Absolute Stereostructures of New Arborinane-Type Triterpenoids and Inhibitors of Nitric Oxide Production from *Rubia yunnanensis*[1]

Toshio Morikawa, Jing Tao, Shin Ando, Hisashi Matsuda, and Masayuki Yoshikawa*

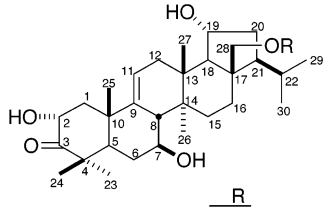
Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan

Received December 11, 2002

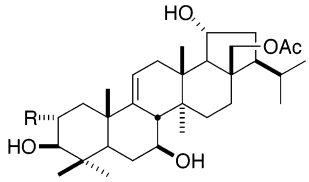
The aqueous acetone extract from the roots of a Chinese herbal medicine, *Rubia yunnanensis*, showed a potent inhibitory effect on nitric oxide production in lipopolysaccharide-activated macrophages. Five new arborinane-type triterpenes, rubianols-a (**1**), -b (**2**), -c (**3**), -d (**4**), and -e (**5**), and a new arborinane-type triterpene glycoside, rubianoside I (**6**), were isolated from the herbal crude extract together with 10 known compounds. The absolute stereostructures of **1**−**6** were determined on the basis of chemical and physicochemical evidence, including the application of the modified Mosher's method. The effects of the isolated constituents on nitric oxide production in lipopolysaccharide-activated macrophages were examined, and several triterpenes were found to show inhibitory activity.

The plant *Rubia yunnanensis* Diels (Rubiaceae) is cultivated in Yunnan Province of the People's Republic of China (Chinese name "Xiao Hong Sheng"), and the roots of this plant are used for the treatment of vertigo, insomnia, rheumatism, tuberculosis, hematemesis, menstrual disorders, and contusions. Previously, triterpenoids,[2−5] anthraquinones,[6] naphthohydroquinones,[7] and cyclic peptides[2,8,9] have been isolated from the roots of *R. yunnanensis*.

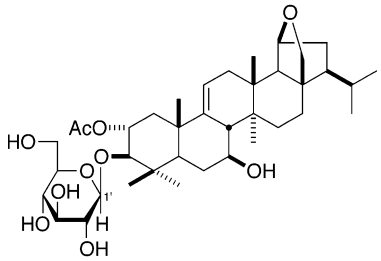
In the course of our characterization studies on bioactive constituents of Chinese natural medicines,[1,10] it was found that the aqueous acetone extract and ethyl acetate (EtOAc)-soluble fraction from the roots of *R. yunnanensis* showed inhibitory activities on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophages. From the EtOAc-soluble fraction, we have isolated five new arborinane-type triterpenes named rubianols-a (**1**), -b (**2**), -c (**3**), -d (**4**), and -e (**5**) and a new arborinane-type triterpene glycoside termed rubianoside I (**6**) from the roots of *R. yunnanensis*. This paper deals with the isolation and structure elucidation of six new constituents (**1**−**6**) and 10 known constituents and the inhibitory effects of these isolates on NO production in LPS-activated mouse peritoneal macrophages.



rubianol-a (**1**) : R = H
rubianol-b (**2**) : R = Ac



rubianol-c (**3**) : R = H
rubianol-d (**4**) : R = OH
rubianol-e (**5**) : R = OAc



rubianoside I (**6**)

Results and Discussion

The 80% aqueous acetone extract from the roots of *R. yunnanensis* purchased in Kunming, Yunnan Province, People's Republic of China, was partitioned into an EtOAc−water mixture to furnish EtOAc- and H_2O-soluble fractions. As shown in Table S1 (Supporting Information), the aqueous acetone extract and EtOAc- and H_2O-soluble fractions from *R. yunnanensis* were found to show inhibitory effects on NO production. In particular, the EtOAc-soluble fraction exhibited potent inhibitory activity.

The EtOAc-soluble portion was subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give **1** (0.0039% from the dried material), **2** (0.0011%), **3** (0.0092%), **4** (0.0041%), **5** (0.0053%), and **6** (0.0018%) together with rubiarbonols A[3,5,11,12] (0.011%) and F[5,11,12] (0.0020%), rubiarbonones B[4,5] (0.0041%) and C[4] (0.012%), (+)-lariciresinol[13,14] (0.018%), (+)-isolariciresinol[14] (0.0050%), (−)-secoisolariciresinol[14] (0.0054%), 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone[6] (0.025%), 4-hydroxy-3,5-dimethoxybenzoic acid[15] (0.0021%), and vanillic acid[15] (0.0019%).

Rubianol-a (**1**) was isolated as a white powder with a positive optical rotation ($[\alpha]_D^{25}$ +10.0°). The positive-ion FABMS of **1** showed a quasimolecular ion peak at *m*/*z* 511 $[M + Na]^+$, and the molecular formula $C_{30}H_{48}O_5$ was determined by HRFABMS. The IR spectrum of **1** showed absorption bands at 3400, 1716, 1655, and 1076 cm^{-1} ascribable to hydroxyl, carbonyl, and olefinic functions, respectively. Acetylation of **1** with acetic anhydride (Ac_2O) in pyridine furnished a tetraacetate (**1a**). The ^1H and ^{13}C NMR (pyridine-d_5, Table 1) spectra[16] of **1** and **1a** showed signals assignable to two secondary methyls [δ **1**: 0.92, 1.10 (3H each, both d, J = 5.8 Hz); **1a**: 0.83, 0.91 (3H each,

* To whom correspondence should be addressed. Tel: +81-75-595-4633. Fax: +81-75-595-4768. E-mail: shoyaku@mb.kyoto-phu.ac.jp.

10.1021/np0205710 CCC: $25.00
Published on Web 05/03/2003

Table 1. ^{13}C NMR Data for Rubianols-a−e (**1**−**5**), Rubianoside I (**6**), and Their Derivatives (**1a**, **3a**, **4a**, **7**)[a]

	1	**1a**	**2**	**3**	**3a**	**4**	**4a**	**5**		**6**	**7**
C-1	48.1	43.5	48.0	37.0	36.3	45.9	42.2	42.5		43.0	46.0
C-2	70.4	72.6	70.4	28.7	24.4	69.2	70.6	74.0		70.5	69.2
C-3	216.0	208.1	216.0	78.0	80.3	83.5	80.0	79.4		88.4	83.5
C-4	47.4	47.9	47.3	39.5	38.0	39.7	39.4	40.2		41.4	41.3
C-5	50.7	49.4	50.7	49.0	47.9	49.1	47.4	48.8		48.6	49.3
C-6	34.1	28.7	34.1	33.8	28.3	33.8	28.2	33.7		33.3	33.9
C-7	71.3	73.2	71.4	72.2	74.0	72.0	73.6	71.9		72.2	72.3
C-8	49.1	44.9	48.8	49.3	45.3	49.0	45.0	48.9		48.4	48.5
C-9	145.7	144.0	145.4	147.8	145.8	147.4	144.8	146.8		146.7	147.5
C-10	40.3	40.2	40.3	39.9	39.4	41.0	40.5	41.0		40.8	39.7
C-11	118.5	118.9	118.0	117.1	118.1	117.2	118.4	117.4		116.7	116.5
C-12	37.6	35.9	37.2	37.3	36.0	37.3	35.9	37.2		37.8	37.8
C-13	38.4	37.6	38.2	38.2	37.6	38.2	37.6	38.2		35.9	36.0
C-14	40.3	40.0	40.1	40.0	39.9	40.1	39.9	40.1		39.2	39.2
C-15	33.1	32.0	32.4	32.4	32.0	32.4	32.0	32.3		30.3	30.3
C-16	33.4	32.9	32.5	32.6	33.0	32.6	32.9	32.5		26.0	26.0
C-17	49.0	46.3	47.4	47.3	46.3	47.3	46.3	47.3		48.5	48.5
C-18	60.0	55.7	59.6	59.6	55.7	59.6	55.8	59.5		57.8	57.9
C-19	70.7	73.4	69.9	69.9	73.4	69.9	73.3	69.9		77.4	77.4
C-20	43.5	39.7	42.6	42.6	39.7	42.6	39.6	42.6		41.3	41.2
C-21	58.1	56.7	57.5	57.5	56.7	57.5	56.8	57.5		54.4	54.3
C-22	30.8	30.5	31.1	31.1	30.5	31.1	30.5	31.1		31.1	31.1
C-23	25.0	24.6	25.0	28.7	27.8	29.3	28.1	29.0		28.4	29.3
C-24	22.0	21.3	22.0	16.4	16.7	17.6	17.6	17.4		18.0	17.6
C-25	22.2	21.9	22.2	22.1	21.8	23.1	22.5	22.7		22.4	22.8
C-26	17.3	17.2	17.2	17.2	17.2	17.3	17.2	17.2		15.7	15.8
C-27	16.8	16.2	16.6	16.6	16.1	16.6	16.1	16.6		16.2	16.2
C-28	62.9	65.1	64.8	64.8	65.2	64.8	65.1	64.8		68.5	68.4
C-29	23.4[b]	22.6[b]	22.9[b]	22.9[b]	22.6[b]	22.9[b]	22.6[b]	22.9[b]		22.5[b]	22.5[b]
C-30	23.6[b]	23.1[b]	23.5[b]	23.5[b]	23.1[b]	23.5[b]	23.1[b]	23.5[b]		23.0[b]	23.0[b]
−OCOCH$_3$		170.1	170.7	170.6	170.3	170.7	170.3	170.7	−OCOCH$_3$	171.0	
		170.4			170.5		170.5	170.8	−OCO*C*H$_3$	21.8	
		170.5			170.6		170.6				
		170.8			170.8		170.6		Glc-1′	106.3	
							170.8		2′	75.9	
−OCO*C*H$_3$		20.8	21.1	21.0	21.1	21.0	20.8	21.0	3′	78.8	
		21.1			21.1		21.1	21.4	4′	72.1	
		21.6			21.6		21.1		5′	78.3	
		21.8			21.9		21.6		6′	63.3	
							21.9				

[a] Measured in pyridine-d_5 at 125 MHz. [b] May be interchangeable within the same column.

both d, J = 6.1 Hz), H$_3$-30, 29], five tertiary methyls [δ **1**: 1.04, 1.22, 1.27, 1.41, 1.44 (3H each, all s, H$_3$-24, 23, 26, 25, 27); **1a**: 0.95, 1.04, 1.14, 1.17, 1.47 (3H each, all s, H$_3$-27, 26, 24, 23, 25)], a methylene and three methines bearing an oxygen function [δ **1**: 4.10, 4.23 (1H each, both d, J = 11.3 Hz, H$_2$-28), 4.05 (1H, m, H-7), 5.01 (1H, dd, J = 5.8, 12.8 Hz, H-2), 5.06 (1H, m, H-19); **1a**: 4.32, 4.42 (1H each, both d, J = 12.2 Hz, H$_2$-28), 5.24 (1H, m, H-7), 5.59 (1H, m, H-19), 5.99 (1H, dd, J = 5.8, 13.8 Hz, H-2)], and an olefin [δ **1**: 5.54 (1H, br d, J = ca. 6 Hz); **1a**: 5.33 (1H, br d, J = ca. 5 Hz), H-11] together with six methylenes (C-1, 6, 12, 15, 16, 20), five methines (C-5, 8, 18, 21, 22), and seven quaternary carbons (C-3, 4, 9, 10, 13, 14, 17). The ^1H and ^{13}C NMR spectral data of **1** were very similar to those of rubiarbonone B, except for the signals due to the 2-hydroxyl group of **1**. The planar structure of **1** was clarified by $^1H-^1H$ correlation spectroscopy ($^1H-^1H$ COSY) and heteronuclear multiple bond connectivity (HMBC) experiments. As shown in Figure 1, the $^1H-^1H$ COSY experiment on **1** indicated the presence of five partial structures drawn with bold lines (C-1−C-2, C-5−C-8, C-11−C-12, C-15−C-16, C-18−C-22−C-29, 30). In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs of **1** (H-2 and C-3; H$_3$-23, 24 and C-3−5; H$_3$-25 and C-1, 5, 9, 10; H$_3$-26 and C-8, 13−15; H$_3$-27 and C-12−14, 18; H$_2$-28 and C-16−18, 21; H$_3$-29, 30 and C-21, 22), so that the connectivities of the quaternary carbons and the positions of seven methyl groups in **1** could be clarified. The relative stereochemistry of **1** was elucidated using a NOESY experiment, which showed NOE correlations between the following proton pairs: H-2 and H$_3$-24, 25; H-5 and H-7, H$_3$-23; H-7 and H$_3$-26; H-8 and H$_3$-25, 27; H-18 and H-21, H$_3$-26; H-19 and H$_3$-27; H$_3$-24 and H$_3$-25; H$_3$-27 and H$_2$-28. The above observations were used to confirm the similar stereochemistry of rubianol-a (**1**) as 2α,7β,19α,28-tetrahydroxy-9(11)-arborinen-3-one.

Rubianol-b (**2**) was also isolated as a white powder and showed absorption bands at 3400, 1740, 1655, and 1076 cm^{-1} assignable to hydroxyl, ester carbonyl, and olefinic functions, respectively. The proton and carbon signals of the ^1H and ^{13}C NMR (pyridine-d_5, Table 1) spectra of **2** were superimposable on those of **1**, except for the signals due to the 28-*O*-acetyl group [δ 2.08 (3H, s, −OAc), 4.30, 4.64 (1H each, both d, J = 12.2 Hz, H$_2$-28)]. Comparison of the ^1H and ^{13}C NMR spectral data for **2** with those for **1** revealed an acetylation shift around the 28-position in **2**. Furthermore, the HMBC experiment showed a long-range correlation between the H$_2$-28 and the acetyl carbonyl carbon. In addition, acetylation of **2** yielded the tetraacetate (**1a**). This evidence led us to formulate the structure of rubianol-b (**2**) as 28-*O*-acetylrubianol-a. The absolute stereostructures of **1** and **2** were characterized by the application of the modified Mosher's method.[17] Briefly, treatment of **2** with (*R*)- or (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) and 4-(dimethylamino)pyridine (4-DMAP) selectively yielded the 19-mono-

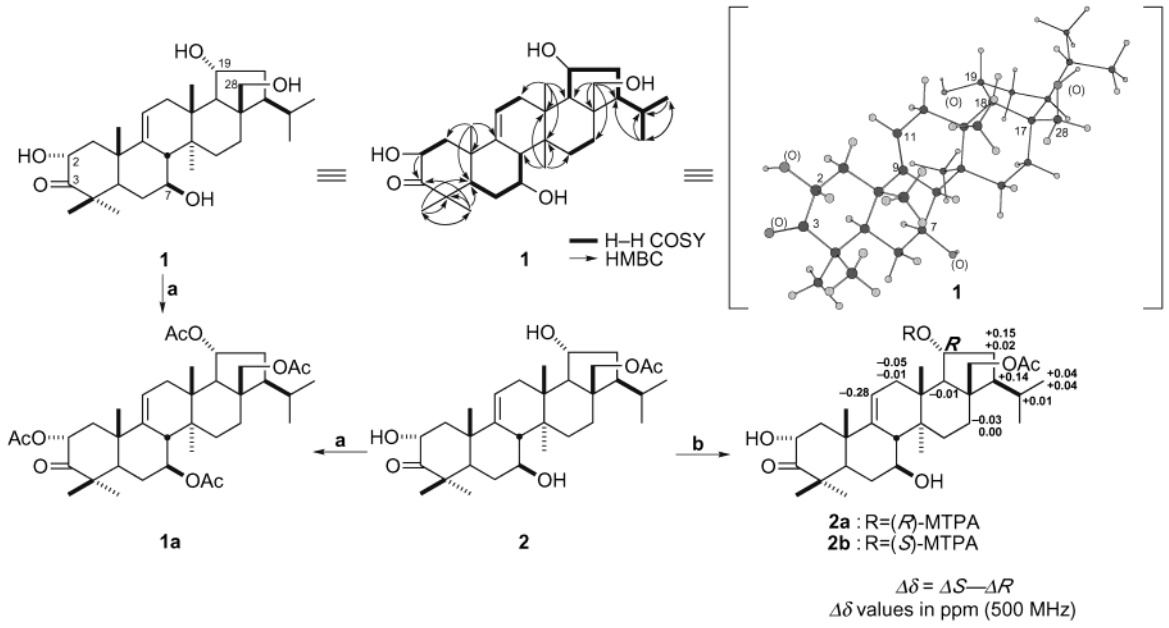


Figure 1. Reagents and conditions: (a) Ac_2O−pyridine, (b) (*R*)- or (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2.

MTPA esters (**2a**, **2b**), respectively (Figure 1). On the basis of conformational analysis of **1**,[18] the selectivity of the 19-esterification reaction with the bulky MTPA seemed to be responsible for its lesser steric hindrance than the other secondary hydroxyl groups. As shown in Figure 1, the signals due to protons attached to C-20, 21, 22, 29, and 30 in the 19-mono-(*S*)-MTPA ester (**2b**) were observed at lower fields compared with those of the 19-mono-(*R*)-MTPA ester (**2a**) [$\Delta\delta$: positive], while signals due to protons of C-11, 12, 16, and 18 in **2b** were observed at higher fields compared with those of **2a** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 19-position of **2** was determined to be in the *R* configuration, and thus absolute stereostructures of **1** and **2** were elucidated as shown.

Rubianol-c (**3**) was isolated as a white powder with a positive optical rotation ($[\alpha]_D^{25}$ +36.4°). The positive-ion FABMS of **3** showed a quasimolecular ion peak at *m*/*z* 539 $[M + Na]^+$, and the molecular formula $C_{32}H_{52}O_5$ of **3** was determined by HRFABMS. The IR spectrum of **3** showed absorption bands at 3400, 1740, and 1655 cm^{-1} assignable to hydroxyl, ester carbonyl, and olefinic functions, respectively. The ^1H and ^{13}C NMR (pyridine-d_5, Table 1) spectra[16] of **3** showed signals assignable to seven methyls [δ 0.88, 0.99 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.11, 1.14, 1.19, 1.25, 1.28 (3H each, all s, H_3-24, 27, 25, 23, 26)], an acetyl group [δ 2.07 (3H, s, −OAc)], a methylene and three methines bearing an oxygen function [δ 4.31, 4.62 (1H each, both d, J = 12.2 Hz, H_2-28), 3.48 (1H, dd, J = 6.1, 9.8 Hz, H-3), 4.02 (1H, m, H-7), 4.66 (1H, m, H-19)], and an olefin [δ 5.48 (1H, br d, J = ca. 6 Hz, H-11)] together with seven methylenes (C-1, 2, 6, 12, 15, 16, 20), five methines (C-5, 8, 18, 21, 22), and six quaternary carbons (C-4, 9, 10, 13, 14, 17). These ^1H and ^{13}C NMR spectral data of **3** resembled those of rubiarbonol A, except for the signals due to the 28-acetyl group. Acetylation of **3** with Ac_2O in pyridine yielded a tetraacetate (**3a**), which was also obtained by acetylation of rubiarbonol A. The position of an acetyl group in **3** was characterized by a HMBC experiment, which showed a long-range correlation between the H_2-28 and the acetyl carbonyl carbon. In addition, an acetylation shift was observed around the 28-position by comparison of the ^1H and ^{13}C NMR data for **3** with those for rubiarbonol A. This evidence led us to formulate the structure of **3** as the 28-acetyl derivative of rubiarbonol A. The absolute stereostructure of **3** was characterized by the application of the modified Mosher's method,[17] as shown in Figure 2. Consequently, the absolute configuration at the 19-position of **3** was determined to be the *R* configuration, and the absolute stereostructures of **3** and rubiarbonol A[19] were elucidated as shown.

Rubianols-d (**4**) and -e (**5**) were also obtained as a white powder with positive optical rotation (**4**: $[\alpha]_D^{25}$ +63.6°; **5**: $[\alpha]_D^{25}$ +18.1°), respectively. The molecular formulas of **4** and **5** were determined from the positive-ion FABMS and by HRFABMS analyses to be $C_{32}H_{52}O_6$ and $C_{34}H_{54}O_7$, respectively. The IR spectra of **4** and **5** showed absorption bands due to hydroxyl, ester carbonyl, and olefinic functions (**4**: 3400, 1740, 1655 cm^{-1}; **5**: 3400, 1740, 1655 cm^{-1}). The proton and carbon signals in the ^1H and ^{13}C NMR (pyridine-d_5, Table 1) spectra of **4** and **5** were found to be similar to those of rubiarbonol F, except for the signals due to an acetyl group for **4** and two acetyl groups for **5**. Acetylation of **4** and **5** with Ac_2O in pyridine yielded a pentaacetate (**4a**), which was also obtained by acetylation of rubiarbonol F. The positions of acetyl groups in **4** and **5** were also determined by a HMBC experiment, which showed long-range correlations between the H_2-28 and the acetyl carbon in **4** and between the H-2, H_2-28 and the acetyl carbons in **5**. The absolute stereostructures of **4** and **5** were determined by application of Mosher's method,[17] as shown in Figure 2. Thus, the absolute stereostructures of **4**, **5**, and rubiarbonol F[19] were determined as shown.

Rubianoside I (**6**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{25}$ +10.9°). The positive-ion FABMS of **6** showed a quasimolecular ion peak at *m*/*z* 699 $[M + Na]^+$, while a quasimolecular ion peak was observed at *m*/*z* 675 $[M - H]^-$ in the negative-ion FABMS. The molecular formula $C_{38}H_{60}O_{10}$ of **6** was determined by HRFABMS. The IR spectrum of **6** showed absorption bands at 1718 and 1655 cm^{-1} assignable to carbonyl and olefinic functions, and strong absorption bands at 3400 and 1078 cm^{-1} were suggestive of a glycoside moiety. The ^1H and ^{13}C NMR (pyridine-d_5, Table 1) spectra of **6** showed signals assignable to seven methyls [δ 0.88, 0.88 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.16, 1.19, 1.21, 1.26, 1.45 (3H each, all s, H_3-24, 26, 25, 27, 23)], an acetyl group [δ 2.45 (3H, s, −OAc)], a methylene and four methines bearing an oxygen function [δ 3.74, 3.97 (1H each, both d, J = 7.6 Hz, H_2-28),

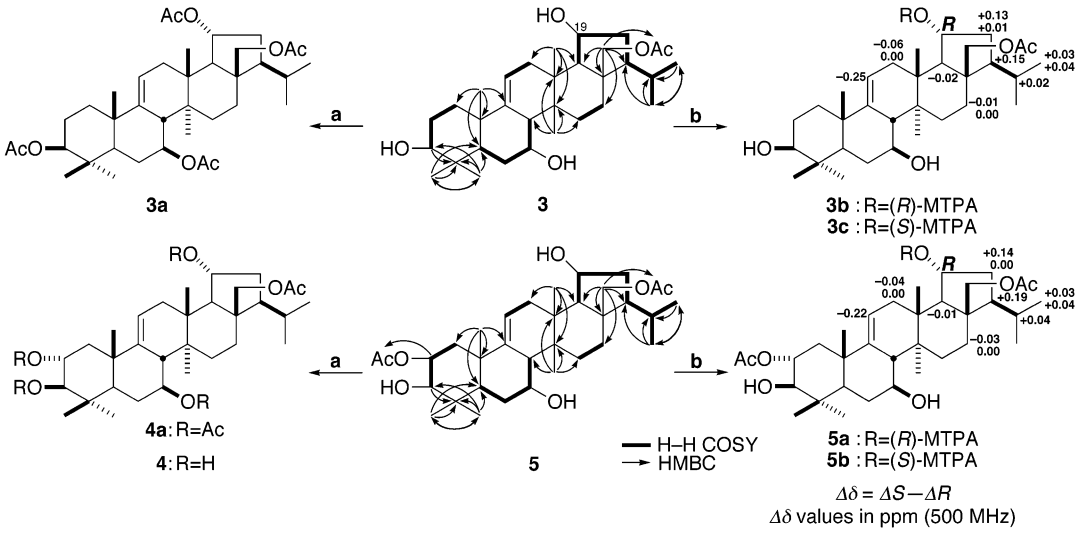


Figure 2. Reagents and conditions: (a) Ac_2O−pyridine, (b) (*R*)- or (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2.

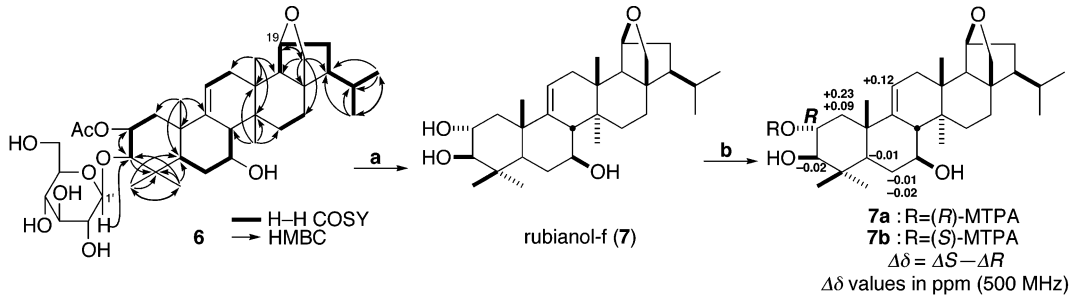


Figure 3. Reagents and conditions: (a) 2 M HCl−1,4-dioxane (1:1, v/v), (b) (*R*)- or (*S*)-MTPA, EDC·HCl, 4-DMAP−CH_2Cl_2.

Table 2. Inhibitory Effects of Constituents from *R. yunnanensis* on NO Production in LPS-Activated Mouse Peritoneal Macrophages

	inhibition (%)[a]					
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM
rubianol-a (**1**)	0.0 ± 2.2	−9.6 ± 2.3	−10.3 ± 6.0	2.1 ± 1.2	1.8 ± 5.0	40.3 ± 7.2[c]
compound **1a**	0.0 ± 0.5	1.7 ± 0.5	7.3 ± 2.0	12.5 ± 1.0[c]	18.7 ± 1.7[c]	23.0 ± 1.4[c]
rubianol-b (**2**)	0.0 ± 1.2	−1.1 ± 1.8	−1.5 ± 4.4	0.0 ± 2.3	−5.8 ± 3.4	15.4 ± 1.0[c]
rubianol-c (**3**)	0.0 ± 5.5	5.2 ± 7.7	11.1 ± 5.5	4.7 ± 7.6	−7.4 ± 5.3	25.5 ± 6.4[c,d]
compound **3a**	0.0 ± 3.2	5.3 ± 3.9	10.6 ± 5.0	−1.2 ± 4.1	0.0 ± 5.0	6.6 ± 3.0
rubianol-d (**4**)	0.0 ± 2.8	17.0 ± 3.6	5.3 ± 7.8	4.3 ± 4.9	−11.1 ± 3.8	76.5 ± 1.1[c]
compound **4a**	0.0 ± 2.4	7.7 ± 0.3[c]	9.5 ± 0.1[c]	18.7 ± 1.6[c]	25.5 ± 1.6[c]	28.4 ± 1.1[c]
rubianol-e (**5**)	0.0 ± 5.0	20.2 ± 4.7[b]	21.3 ± 5.2[b]	−12.0 ± 5.7	−7.9 ± 4.5	77.1 ± 4.4[c]
rubianoside I (**6**)	0.0 ± 2.1	5.1 ± 2.9	−0.3 ± 2.8	−8.0 ± 1.2	1.5 ± 7.0	−3.2 ± 5.0
rubiarbonol A	0.0 ± 3.5	6.3 ± 4.5	4.2 ± 3.1	−4.2 ± 2.2	2.9 ± 4.9[d]	86.3 ± 1.4[c,d]
rubiarbonol F	0.0 ± 5.5	9.6 ± 8.4	7.9 ± 5.6	15.4 ± 4.0	22.0 ± 6.2	60.4 ± 7.8[c,d]
rubiarbonone B	0.0 ± 4.9	4.3 ± 6.8	−9.7 ± 6.3	−13.7 ± 6.4	16.9 ± 5.3	19.7 ± 5.1
rubiarbonone C	0.0 ± 1.7	−2.1 ± 2.3	3.7 ± 6.7	−2.3 ± 4.2	8.0 ± 8.8	90.3 ± 2.9[c,d]
(+)-lariciresinol	0.0 ± 0.7	4.0 ± 4.4	3.0 ± 4.4	5.5 ± 3.4	−8.2 ± 3.9	11.9 ± 9.4
(+)-isolariciresinol	0.0 ± 6.9	−0.2 ± 4.8	−0.6 ± 1.9	0.6 ± 6.8	5.1 ± 2.0	7.5 ± 7.1
(+)-secoisolariciresinol	0.0 ± 3.2	5.9 ± 4.2	−7.3 ± 3.3	6.5 ± 5.7	0.9 ± 6.2	−12.5 ± 4.8
2-methyl-1,3,6-trihydroxy-9,10-anthraquinone	0.0 ± 6.9	4.9 ± 2.4	7.9 ± 4.4	37.5 ± 3.1[c]	99.5 ± 0.2[c,d]	99.6 ± 0.2[c,d]
4-hydroxy-2,6-dimethoxybenzoic acid	0.0 ± 9.9	9.5 ± 3.4	9.3 ± 5.4	7.5 ± 6.8	7.2 ± 6.1	28.0 ± 4.4[b]
vanillic acid	0.0 ± 7.3	5.9 ± 7.1	16.2 ± 6.0	8.2 ± 8.2	−3.6 ± 12.6	5.9 ± 6.6
L-NMMA	0.0 ± 4.0	5.9 ± 0.9	10.3 ± 3.7	15.0 ± 1.6[c]	34.1 ± 3.2[c]	63.1 ± 1.2[c]

[a] Each value represents the mean ± SEM ($N = 4$). [b] Significantly different from the control. $p < 0.05$. [c] Significantly different from the control. $p < 0.01$. [d] Cytotoxic effect was observed.

3.66 (1H, d, $J = 10.1$ Hz, H-3), 3.97 (1H, m, H-7), 4.21 (1H, m, H-19), 5.66 (1H, m, H-2)], and an olefin [δ 5.36 (1H, br d, J = ca. 5 Hz, H-11)] together with a β-D-glucopyranosyl signal [δ 5.02 (1H, d, $J = 7.6$ Hz, H-1′)]. Acid hydrolysis with 2 M hydrochloric acid (HCl) of **6** yielded D-glucose, which was identified by HPLC analysis using an optical rotation detector,[1b] and a new triterpene termed rubianol-f (**7**) as its aglycon. The ^{1}H−^{1}H COSY and the HMBC experiments on **6** unambiguously characterized the planar structure of **6** as shown in Figure 3. The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **7** were similar to those of rubiarbonol F, except for the signals due to the 19,28-oxide ring, which was confirmed by the HMBC experiment on **6** and **7**. Namely, long-range correlations were observed between the H-19 and C-28 and between the H_2-28 and C-17, 18, 19, 21. Comparison of the ^{1}H and ^{13}C NMR spectra of **6** with those of **7** showed an acetylation shift and a glycosylation shift around the 2- and 3-positions of **6**, respectively. Furthermore, long-range correlations were observed between the H-2 and acetyl carbonyl carbon

and between the anomeric proton of the D-glucopyranosyl moiety and the C-3. Thus, the positions of the acetyl group and the β-D-glucopyranosyl part in **6** were confirmed on C-2 and C-3, respectively. On the basis of these findings, the structures of **6** and **7** were characterized as shown. The absolute stereostructures of **6** and **7** were determined by an application of the modified Mosher's method,[17] in which **7** gave the 2-(*R*)- and 2-(*S*)-MTPA esters (**7a** and **7b**). As shown in Figure 3, the signals due to the protons attached to the 1- and 11-carbons in **7b** were observed at lower fields compared with those of **7a** [$\Delta\delta$: positive], while signals due to the protons of the 3-, 5-, and 6-carbons in **7b** were observed at higher fields compared with those of **7a** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 2-position of **7** has been determined to be in the *R* configuration, so that the absolute stereostructures of **6** and **7** could be determined.

The effects of the constituents from the roots of *R. yunnanensis* on NO production from LPS-activated macrophages were examined, and the results are summarized in Table 2. Among them, rubianols-d (**4**) and -e (**5**) exhibited inhibitory activity without cytotoxic effects in the MTT assay. Their inhibitory activities were equivalent to that of N^G-monomethyl-L-arginine (L-NMMA), a nonselective NOS inhibitor (IC_{50} = 57 μM). On the other hand, the following constituents were found to show a cytotoxic effect: rubianol-C (**3**), rubiarbonols A and F, rubiarbonone C, and 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone. These compounds also inhibited NO production.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JEOL LNM-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL LNM-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index detector and Shodex OR-2 optical rotation detector.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); HPLC column, YMC-Pack ODS-A (250 × 20 mm i.d.) and Asahipak NH-2P-50-4E (250 × 4.6 mm i.d.); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The roots of *Rubia yunnanensis* were purchased in Kunming, Yunnan Province, People's Republic of China, in September 2001, and identified by one of the authors (M.Y.). A voucher of the plant is on file in our laboratory (2001.09.Yunnan-19).

Extraction and Isolation. The dried roots of *R. yunnanensis* (1.6 kg) were cut and extracted overnight three times with 80% aqueous acetone at room temperature. Evaporation of the solvent under reduced pressure provided the aqueous acetone extract (453 g, 28.3%). The aqueous acetone extract (382.5 g) was partitioned using EtOAc−H_2O (1:1, v/v), and removal of the solvent in vacuo from the EtOAc- and H_2O-soluble portions yielded 57.5 g (4.3%) and 325.0 g (24.0%) of the residue, respectively. Normal-phase silica gel column chromatography [750 g, *n*-hexane−EtOAc (5:1−1:1, v/v)−$CHCl_3$−MeOH (1:1, v/v)−MeOH] of the EtOAc-soluble portion (45 g) gave six fractions [Fr. 1 (11.0 g), 2 (16.7 g), 3 (2.8 g), 4 (3.9 g), 5 (8.9 g), 6 (1.7 g)]. Fraction 2 (16.0 g) was separated by reversed-phase silica gel column chromatography [480 g, MeOH−H_2O (30:70−50:50−70:30, v/v)−MeOH] to furnish 10 fractions [Fr. 2-1 (274 mg), Fr. 2-2 (167 mg), Fr. 2-3 (404 mg), Fr. 2-4 (822 mg), Fr. 2-5 (1.85 g), Fr. 2-6 (783 mg), Fr. 2-7 (1.10 g), Fr. 2-8 (1.33 g), Fr. 2-9 (1.79 g), Fr. 2-10 (7.48 g)]. Fraction 2-2 (167 mg) was further separated by HPLC [YMC-Pack ODS-A, MeOH−H_2O (30:70, v/v)] to give 4-hydroxy-3,5-dimethoxybenzoic acid (20 mg, 0.0021%) and vanillic acid (18 mg, 0.0019%). Fraction 2-4 (822 mg) was separated by HPLC [MeOH−H_2O (50:50, v/v)] to give (+)-lariciresinol (178 mg, 0.018%), (+)-isolariciresinol (50 mg, 0.0050%), and (−)-sec-oisolariciresinol (54 mg, 0.0054%). Fraction 2-6 (783 mg) was separated by HPLC [MeOH−H_2O (65:35, v/v)] to give rubianol-a (**1**, 39 mg, 0.0039%). Fraction 2-7 (1.10 g) was purified by HPLC [MeOH−H_2O (65:35, v/v)] to give rubianols-b (**2**, 12 mg, 0.0011%) and -d (**4**, 41 mg, 0.0041%), rubiarbonol A (111 mg, 0.011%), and rubiarbonone B (41 mg, 0.0041%). Fraction 2-8 (1.33 g) was subjected to HPLC [CH_3CN−H_2O (45:55, v/v)] to furnish rubianols-c (**3**, 93 mg, 0.0092%) and -e (**5**, 54 mg, 0.0053%). Fraction 2-9 (691 mg) was further purified by HPLC [CH_3CN−H_2O (60:40, v/v)] to give rubiarbonone C (47 mg, 0.012%) and 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone (94 mg, 0.025%). Fraction 3 (2.3 g) was separated by reversed-phase silica gel column chromatography [70 g, MeOH−H_2O (30:70−50:50−70:30, v/v)−MeOH] and then HPLC [MeOH−H_2O (60:40, v/v)] to furnish rubiarbonol F (17 mg, 0.0020%). Fraction 4 (3.4 g) was subjected to reversed-phase silica gel column chromatography [110 g, MeOH−H_2O (50:50−60:40−70:30 v/v)−MeOH] and finally HPLC [MeOH−H_2O (75:25 v/v)] to give rubianoside I (**6**, 17 mg, 0.0018%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values[3−6,11−14] or commercial samples.[15]

Rubianol-a (1): white powder; $[\alpha]_D^{25}$ +10.0° (*c* 0.30, MeOH); IR (KBr) ν_{max} 3400, 2950, 1716, 1655, 1458, 1375, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.92, 1.10 (3H each, both d, J = 5.8 Hz, H_3-30, 29), 1.04, 1.22, 1.27, 1.41, 1.44 (3H each, all s, H_3-24, 23, 26, 25, 27), 1.48 (1H, m, H-5), 1.59 (1H, m, H-21), 1.85, 2.64 (1H each, both m, H_2-1), 1.57, 2.03 (1H each, both m, H_2-16), 2.09, 2.17 (1H each, both m, H_2-6), 2.09, 2.75 (1H each, both m, H_2-15), 2.12 (1H, m, H-22), 2.16, 2.62 (1H each, both m, H_2-20), 2.32 (1H, d, J = 10.3 Hz, H-18), 2.47, 2.53 (1H each, both m, H_2-12), 2.57 (1H, d-like, H-8), 4.05 (1H, m, H-7), 4.10, 4.23 (1H each, both d, J = 11.3 Hz, H_2-28), 5.01 (1H, dd, J = 5.8, 12.8 Hz, H-2), 5.06 (1H, m, H-19), 5.54 (1H, br d, J = ca. 6 Hz, H-11); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 511 $[M + Na]^+$; HRFABMS m/z 511.3394 (calcd for $C_{30}H_{48}O_5Na$ $[M + Na]^+$, 511.3399).

Rubianol-b (2): white powder; $[\alpha]_D^{25}$ +16.8° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400, 2950, 1740, 1655, 1458, 1375, 1076, 1034 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.99 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.08, 1.14, 1.20, 1.23, 1.41 (3H each, all s, H_3-24, 27, 26, 23, 25), 1.45 (1H, m, H-5), 1.52 (1H, m, H-21), 1.55, 1.92 (1H each, both m, H_2-16), 1.59 (1H, m, H-22), 1.83, 2.62 (1H each, both m, H_2-1), 1.92, 2.76 (1H each, both m, H_2-15), 2.03, 2.14 (1H each, both m, H_2-6), 2.08 (3H, s, −OAc), 2.12, 2.22 (1H each, both m, H_2-20), 2.26 (1H, d, J = 10.1 Hz, H-18), 2.34, 2.48 (1H each, both m, H_2-12), 2.44 (1H, d-like, H-8), 4.00 (1H, m, H-7), 4.30, 4.64 (1H each, both d, J = 12.2 Hz, H_2-28), 4.68 (1H, m, H-19), 5.01 (1H, dd, J = 5.8, 12.8 Hz, H-2), 5.49 (1H, br d, J = ca. 6 Hz, H-11); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 553 $[M + Na]^+$; HRFABMS m/z 553.3498 (calcd for $C_{32}H_{50}O_6Na$ $[M + Na]^+$, 553.3505).

Rubianol-c (3): white powder; $[\alpha]_D^{25}$ +36.4° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400, 2950, 1740, 1655, 1458, 1375, 1257, 1051 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.99 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.11, 1.14, 1.19, 1.25, 1.28 (3H each, all s, H_3-24, 27, 25, 23, 26), 1.10 (1H, m, H-5), 1.50 (1H, m, H-21), 1.52, 1.73 (1H each, both m, H_2-1), 1.56, 1.91 (1H each, both m, H_2-16), 1.59 (1H, m, H-22), 1.89, 2.82 (1H each, both m, H_2-15), 1.96 (2H, m, H_2-2), 1.98, 2.28 (1H each, both m, H_2-6), 2.07 (3H, s, −OAc), 2.13, 2.21 (1H each, both m, H_2-20), 2.29 (1H, d, J = 10.3 Hz, H-18), 2.35, 2.50 (1H each, both

m, H_2-12), 2.42 (1H, d, J = 7.0 Hz, H-8), 3.48 (1H, dd, J = 6.1, 9.8 Hz, H-3), 4.02 (1H, m, H-7), 4.31, 4.62 (1H each, both d, J = 12.2 Hz, H_2-28), 4.66 (1H, m, H-19), 5.48 (1H, br d, J = ca. 6 Hz, H-11); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 539 $[M + Na]^+$; HRFABMS *m/z* 539.3717 (calcd for $C_{32}H_{52}O_5Na$ $[M + Na]^+$, 539.3712).

Rubianol-d (4): white powder; $[\alpha]_D^{25}$ +63.6° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400, 2943, 1740, 1655, 1458, 1375, 1259, 1051, 1034 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.99 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.10, 1.15, 1.27, 1.27, 1.28 (3H each, all s, H_3-27, 24, 25, 26, 23), 1.24 (1H, m, H-5), 1.52 (1H, m, H-21), 1.57, 1.92 (1H each, both m, H_2-16), 1.58 (1H, m, H-22), 1.78, 2.38 (1H each, both m, H_2-1), 1.92, 2.82 (1H each, both m, H_2-15), 2.00, 2.27 (1H each, both m, H_2-6), 2.07 (3H, s, −OAc), 2.12, 2.21 (1H each, both m, H_2-20), 2.28 (1H, d, J = 10.0 Hz, H-18), 2.35, 2.48 (1H each, both m, H_2-12), 2.46 (1H, d-like, H-8), 3.42 (1H, d, J = 10.1 Hz, H-3), 4.05 (1H, m, H-7), 4.22 (1H, ddd, J = 4.0, 10.1, 10.1 Hz, H-2), 4.30, 4.63 (1H each, both d, J = 11.9 Hz, H_2-28), 4.67 (1H, m, H-19), 5.61 (1H, br d, J = ca. 6 Hz, H-11); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 555 $[M + Na]^+$; HRFABMS *m/z* 555.3668 (calcd for $C_{32}H_{52}O_6Na$ $[M + Na]^+$, 555.3662).

Rubianol-e (5): white powder; $[\alpha]_D^{25}$ +18.1° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400, 2950, 1740, 1655, 1458, 1375, 1257, 1051, 1031 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.99 (3H each, both d, J = 6.1 Hz, H_3-30, 29), 1.13, 1.15, 1.26, 1.28, 1.29 (3H each, all s, H_3-27, 24, 23, 25, 26), 1.21 (1H, m, H-5), 1.55 (1H, m, H-21), 1.57, 2.26 (1H each, both m, H_2-1), 1.59, 1.92 (1H each, both m, H_2-16), 1.59 (1H, m, H-22), 1.91, 2.82 (1H each, both m, H_2-15), 1.97, 2.20 (1H each, both m, H_2-6), 2.01, 2.07 (3H each, both s, −OAc), 2.13, 2.21 (1H each, both m, H_2-20), 2.29 (1H, d, J = 10.3 Hz, H-18), 2.37, 2.50 (1H each, both m, H_2-12), 2.39 (1H, d-like, H-8), 3.55 (1H, d, J = 10.1 Hz, H-3), 4.02 (1H, m, H-7), 4.30, 4.67 (1H each, both d, J = 12.3 Hz, H_2-28), 4.68 (1H, m, H-19), 5.43 (1H, br d, J = ca. 6 Hz, H-11), 5.53 (1H, ddd, = 4.0, 10.1, 10.1 Hz, H-2); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 597 $[M + Na]^+$; HRFABMS *m/z* 597.3776 (calcd for $C_{34}H_{54}O_7Na$ $[M + Na]^+$, 597.3767).

Rubianoside I (6): white powder; $[\alpha]_D^{25}$ +10.9° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400, 2953, 1718, 1655, 1458, 1387, 1078, 1039 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.88 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.16, 1.19, 1.21, 1.26, 1.45 (3H each, all s, H_3-24, 26, 25, 27, 23), 1.11 (1H, m, H-5), 1.28, 1.93 (1H each, both m, H_2-20), 1.49 (1H, m, H-21), 1.55 (1H, br s, H-18), 1.62, 2.12 (1H each, both m, H_2-1), 1.70, 1.82 (1H each, both m, H_2-16), 1.82, 2.95 (1H each, both m, H_2-15), 1.87, 2.21 (1H each, both m, H_2-6), 1.88, 1.96 (1H each, both m, H_2-12), 2.14 (1H, m, H-22), 2.33 (1H, d, J = 9.8 Hz, H-8), 2.45 (3H, s, −OAc), 3.66 (1H, d, J = 10.1 Hz, H-3), 3.74, 3.97 (1H each, both d, J = 7.6 Hz, H_2-28), 3.97 (1H, m, H-7), 4.21 (1H, m, H-19), 5.02 (1H, d, J = 7.6 Hz, H-1′), 5.36 (1H, br d, J = ca. 5 Hz, H-11), 5.66 (1H, m, H-2); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 699 $[M + Na]^+$; negative-ion ion FABMS *m/z* 675 $[M - H]^-$; HRFABMS *m/z* 699.4095 (calcd for $C_{38}H_{60}O_{10}Na$ $[M + Na]^+$, 699.4084).

Acetylation of Rubianols-a−e (1−5) and Rubiarbonols A and F. A solution of **1** (4.4 mg) in pyridine (1.0 mL) was treated with acetic anhydride (Ac_2O, 0.8 mL), and the mixture was stirred at room temperature for 8 h. The reaction mixture was poured into ice−water, and the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtrated. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1.0 g, *n*-hexane−EtOAc (3:1, v/v)] to give **1a** (4.1 mg, 69%).

Through a similar procedure, **1a** (1.5 mg, 76% from **2**), **3a** (4.3 mg, 52% from **3**; 3.0 mg, 52% from rubiarbonol A), and **4a** (3.3 mg, 50% from **4**; 3.0 mg, 82% from **5**; 3.6 mg, 51% from rubiarbonol F) were obtained from **2** (1.6 mg), **3** (6.7 mg), **4** (5.0 mg), **5** (3.0 mg), rubiarbonols A (4.3 mg), or F (4.9 mg) using Ac_2O (0.8 mL) in pyridine (1.0 mL).

Compound 1a: white powder; $[\alpha]_D^{25}$ −3.8° (*c* 0.20, MeOH); IR (KBr) ν_{max} 2961, 1735, 1470, 1375, 1239, 1034 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.91 (3H each, both d, J = 6.1 Hz, H_3-30, 29), 0.95, 1.04, 1.14, 1.17, 1.47 (3H each, all s, H_3-27, 26, 24, 23, 25), 1.35 (1H, m, H-21), 1.39, 1.73 (1H each, both m, H_2-16), 1.49 (1H, m, H-22), 1.51 (1H, m, H-5), 1.52, 1.75 (1H each, both m, H_2-15), 1.81, 2.28 (1H each, both m, H_2-20), 1.87, 2.19 (1H each, both m, H_2-6), 1.95, 2.41 (1H each, both m, H_2-1), 2.11, 2.22, 2.22, 2.22 (3H each, all s, −OAc), 2.10, 2.15 (1H each, both m, H_2-12), 2.20 (1H, d, J = 12.5 Hz, H-18), 2.56 (1H, d, J = 11.6 Hz, H-8), 4.32, 4.42 (1H each, both d, J = 12.2 Hz, H_2-28), 5.24 (1H, m, H-7), 5.59 (1H, m, H-19), 5.33 (1H, br d, J = ca. 5 Hz, H-11), 5.99 (1H, dd, J = 5.8, 13.8 Hz, H-2); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 679 $[M + Na]^+$; HRFABMS *m/z* 679.3828 (calcd for $C_{38}H_{56}O_9Na$ $[M + Na]^+$, 679.3822).

Compound 3a: a white powder; $[\alpha]_D^{25}$ −2.0° (*c* 0.20, MeOH); IR (KBr) ν_{max} 2940, 2361, 1739, 1470, 1370, 1244, 1030 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.91 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 0.91, 0.95, 0.95, 1.09, 1.13 (3H each, all s, H_3-24, 23, 27, 26, 25), 1.12 (1H, m, H-5), 1.35 (1H, m, H-21), 1.38, 1.74 (1H each, both m, H_2-16), 1.47, 1.71 (1H each, both m, H_2-1), 1.49 (1H, m, H-22), 1.56, 1.73 (1H each, both m, H_2-15), 1.62, 2.12 (1H each, both m, H_2-12), 1.72, 2.23 (1H each, both m, H_2-6), 1.73, 1.87 (1H each, both m, H_2-2), 1.80, 2.27 (1H each, both m, H_2-20), 2.08, 2.12, 2.16, 2.22 (3H each, all s, −OAc), 2.24 (1H, d, J = 12.0 Hz, H-18), 2.47 (1H, d, J = 11.3 Hz, H-8), 4.32, 4.42 (1H each, both d, J = 12.2 Hz, H_2-28), 4.70 (1H, dd, J = 4.0, 12.2 Hz, H-3), 5.23 (1H, m, H-7), 5.31 (1H, br d, J = ca. 6 Hz, H-11), 5.57 (1H, m, H-19); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 665 $[M + Na]^+$; HRFABMS *m/z* 665.4026 (calcd for $C_{38}H_{58}O_8Na$ $[M + Na]^+$, 665.4029).

Compound 4a: white powder; $[\alpha]_D^{25}$ −17.2° (*c* 0.20, MeOH); IR (KBr) ν_{max} 2983, 1743, 1465, 1380, 1247, 1038 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.91 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 0.92, 0.93, 0.97, 1.08, 1.23 (3H each, all s, H_3-27, 24, 23, 26, 25), 1.27 (1H, m, H-5), 1.34 (1H, m, H-21), 1.45, 1.74 (1H each, both m, H_2-16), 1.50 (1H, m, H-22), 1.55, 1.71 (1H each, both m, H_2-15), 1.56, 2.24 (1H each, both m, H_2-1), 1.58, 2.11 (1H each, both m, H_2-12), 1.69, 2.20 (1H each, both m, H_2-6), 1.82, 2.27 (1H each, both m, H_2-20), 2.12, 2.15, 2.15, 2.22, 2.24 (3H, s, −OAc), 2.23 (1H, d, J = 10.0 Hz, H-18), 2.45 (1H, d, J = 10.4 Hz, H-8), 4.33, 4.40 (1H each, both d, J = 12.5 Hz, H_2-28), 5.10 (1H, d, J = 10.7 Hz, H-3), 5.21 (1H, m, H-7), 5.22 (1H, br d, J = ca. 7 Hz, H-11), 5.49 (1H, m, H-2), 5.62 (1H, m, H-19); ^{13}C NMR data, see Table 1; positive-ion FABMS *m/z* 723 $[M + Na]^+$; HRFABMS *m/z* 723.4080 (calcd for $C_{40}H_{60}O_{10}Na$ $[M + Na]^+$, 723.4084).

Preparation of the (*R*)-MTPA Esters (2a, 3b, 5a) and (*S*)-MTPA Esters (2b, 3c, 5b) from Rubianols-b (2), -c (3), and -e (5). A solution of **2**, **3**, or **5** (3.0 mg each) in CH_2Cl_2 (1.0 mL) was treated with (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA, 7.5 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl, 6.2 mg) and 4-(dimethylamino)pyridine (4-DMAP, 2.0 mg), and the mixture was stirred under reflux for 8 h. After cooling, the reaction mixture was poured into ice−water, and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (2:1, v/v)] to give **2a** (2.1 mg, 50%), **3b** (1.3 mg, 30%), or **5a** (3.0 mg, 73%), respectively. Using a similar procedure, (*S*)-MTPA esters [**2b** (2.1 mg, 50%), **3c** (1.3 mg, 31%), or **5b** (1.4 mg, 34%)] were obtained from **2**, **3**, or **5** (3.0 mg each), respectively, using (*S*)-MTPA (7.5 mg), EDC·HCl (6.2 mg), and 4-DMAP (2.0 mg).

Compound 2a: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.79, 0.87 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.03, 1.09, 1.09, 1.24, 1.43 (3H each, all s, CH_3), 1.34 (1H, m, H-21), 1.46 (1H, m, H-5), 1.49, 1.87 (1H each, both m, H_2-16), 1.51 (1H, m, H-22), 1.87, 2.45 (1H each, both m, H_2-20), 1.87, 2.71 (1H each, both m, H_2-15), 2.04, 2.15 (1H each, both m, H_2-6), 2.16 (3H, s, −OAc), 2.38 (1H, d, J = 10.7 Hz, H-18), 2.40 (1H, d, m, H-8), 2.40, 2.45 (1H each, both m, H_2-12), 2.65 (2H, m, H_2-1), 3.80

(3H, s, −OCH_3), 3.97 (1H, m, H-7), 4.37, 4.45 (1H each, both d, J = 12.5 Hz, H_2-28), 5.04 (1H, m, H-2), 5.40 (1H, br d, J = ca. 7 Hz, H-11), 5.79 (1H, m, H-19), 7.45 (3H, m, Ph-H), 7.88 (2H, m, Ph-H).

Compound 2b: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.91 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.03, 1.09, 1.09, 1.24, 1.43 (3H each, all s, CH_3), 1.48 (1H, m, H-21), 1.41 (1H, m, H-5), 1.49, 1.84 (1H each, both m, H_2-16), 1.52 (1H, m, H-22), 2.02, 2.47 (1H each, both m, H_2-20), 1.84, 2.69 (1H each, both m, H_2-15), 2.01, 2.08 (1H each, both m, H_2-6), 2.16 (3H, s, −OAc), 2.35 (1H, d, m, H-8), 2.35, 2.44 (1H each, both m, H_2-12), 2.37 (1H, d, J = 10.7 Hz, H-18), 2.63 (2H, m, H_2-1), 3.80 (3H, s, −OCH_3), 3.92 (1H, m, H-7), 4.37, 4.45 (1H each, both d, J = 12.5 Hz, H_2-28), 5.04 (1H, m, H-2), 5.12 (1H, br d, J = ca. 7 Hz, H-11), 5.79 (1H, m, H-19), 7.45 (3H, m, Ph-H), 7.88 (2H, m, Ph-H).

Compound 3b: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.80, 0.88 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.05, 1.13, 1.16, 1.20, 1.26 (3H each, all s, CH_3), 1.10 (1H, m, H-5), 1.36 (1H, m, H-21), 1.50, 1.83 (1H each, both m, H_2-16), 1.51 (1H, m, H-22), 1.52 (2H, m, H_2-1), 1.83, 2.80 (1H each, both m, H_2-15), 1.89, 2.46 (1H each, both m, H_2-20), 1.99, 2.30 (1H each, both m, H_2-6), 2.02 (2H, m, H_2-2), 2.16 (3H, s, −OAc), 2.36 (1H, d, m, H-8), 2.36, 2.48 (1H each, both m, H_2-12), 2.44 (1H, d, J = 9.8 Hz, H-18), 3.52 (1H, m, H-3), 3.79 (3H, s, −OCH_3), 4.00 (1H, m, H-7), 4.38, 4.46 (1H each, both d, J = 12.8 Hz, H_2-28), 5.38 (1H, br d, J = ca. 7 Hz, H-11), 5.80 (1H, m, H-19), 7.49 (3H, m, Ph-H), 7.89 (2H, m, Ph-H).

Compound 3c: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.84, 0.91 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.05, 1.13, 1.16, 1.20, 1.26 (3H each, all s, CH_3), 1.06 (1H, m, H-5), 1.50, 1.82 (1H each, both m, H_2-16), 1.51 (1H, m, H-21), 1.52 (2H, m, H_2-1), 1.53 (1H, m, H-22), 1.82, 2.79 (1H each, both m, H_2-15), 1.96, 2.30 (1H each, both m, H_2-6), 2.02, 2.47 (1H each, both m, H_2-20), 2.02 (2H, m, H_2-2), 2.16 (3H, s, −OAc), 2.30 (1H, d, m, H-8), 2.30, 2.48 (1H each, both m, H_2-12), 2.42 (1H, d, J = 9.8 Hz, H-18), 3.50 (1H, m, H-3), 3.79 (3H, s, −OCH_3), 3.95 (1H, m, H-7), 4.39, 4.46 (1H each, both d, J = 12.8 Hz, H_2-28), 5.13 (1H, br d, J = ca. 7 Hz, H-11), 5.80 (1H, m, H-19), 7.49 (3H, m, Ph-H), 7.89 (2H, m, Ph-H).

Compound 5a: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.80, 0.88 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 1.05, 1.17, 1.17, 1.27, 1.31 (3H each, all s, CH_3), 1.21 (1H, m, H-5), 1.31 (1H, m, H-21), 1.49, 1.88 (1H each, both m, H_2-16), 1.50 (1H, m, H-22), 1.60, 2.32 (1H each, both m, H_2-1), 1.88, 2.48 (1H each, both m, H_2-20), 1.88, 2.79 (1H each, both m, H_2-15), 2.01, 2.27 (1H each, both m, H_2-6), 2.02, 2.16 (3H each, both s, −OAc), 2.35 (1H, d, m, H-8), 2.45 (1H, d, J = 9.8 Hz, H-18), 2.35, 2.48 (1H each, both m, H_2-12), 3.59 (1H, m, H-3), 3.79 (3H, s, −OCH_3), 4.00 (1H, m, H-7), 4.39, 4.45 (1H each, both d, J = 12.8 Hz, H_2-28), 5.40 (1H, br d, J = ca. 7 Hz, H-11), 5.57 (1H, m, H-2), 5.83 (1H, m, H-19), 7.49 (3H, m, Ph-H), 7.89 (2H, m, Ph-H).

Compound 5b: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.83, 0.92 (3H each, both d, J = 6.4 Hz, H_3-30, 29), 0.99, 1.12, 1.16, 1.26, 1.29 (3H each, all s, CH_3), 1.21 (1H, m, H-5), 1.49, 1.85 (1H each, both m, H_2-16), 1.50 (1H, m, H-21), 1.54 (1H, m, H-22), 1.60, 2.31 (1H each, both m, H_2-1), 1.85, 2.78 (1H each, both m, H_2-15), 1.98, 2.25 (1H each, both m, H_2-6), 2.02, 2.48 (1H each, both m, H_2-20), 2.04, 2.15 (3H each, both s, −OAc), 2.30 (1H, d, m, H-8), 2.44 (1H, d, J = 9.8 Hz, H-18), 2.31, 2.48 (1H each, both m, H_2-12), 3.59 (1H, m, H-3), 3.79 (3H, s, −OCH_3), 3.96 (1H, m, H-7), 4.39, 4.45 (1H each, both d, J = 12.8 Hz, H_2-28), 5.18 (1H, br d, J = ca. 7 Hz, H-11), 5.57 (1H, m, H-2), 5.83 (1H, m, H-19), 7.49 (3H, m, Ph-H), 7.89 (2H, m, Ph-H).

Acid Hydrolysis of Rubianoside I (6). A solution of **6** (4.5 mg) in 2 M HCl-1,4-dioxane (1:1, v/v, 0.5 mL) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice−water and then extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Shodex Asahipak NH-2P-50-4E; detection, optical rotation; mobile phase, CH_3CN−H_2O (75:25, v/v); flow rate, 0.8 mL/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with that of an authentic sample. t_R: 11.1 min (D-glucose, positive optical rotation).

The EtOAc layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (3:1, v/v)] to give rubianol-f (**7**) (3.1 mg, 99%).

Rubianol-f (7): white powder; $[\alpha]_D^{25}$ +16.9° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3431, 2961, 1640, 1465, 1375, 1063, 1034 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.88, 0.88 (3H each, both d, J = 6.5 Hz, H_3-30, 29), 1.15, 1.17, 1.26, 1.28, 1.29 (3H each, all s, H_3-24, 26, 25, 27, 23), 1.25 (1H, m, H-5), 1.29, 1.89 (1H each, both m, H_2-20), 1.22 (1H, m, H-21), 1.53 (1H, br s, H-18), 1.80, 2.41 (1H each, both m, H_2-1), 1.70, 1.82 (1H each, both m, H_2-16), 1.82, 2.99 (1H each, both m, H_2-15), 2.05, 2.30 (1H each, both m, H_2-6), 1.83, 1.92 (1H each, both m, H_2-12), 1.66 (1H, m, H-22), 2.41 (1H, d-like, H-8), 3.44 (1H, d, J = 9.5 Hz, H-3), 3.72, 3.87 (1H each, both d, J = 7.3 Hz, H_2-28), 4.03 (1H, m, H-7), 4.24 (1H, m, H-2), 4.24 (1H, m, H-19), 5.57 (1H, br d, J = ca. 6 Hz, H-11); ^{13}C NMR data, see Table 1; positive-ion FABMS *m*/*z* 495 $[M + Na]^+$; HRFABMS *m*/*z* 495.3459 (calcd for $C_{30}H_{48}O_4Na$ $[M + Na]^+$, 495.3450).

Preparation of the (*R*)-MTPA Ester (7a) and (*S*)-MTPA Ester (7b) from 7. A solution of **7** (1.5 mg) in CH_2Cl_2 (1.0 mL) was treated with (*R*)-MTPA (3.7 mg) in the presence of EDC·HCl (3.1 mg) and 4-DMAP (1.0 mg), and the mixture was stirred under reflux for 8 h. After cooling, the reaction mixture was poured into ice−water, and the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [0.5 g, *n*-hexane−EtOAc (3:1, v/v)] to give **7a** (1.1 mg, 50%). Using a similar procedure, (*S*)-MTPA esters **7b** (1.4 mg, 64%) was obtained from **7** (1.5 mg) using (*S*)-MTPA (3.7 mg), EDC·HCl (3.1 mg), and 4-DMAP (1.0 mg).

Compound 7a: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.91, 0.93 (3H each, both d, J = 7.2 Hz, H_3-30, 29), 0.92, 0.93, 1.02, 1.04, 1.21(3H each, all s, CH_3), 1.02 (1H, m, H-5), 1.28 (1H, m, H-21), 1.29, 2.03 (1H each, both m, H_2-1), 1.58, 1.75 (1H each, both m, H_2-16), 1.58, 2.33 (1H each, both m, H_2-15), 1.68 (1H, m, H-22), 1.69, 1.92 (1H each, both m, H_2-6), 1.80 (2H, m, H_2-12), 1.95 (1H, br s, H-18), 1.95, 2.03 (1H each, both m, H_2-20), 2.10 (1H, d, m, H-8), 3.21 (1H, d, J = 10.4 Hz, H-3), 3.56 (3H, s, −OCH_3), 3.64 (1H, m, H-7), 3.70, 3.79 (1H each, both d, J = 7.3 Hz, H_2-28), 4.15 (1H, m, H-19), 5.22 (1H, br d, J = ca. 6 Hz, H-11), 5.32 (1H, m, H-2), 7.43 (3H, m, Ph-H), 7.59 (2H, m, Ph-H).

Compound 7b: ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.91, 0.94 (3H each, both d, J = 7.2 Hz, H_3-30, 29), 0.92, 0.96, 1.01, 1.05, 1.23 (3H each, all s, CH_3), 1.01 (1H, m, H-5), 1.28 (1H, m, H-21), 1.52, 2.12 (1H each, both m, H_2-1), 1.58, 1.78 (1H each, both m, H_2-16), 1.58, 2.35 (1H each, both m, H_2-15), 1.68 (1H, m, H-22), 1.68, 1.90 (1H each, both m, H_2-6), 1.83 (2H, m, H_2-12), 1.96 (1H, br s, H-18), 1.95, 2.04 (1H each, both m, H_2-20), 2.10 (1H, d, m, H-8), 3.19 (1H, d, J = 10.4 Hz, H-3), 3.56 (3H, s, −OCH_3), 3.63 (1H, m, H-7), 3.70, 3.79 (1H each, both d, J = 7.3 Hz, H_2-28), 4.16 (1H, m, H-19), 5.34 (1H, br d, J = ca. 6 Hz, H-11), 5.32 (1H, m, H-2), 7.43 (3H, m, Ph-H), 7.59 (2H, m, Ph-H).

NO Production from Macrophages Stimulated by Lipopolysaccharide. Peritoneal exudate cells were collected from the peritoneal cavities of male ddY mice, which had been injected intraperitoneally with 4% thioglycolate medium (TGC) 4 days previously, by washing with 6−7 mL of ice-cold phosphate-buffered saline (PBS), and the cells (5×10^5 cells/well) were suspended in 200 μL of RPMI 1640 supplemented with 5% fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) and precultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing the cells with PBS, and the adherent cells (more than 95% macrophages as determined by Giemsa staining) were cultured in fresh medium containing 10 μg/mL

lipopolysaccharide (LPS) and test compound (1−100 μM) for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite in the culture medium using Griess reagent.

Cytotoxicity was determined using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay. Briefly, after 20 h incubation with test compounds, MTT (10 μL, 5 mg/mL in PBS) solution was added to the wells. After a 4 h culture, the medium was removed, and 2-propanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm (reference, 655 nm). N^G-Monomethyl-L-arginine (L-NMMA) was used as a reference compound. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated using the following formula, and the IC_{50} was determined graphically ($N = 4$):

$$\text{inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

$A-C$: NO_2^- concentration (μM) [*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)].

Statistics. Values were expressed as means ± SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

Supporting Information Available: Table of data on NO production in LPS-activated mouse peritoneal macrophages for the 80% aqueous acetone extract and its EtOAc- and H_2O-soluble fractions. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Part 10 in our series Bioactive Constituents of Chinese Natural Medicines. (b) For part 9: see Tao, J.; Morikawa, T.; Toguchida, I.; Ando, S.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2002**, *10*, 4005−4012.
(2) Zou, C.; Hao, X.-J.; Chen, C.-X.; Zhou, J. *Acta Bot. Yunnan.* **1992**, *14*, 114.
(3) Zou, C.; Hao, X.-J.; Chen, C.-X.; Zhou, J. *Acta Bot. Yunnan.* **1993**, *15*, 89−91.
(4) Zou, C.; Hao, X.-J.; Chen, C.-X.; Zhou, J. *Acta Bot. Yunnan.* **1999**, *21*, 256−259.
(5) Liou, M.-J.; Wu, T.-S. *J. Nat. Prod.* **2002**, *65*, 1283−1287.
(6) Chen, Y.-Q.; Luo, Y.-R. *Youji Huaxue* **1991**, *11*, 523−524.
(7) Liou, M.-J.; Wu, P.-L.; Wu, T.-S. *Chem. Pharm. Bull.* **2002**, *50*, 276−279.
(8) Zou, C.; Hao, X.-J.; Zhou, J. *Acta Bot. Yunnan.* **1993**, *15*, 399−402.
(9) Shen, X.-Y.; Wu, H.-M.; He, M.; Hao, X.-J.; Zhou, J. *Acta Chem. Sin.* **1996**, *54*, 1194−1199.
(10) Morikawa, T.; Matsuda, H.; Toguchida, I.; Ueda, K.; Yoshikawa, M. *J. Nat. Prod.* **2002**, *65*, 1468−1474.
(11) Itokawa, H.; Qiao, Y.-F.; Takeya, K. *Chem. Pharm. Bull.* **1990**, *38*, 1435−1437.
(12) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(13) Duh, C.-Y.; Phoebe, C. H., Jr.; Pezzuto, J. M.; Kinghorn, A. D.; Farnsworth, N. R. *J. Nat. Prod.* **1986**, *49*, 706−709.
(14) Fonseca, S. F.; Campello, J. P.; Barata, L. E. S.; Rúveda, E. A. *Phytochemistry* **1978**, *17*, 499−502.
(15) The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS) with commercial samples.
(16) The ^{1}H and ^{13}C NMR spectra of **1**−**7** were assigned with the aid of homo- and heterocorrelation spectroscopy (^{1}H−^{1}H, ^{13}C−^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
(17) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(18) CS Chem 3D (ver. 5.0, Cambridge Soft Corporation, Cambridge, MA) was used to build and optimize the conformation of **1** using MOPAC (AM1) program (Figure 1).
(19) Since the absolute stereostructures of rubiarbonols A and F have not been described, this paper represents the first report of the absolute stereostructures of rubiarbonols A and F.

NP0205710