.9 q	0.83 d (6.5)	C-21, 22, 29	H-20α, 20β, 21, 22

by the correlations of H-11 with C-8 (δ 48.9), C-10 (δ 39.0), and C-13 (δ 37.6) in the HMBC spectrum. An isopropyl group connected to C-21 was suggested by the ^{13}C−^{1}H long-range correlations between two methyls at δ 0.88 and δ 0.83 (each 3H, d, J = 6.5 Hz), and C-21 (δ 57.2) and C-22 (δ 30.3), and between H-22 (δ 1.43) and C-21 in the HMBC experiment. The ^3J correlation of the carbonyl carbon signal at δ 216.2 (C-3) with the protons at δ 1.06 (23-Me) and 1.07 (24-Me) revealed the presence of a keto group at C-3 in the HMBC experiment (Table 1). The ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY NMR experiments (Table 1) enabled all connectivities and assignments of the ^{1}H and ^{13}C NMR spectral data to be made (Table 1). Therefore, the structure 7β,19α-dihydroxyarbor-9(11)-en-3-one was assigned for rubiarbonone D (**1**).

Rubiarbonone F (**2**) was isolated as colorless needles, and its molecular formula was determined to be $C_{30}H_{46}O_5$ (*m*/*z* 486.3342) by its HREIMS. The IR spectrum showed the absorption bands at 3650 cm^{-1} (OH), 1698 cm^{-1} (C=O), and 1646 cm^{-1} (C=C). The ^{13}C and DEPT NMR spectra exhibited seven methyl groups (one methyl less than **1**) at δ 15.8 (C-27), 17.0 (C-26), 21.3 (C-25), 22.5 (C-24), 22.6 (C-29), 23.2 (C-30), and 25.5 (C-23), two carbons bearing hydroxyl groups at δ 72.0 (C-19) and 72.7 (C-7), two olefinic carbons at δ 119.4 (C-11) and 146.1 (C-9), five aliphatic quaternary carbons at δ 39.3 (C-13), 40.2 (C-10), 40.4 (C-14), 48.4 (C-4), and 56.2 (C-17), and one keto group at δ 218.8 (C-3), which were very similar to those of **1**. The ^{1}H and ^{13}C NMR spectra (Table 2) of **2** differed from those of **1** only in the presence of a carboxylic group at δ_C 179.1 instead of a methyl group at δ_H 0.81 (3H, s) and δ_C 15.5 in **1**. The location of the carboxylic group was confirmed to be attached to C-17 on the basis of the ^{13}C−^{1}H long-range correlations between C-28 (δ 179.1) and H-18 (δ 2.09), and H-21 (δ 1.57) in the HMBC experiment (Table 4, Supporting Information). The orientation of the methyls and the hydroxyls was proved by a ROESY NMR experiment (Table 5, Supporting Information). The ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY NMR data supported the complete ^{1}H and ^{13}C NMR signal assignments (Table 2). According to the above analysis, rubiarbonone F (**2**) was assigned as 28-carboxy-7β,19α-dihydroxyarbon-9(11)-en-3-one.

Rubiarboside F (**3**) was isolated as a colorless powder. The HRFABMS showed a $[M]^+$ peak at *m*/*z* 652.4186, corresponding to the molecular formula $C_{36}H_{60}O_{10}$. The IR spectrum exhibited the absorption bands at 3401 cm^{-1} (OH) and 1646 cm^{-1} (C=C). The ^{1}H and ^{13}C NMR spectra revealed that **3** was a glucoside of the known arborane triterpenoid, rubiarbonol F (**10**). The signals at δ_H 4.98 (d, J = 7.8 Hz) for an anomeric proton H-1′ and δ_C 62.4, 71.4, 75.5, 78.5, 78.6, and 106.5 were attributed to a β-D-glucopyranosyl unit. The linkage of the β-D-glucopyranosyl group at C-3 was further confirmed by the NOE between H-1′ (δ 4.98) and H-3 (δ 3.34) (Table 5, Supporting Information) and the HMBC correlations between C-3 (δ 95.3) and H-1′, and between C-1′ (δ 106.5) and H-3 (Table 4, Supporting Information), together with the downfield shift of the C-3 signal at δ 83.58 in rubiarbonol F[13] to δ 95.3 in **3**. Therefore, rubiarboside F (**3**) was determined as 3β-(β-D-glucopyranosyl)-2α,7β,19α,28-tetrahydroxyarbor-9(11)-ene.

Rubiarboside G (**4**) was obtained as colorless powder, and its molecular formula was established as $C_{42}H_{70}O_{14}$ (*m*/*z* 821.4659, $[M + Na]^+$) from its HRFABMS. The ^{1}H,^{13}C NMR (Table 2), HMQC, HMBC, and ROESY NMR spectra (Tables 4 and 5, Supporting Information) showed that the structure of **4** was close to that of the known triterpenoid rubiarbonol A (**8**). Except for the arborane nucleus with five methyls, two hydroxyls, one isopropyl, and one hydroxymethyl, the remaining ^{1}H and ^{13}C NMR signals represented two β-D-glucopyranosyl units. Hence, **4** is a glucosylated rubiarbonol A derivative (**8**). The glucosidic linkage of **4** was formed between the anomeric carbon of one glucose and C-3, on the basis of the ^{13}C−^{1}H long-range correlation between C-3 (δ 89.0) and H-1′ (δ 4.88), and the NOE correlation between H-1′ and H-3 (δ 3.40), together with the downfield shifted signal of C-3 at δ 77.9 in rubiarbonol A[13] to δ 89.0 in **4**. The downfield shift of the carbon signal C-6′ to δ 70.4 implied that the two glucose units are linked together by a 6 → 1 ether linkage. The NOEs between H-6′ (δ 4.23 and 4.84) and H-1″ (δ 5.12), together with the ^{13}C−^{1}H long-range correlation between C-6′ (δ 70.4) and H-1″, confirmed this linkage. On the basis of the above analysis, the structure of 3β-[β-D-glucopyranosyl-(6→1)-β-D-glucopyransyl]-7β,19α,28-trihydroxyarbor-9(11)-ene (**4**) was assigned to rubiarboside G.

Rubiarbonone E (**5**) was isolated as a colorless powder, and its molecular formula was determined to be $C_{30}H_{46}O_4$ (*m*/*z* 470.3395) from its HREIMS. The UV spectrum showed

Table 2. ^{1}H and ^{13}C NMR Spectral Data of Compounds **2**−**5**

	2[a]		**3**[b]		**4**[b]		**5**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	1.69 m	38.2 t	1.62 m	45.3 t	1.56 m	36.8 t	7.37 d (10.5)	157.7 d
1*β*	2.10 m		2.37 dd (12.7, 3.8)		1.79 m			
2α	2.34 ddd (15.0, 4.6, 3.1)	35.9 t		67.2 d	2.54 m	27.2 t	5.92 d (10.5)	125.7 d
2*β*	2.84 td (15.0, 5.8)		4.11 m		1.97 m			
3		218.8 s	3.34 d (9.3)	95.3 d	3.40 dd (11.5, 3.9)	89.0 d		206.8 s
4		48.4 s		40.5 s		39.5 s		45.0 s
5	1.34 dd (12.6, 2.9)	50.9 d	1.14 m	48.8 d	1.01 dd (12.0, 9.6)	49.1 d	1.67 dd (13.1, 1.7)	47.2 d
6α	1.82 m	34.4 t	2.21 m	33.5 t	2.19 m	33.5 t	2.02 m	33.1 t
6*β*	1.77 m		1.90 br q (12.3)		1.88 br q (12.0)		1.82 m	
7	3.68 td (10.4, 5.3)	72.7 d	4.04 td (10.4, 4.6)	71.9 d	3.98 m	72.1 d	3.81 td (10.1, 6.2)	71.4 d
8	2.11 br d (10.4)	50.0 d	2.49 br d (10.4)	49.0 d	2.44 br d (9.6)	49.5 d	2.30 br d (10.1)	49.3 d
9		146.1 s		146.9 s		147.4 s		143.7 s
10		40.2 s		40.2 s		39.4 s		43.0 s
11	5.43 d (6.4)	119.4 d	5.61 d (5.0)	117.6 d	5.52 d (5.8)	117.4 d	5.54 d (6.1)	119.1 s
12α	2.10 m	36.2 t	2.58 m	37.5 t	2.52 m	37.6 t	2.05 m	37.8 t
12*β*	1.80 m		2.48 m		2.45 m		1.96 m	
13		39.3 s		38.3 s		38.3 s		38.7 s
14		40.4 s		40.2 s		40.2 s		40.9 s
15α	2.19 m	34.4 t[c]	2.80 br d (14.0)	33.0 t	2.76 br d (14.8)	33.0 t	2.05 m	33.4 t
15*β*	1.85 m		2.01 m		2.01 m		1.62 td (13.3, 4.1)	
16α	1.37 td (13.3, 4.2)	34.2 t[c]	1.58 m	33.3 t	1.60 m	33.3 t	1.39 td (13.3, 3.6)	33.6 t
16*β*	2.45 br dt (13.3, 3.1)		2.01 m		1.99 m		1.76 m	
17		56.2 s		48.9 s		49.1 s		49.6 s
18	2.09 d (9.4)	60.5 d	2.33 d (8.8)	59.9 d	2.30 d (9.6)	60.3 d	1.90 d (9.7)	60.1 d
19	4.55 td (9.4, 2.9)	72.0 d	5.05 br t (8.8)	70.5 d	5.00 br t (9.6)	70.7 d	4.39 td (9.7, 2.9)	71.7 d
20α	1.69 m	42.8 t	2.15 m	43.4 t	2.14 m	43.3 t	1.64 m	43.0 t
20*β*	2.17 m		2.62 m		2.59 m		2.15 td (10.0, 2.9)	
21	1.57 br q (9.8)	57.5 d	1.56 m	58.0 d	1.58 m	58.1 d	1.35 m	58.6 d
22	1.35 m	32.7 d	2.12 m	30.7 d	2.14 m	30.7 d	1.76 m	31.4 d
23	1.05 s	25.5 q	1.43 s	28.4 q	1.29 s	28.1 q	1.14 s	25.3 q
24	1.08 s	22.5 q	1.10 s	18.0 q	1.06 s	16.8 q	1.06 s	22.3 q
25	1.29 s	21.3 q	1.17 s	22.8 q	1.11 s	21.9 q	1.33 s	22.6 q
26	1.02 s	17.0 q	1.32 s	17.2 q	1.30 s	17.2 q	1.03 s	17.6 q
27	0.92 s	15.8 q	1.40 s	16.7 q	1.38 s	16.7 q	1.10 s	16.8 q
28		179.1 s	4.09, 4.21 d (11.9)	62.8 t	4.10, 4.19 d (10.4)	62.8 t	3.75, 3.80 d (11.9)	63.4 t
29	0.96 d (6.5)	22.6 q	1.08 d (6.3)	23.3 q	1.10 d (6.7)	23.3 q	0.97 d (6.5)	23.5 q
30	0.85 d (6.5)	23.2 q	0.95 d (6.3)	23.6 q	0.97 d (6.7)	23.5 q	0.88 d (6.5)	23.7 q
1′			4.98 d (7.8)	106.5 d	4.88 d (7.8)	106.8 d		
2′			4.08 m	75.5 d	3.96 m	75.5 d		
3′			4.24 m	78.6 d	4.22 m	78.5 d		
4′			4.25 m	71.4 d	4.21 m	71.8 d		
5′			4.10 m	78.5 d	4.12 m	77.0 d		
6′			4.36 dd (11.5, 5.5)	62.4 t	4.23 dd (11.6, 6.3)	70.4 t		
6′			4.60 bd (11.5)		4.84 br t (11.6)			
1″					5.12 d (7.7)	105.3 d		
2″					4.03 m	75.2 d		
3″					4.18 m	78.4 d		
4″					4.05 m	71.8 d		
5″					3.93 m	78.4 d		
6″					4.34 dd (11.5, 6.3)	62.9 t		
6″					4.49 dd (11.5, 2.1)			

[a] In CD_3OD. [b] In C_5D_5N. [c] May be changed.

an absorption at λ_{max} 224.6 nm, and an IR absorption was observed at 1667 cm^{-1}, characteristic of the presence of an α,*β*-unsaturated carbonyl chromophore in the molecule. Two mutually coupled olefinic protons at δ 5.92 and 7.37 (each 1H, d, J = 10.5 Hz) were assigned to H-2 and H-1, respectively. The remaining ^{1}H and ^{13}C NMR signals of **5** were very similar to those of **1** and **2**, revealing an arborane-type skeleton with five tertiary methyls at C-4 (two methyls), C-10, C-13, and C-14, two hydroxyls at C-7 and C-19, one hydroxymethyl group at C-17, and an isopropyl group at C-21. Complete NMR assignments (Table 2) were determined by the analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra (Table 4, Supporting Information). The relative stereochemistry of all of the substituents was assigned by the ROESY experiment (Table 5, Supporting Information), which showed correlations similar to those of **1**−**4**. On the basis of these data, rubiarbonone E (**5**) was assigned as 7*β*,19α,28-trihydroxyarbor-1(2),9(11)-dien-3-one.

Compounds **6**, **8**, and **9** were evaluated for their antiplatelet aggregation activity (Table 3). Rubiarbonol B (**9**) exhibited the most potent antiplatelet aggregation activity induced by arachidonic acid and collagen at 150 μM. Rubiarbonone A (**6**) and rubiarbonol A (**8**) promoted platelet aggregation at high doses; however, they also exhibited antiplatelet aggregation activity at lower concentrations. This observation is consistent with a basic tenet of Chinese Traditional Medicine, in that the variation of the dose of a prescription may produce either stimulatory or inhibitory effects.

Experimental Section

General Experimental Procedures. Melting points (Yanagimoto apparatus) are uncorrected. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV

Table 3. Effects on Platelet Aggregation Activity (%) of Compounds **6**, **8**, and **9**[a,b]

compound	dose (μM)	AA (100 μM)	collagen (10 μg/mL)	Thr (0.1 U/mL)	PAF (2 ng/mL)
control		87.1 ± 1.1	91.0 ± 0.8	91.7 ± 0.0	92.6 ± 0.6
rubiarbonone-A (**6**)[c]	150				
	100	85.4 ± 1.5	88.1 ± 1.7	91.1 ± 0.3	90.3 ± 1.7
rubiarbonol-A (**8**)[d]	150				
	100	83.8 ± 2.0	89.6 ± 1.3	90.7 ± 0.5	91.3 ± 0.9
rubiarbonol-B (**9**)	150	80.4 ± 1.5**	79.8 ± 4.8**	89.6 ± 0.6**	90.4 ± 0.5*
	100		82.2 ± 4.7**		

[a] AA = arachidonic acid, PAF = platelet-activating factor, Thr = thrombin. [b] Platelets were incubated with test sample or 0.5% DMSO at 37 °C for 1 min; then AA (100 μM), collagen (10 μg/mL), Thr (0.1 U/mL), or PAF (2 ng/mL) was added to trigger aggregation. Values are presented as mean ±SD * $p < 0.05$, ** $p < 0.01$. [c] Promoted platelet aggregation at a dose of 28.4 ± 4.8 μg/mL. [d] Promoted platelet aggregation at a dose of 30.9 ± 6.0 μg/mL.

spectra in MeOH solution were obtained on a Hitachi UV-3210 spectrophotometer. CD spectra in MeOH solution were recorded on a JASCO J-720 spectropolarimeter. IR spectra in KBr disks were recorded on a Shimadzu FT-IR DR-8501 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were determined on a Bruker AMX-400 or a Varian Unity plus 400 spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a VG 70-250S mass spectrometer.

Plant Material. The roots of *Rubia yunnanensis* used in this investigation were collected in Jiu Jiang Xian,Yunnan Province, People's Republic of China in July, 1996, by Miss S. Zhang and identified by Prof. C. S. Kuoh. A voucher specimen (TSWu 96021) has been deposited at the Herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried roots of *Rubia yunnanensis* (4.2 kg), collected in Yunnan, China, were extracted with MeOH (×3) and then concentrated to dryness under a vacuum. The crude extract was partitioned successively between chloroform and water and then ethyl acetate and water. The $CHCl_3$ layer was subjected to column chromatography on silica gel eluted with a gradient of MeOH in $CHCl_3$ to afford 21 fractions. Fractions 9−11 ($CHCl_3$−MeOH, 70:1) were combined and rechromatographed on a silica gel column [C_6H_6−(i-C_3H_7)$_2$O, 30:1] to give a mixture of stigmasterol and β-sitosterol (10 mg, 0.00024%). Fractions 12−14 ($CHCl_3$−MeOH, 20:1) were combined and then separated on a silica gel column ($CHCl_3$−MeOH, 100:1) to furnish nine subfractions. Subfraction 6 was further purified by preparative TLC [C_6H_6−(i-C_3H_7)$_2$O, 9:1, R_f value 0.07] to give rubiarbonone D (**1**) (3.2 mg, 0.000076%). Fractions 15−17 ($CHCl_3$−MeOH, 10:1) were combined and subjected to Diaion HP-20 column chromatography eluted with a gradient of H_2O and MeOH (H_2O−MeOH, 20:80 to 0:100) to afford rubiarbonone A (**6**) (5.0 mg, 0.00012%), rubiarbonone B (**7**) (10.0 mg, 0.00024%), rubiarbonone E (**5**) (3.0 mg, 0.000071%), rubiarbonone F (**2**) (2.5 mg, 0.00006%), rubiarbonol A (**8**) (30.0 mg, 0.00071%), rubiarbonol B (**9**) (3.0 mg, 0.000071%), and rubiarbonol G (**11**) (3.0 mg, 0.000071%), successively. Fractions 18−21 ($CHCl_3$−MeOH, 3:1) were also combined and subjected to Diaion HP-20 column chromatography eluted with a gradient of H_2O and MeOH (H_2O−MeOH, 30:70 to 0:100) to give rubiarbonol F (**10**) (3.0 mg, 0.000071%).

The EtOAc layer was separated by Diaion HP-20 column chromatography eluted with a gradient of H_2O and MeOH to afford 21 fractions. Fractions 15−17 (H_2O−MeOH, 50:50) were combined and further subjected to RP-18 column chromatography eluting with a gradient of H_2O and MeOH (H_2O−MeOH, 40:60 to 0:100) to afford 17 subfractions. Subfraction 4 (H_2O−MeOH, 40:60) was rechromatographed on preparative TLC ($CHCl_3$−MeOH, 5:1) to furnish rubiarboside A (**12**) (R_f 0.51, 15.0 mg, 0.00036%) and rubiarboside F (**3**) (R_f 0.39, 5.5 mg, 0.00013%). Subfraction 13 (H_2O−MeOH, 30:70) was rechromatographed on silica gel column chromatography ($CHCl_3$−MeOH, 3:1) to give rubiarboside G (**4**) (5.1 mg, 0.00012%).

Rubiarbonone D (1): colorless needles ($CHCl_3$), mp 231−232 °C; $[\alpha]_D$ +94.4° (c 0.03, $CHCl_3$); CD (c 7.0833 × 10^{-4} M, $CHCl_3$) $[\theta]_{257}$ 0, $[\theta]_{300}$ −1385, $[\theta]_{336}$ 0 nm; IR (KBr) ν_{max} 3443 (OH), 1698 (C=O), 1646 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS m/z 456 (M^+, 5), 438 (100), 423 (24), 405 (15), 395 (17), 377 (5), 300 (6), 286 (15), 271 (28), 257 (7), 233 (10), 217 (9), 189 (9), 161 (15), 159 (15), 147 (19), 133 (22), 125 (34), 107 (34), 95 (47); HREIMS m/z 456.3606 $[M]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3603).

Rubiarbonone F (2): colorless needles (MeOH), mp 253−254 °C; $[\alpha]_D$ +26.4° (c 0.06, MeOH); CD (c 9.918 × 10^{-4} M, MeOH) $[\theta]_{219}$ −86.04, $[\theta]_{227}$ −2157, $[\theta]_{251}$ 0, $[\theta]_{253}$ +31.59, $[\theta]_{255}$ 0, $[\theta]_{298}$ −1808, $[\theta]_{332}$ 0, $[\theta]_{338}$ +113.6, $[\theta]_{344}$ 0 nm; IR (KBr) ν_{max} 3650 (OH), 1698 (C=O), 1646 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS m/z 486 (M^+, 11), 468 ($[M - H_2O]^+$, 18), 458 (32), 453 (39), 441 (12), 440 (14), 423 (32), 395 (32), 379 (18), 349 (15), 317 (14), 301 (12), 287 (20), 255 (22), 253 (14), 239 (17), 213 (21), 159 (40), 135 (51), 125 (55), 107 (55), 97 (49), 91 (41), 83 (51), 81 (57), 69 (70), 55 (100); HREIMS m/z 486.3342 $[M]^+$ (calcd for $C_{30}H_{46}O_5$, 486.3345).

Rubiarboside F (3): colorless powder (MeOH), mp 294−295 °C; $[\alpha]_D$ +98.1° (c 0.05, MeOH); IR (KBr) ν_{max} 3401 (OH), 1646 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; FABMS m/z 652 (M^+, 3), 635 (4), 591 (4), 547 (4), 503 (6), 459 (3), 440 (5), 392 (20), 345 (8), 308 (2), 283 (3), 134 (11), 133 (100); HRFABMS m/z 652.4186 $[M]^+$ (calcd for $C_{36}H_{60}O_{10}$, 652.4187).

Rubiarboside G (4): colorless powder (MeOH), mp > 290 °C; $[\alpha]_D$ +56.4° (c 0.05, MeOH); IR (KBr) ν_{max} 3415 (OH), 1646 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; FABMS m/z 821 ($[M + Na]^+$, 10), 789 (9), 745 (15), 701 (15), 657 (18), 635 (16), 613 (21), 569 (22), 547 (20), 525 (21), 503 (20), 481 (15), 459 (17), 415 (12), 437 (12), 371 (12), 327 (9), 277 (18), 239 (19), 185 (100); HRFABMS m/z 821.4659 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{14}Na$, 821.4663).

Rubiarbonone E (5): colorless powder (MeOH), mp 258−259 °C; $[\alpha]_D$ +233.4° (c 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 224.6 (4.03) nm; CD (c 1.9341 × 10^{-4} M, MeOH) $[\theta]_{214}$ −20 230, $[\theta]_{227}$ 0, $[\theta]_{236}$ +8663, $[\theta]_{289}$ +813, $[\theta]_{323}$ +3477, $[\theta]_{356}$ 0 nm; IR (KBr) ν_{max} 3386 (OH), 1667 (C=O) cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5 (Supporting Information); EIMS m/z 470 (M^+, 2), 452 (100), 437 (21), 419 (50), 403 (44), 381 (14), 284 (18), 266 (43), 187 (23), 159 (28), 145 (57), 105 (48), 91 (68); HREIMS m/z 470.3395 $[M]^+$ (calcd for $C_{30}H_{46}O_4$, 470.3396).

Antiplatelet Aggregation Assay. The bioassay methods of antiplatelet aggregation activity were described in a previous paper.[14]

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Supporting Information Available: Tables of HMBC and ROESY spectral data for compounds **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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