Indonesian Medicinal Plants. XVII.[1] Characterization of Quassinoids from the Stems of *Quassia indica*

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Four new quassinoids named samaderines X (1), Y (2), and Z (3), and indaquassin X (5), and a new C_{19} quassinoid glycoside, 2-*O*-glucosylsamaderine C (10), together with five known quassinoids, samaderines B (7), C (8), and E (4), indaquassin C (6), and simarinolide (9), were isolated from the stems of *Quassia indica* (Simaroubaceae), an Indonesian medicinal plant. The chemical structures of these quassinoids have been elucidated on the bases of their chemical and physicochemical properties. Samaderines X (1), Z (3), E (4), and B (7) were shown to exhibit significant growth-inhibitory activity against the cultured malarial parasite *Plasmodium falciparum* (a chloroquine- resistant K1 strain), and 1—8 were shown to exhibit *in vitro* cytotoxicity (IC_{50}: 0.04—1.00 μg/ml) against KB cells. Samaderines X (1), B (7), and C (8), as well as indaquassin X (5), exhibited inhibitory activity in the *in vitro* endothelial cell-neutrophil leukocyte adhesion assay, whereas samaderines X (1) and B (7) were found to exhibit significant anti-inflammatory activity.

Key words Indonesian medicinal plant; *Quassia indica*; Simaroubaceae; samaderine; quassinoid C_{19} glycoside

In our continuing search for new biologically active compounds from Indonesian medicinal plants,[1,3,4] we have been investigating the chemical constituents of the stems of *Quassia indica* (GAERTN.) NOOTEBOOM, a simaroubaceous tree, which was collected in the Poso area of Central Sulawesi, during our fourth expedition in Indonesia in August 1992. In this area, the decoction of the roots, bark or stems of *Quassia indica*, which is locally called Tobelo, has been administered as a folk medicine to cure malaria. In common with other simaroubaceous plants, *Quassia indica* contains various biologically active quassinoids, some of which have been extensively investigated due to their significant antitumor activities.[5] We have examined the stems of this plant and have so far isolated four new quassinoids named samaderines X (**1**),[6] Y (**2**),[6,7] and Z (**3**)[6] and indaquassin X (**5**), together with known samaderines B (**7**),[8] C (**8**),[8] and E (**4**),[9] indaquassin C (**6**),[7] and simarinolide (**9**),[10] and a new quassinoid glucoside, 2-*O*-glucosylsamaderine C (**10**). To our knowledge, **10** is the first C_{19} quassinoid glucoside that has been isolated and reported so far.

The methanol extract (3.2%) of the air-dried stems was partitioned into an ethyl acetate and water mixture to separate the ethyl acetate-soluble portion (1.0%). The water-soluble portion was further partitioned with *n*-butanol to give an *n*-butanol-soluble portion (0.8%) and a water-soluble portion (1.4%). In a preliminary bioassay of cytotoxicity against KB cells, the ethyl acetate and the *n*-butanol extracts showed a significant *in vitro* activity. Separation and purification of the ethyl acetate-soluble portion by successive silica gel and Sephadex LH-20 column chromatography followed by HPLC provided samaderines X (**1**, 0.004% from the air-dried stems), Y (**2**, 0.0004%), and Z (**3**, 0.0006%), together with **4** (0.001%), **7** (0.022%), **8** (0.017%), and **9** (0.01%). The *n*-butanol-soluble portion was also subjected to chromatographic separation to afford indaquassin X (**5**, 0.01%), indaquassin C (**6**, 0.012%), and 2-*O*-glucosylsamaderine C (**10**, 0.011%). Samaderines B (**7**),[8] C (**8**),[8] and E (**4**),[9] indaquassin C (**6**),[7] and simarinolide (**9**)[10] were found to be identical with corresponding authentic samples by comparison of their physical data.

Samaderine X (1) Samaderine X (**1**) was crystallized from a $CHCl_3$–*n*-hexane mixture as colorless prisms of mp 171—172 °C. In its FAB-MS, **1** gave a *quasi*-molecular $(M+H)^+$ ion peak at *m/z* 437 and the molecular composition was defined as $C_{22}H_{28}O_9$ from the high-resolution MS analysis. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3420 cm^{-1}, br), δ-lactone, and acetoxyl (1743 cm^{-1}, br) groups, and an enone moiety (1668 cm^{-1}), while the UV absorption maximum at 237 nm (ε = 8800) suggested the presence of an α,β-unsaturated carbonyl group in **1**.

The ^1H- and ^{13}C-NMR spectra of **1** showed several signals typically assignable to the quassinoid skeleton. In a detailed comparison of the NMR spectra of **1** with those hitherto reported for other quassinoids, we have found that the spectra of samaderine X (**1**) were very similar to those of samaderine E (**4**) except for the following signals. Thus, instead of the C-14 quaternary carbon (δ_C 83.7) and C-15 methylene [δ 3.66, 2.49 (both 1H, d, J = 19 Hz), δ_C 38.0] signals observed in samaderine E (**4**), the C-14 and C-15 of **1** were observed as methine [δ 2.50 (dd, J = 1.5, 13 Hz), δ_C 53.9] and oxymethine [δ 6.28 (br d, J = *ca.* 13 Hz), δ_C 70.2] signals, respectively. In addition, the ^1H-NMR spectrum of **1** showed a signal due to acetoxyl methyl protons at δ 2.11 (s). Finally, detailed ^1H- and ^{13}C-NMR analyses of **1**, including homo- and heteronuclear correlation spectroscopies (COSY) and correlation spectroscopy *via* long-range coupling (COLOC), have enabled us to make a complete assignment of the proton and carbon signals, as shown in Tables 1 and 2.

Furthermore, the relative stereostructure of **1** has been clarified by a nuclear Overhauser enhancement spectroscopy (NOESY) experiment as illustrated in Fig. 1, while the absolute configuration of **1** has been determined

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Table 1. ^{1}H-NMR Data for Samaderines X (**1**), Y (**2**), Z (**3**), and E (**4**), Indaquassin X (**5**), and Indaquassin C (**6**)

Proton (s)	1	2	3	4	5	6
1	4.27 (s)	4.23 (s)	4.22 (s)	4.22 (s)	4.20 (s)	4.18 (s)
3	6.03 (q, 1.5)	6.03 (q, 1.5)	6.02 (q, 1.5)	6.02 (q, 1)	6.04 (q, 1)	6.04 (br s)
5	2.98 (br d, *ca.* 12)	2.87 (br d, *ca.* 12)	2.94 (br d, *ca.* 13)	2.87 (br d, *ca.* 13)	2.97 (br d, *ca.* 11)	2.93 (br d, *ca.* 11)
6α	2.31 (ddd, 3, 3, 15)	2.28 (ddd, 3, 3, 15)	2.26 (ddd, 3, 3, 14)	2.34 (ddd, 3, 3, 14)		
6β	1.92 (ddd, 3, 12, 15)	1.95 (ddd, 3, 12, 15)	1.85 (ddd, 3, 13, 14)	1.86 (ddd, 3, 13, 14)	3.91 (dd, 3, 11)	3.95 (dd, 3, 11)
7	4.77 (t-like, *ca.* 3)	4.68 (t-like, *ca.* 3)	4.70 (t-like, *ca.* 3)	5.15 (t-like, *ca.* 2.5)	4.98 (d, 3)	5.00 (d, 3)
9	2.39 (dd, 1.5, 4)	2.19 (dd, 1.5, 5)	2.30 (br d, *ca.* 5)	2.20 (dd, 1.5, 5)	2.36 (br d, *ca.* 4.5)	2.18 (dd, 1.5, 5)
11	4.66 (br d, *ca.* 4)	4.59 (br d, *ca.* 5)	4.64 (br d, *ca.* 5)	4.50 (br d, *ca.* 5)	4.64 (br d, *ca.* 4.5)	4.57 (br d, *ca.* 5)
12	3.61 (d, 1.5)	3.56 (d, 1.5)	3.61 (br s)	3.69 (br s)	3.75 (d, 1)	3.70 (br s)
14	2.50 (dd, 1.5, 13)	2.17 (m)	2.10 (dd, 1.5, 12.5)			
15α	6.28 (br d, *ca.* 13)	3.43 (dd, 14, 19)	5.18 (d, 12.5)	3.66 (d, 19)	5.23 (s)	3.76 (d, 19)
15β		2.58 (dd, 5.5, 19)		2.49 (d, 19)		2.52 (d, 19)
17a	4.59 (d, 8)	4.62 (d, 8)	4.56 (d, 7.5)	4.57 (d, 8)	4.49 (d, 7.5)	4.54 (d, 7.5)
17b	3.56 (dd, 1.5, 8)	3.49 (dd, 1.5, 8)	3.49 (dd, 1.5, 7.5)	3.79 (dd, 1.5, 8)	3.87 (dd, 1.5, 7.5)	3.82 (dd, 1.5, 7.5)
18	1.96 (s)	1.96 (s)	1.95 (s)	1.96 (s)	2.27 (s)	2.27 (s)
19	1.18 (s)	1.18 (s)	1.17 (s)	1.15 (s)	1.23 (s)	1.23 (s)
20	1.37 (s)	1.34 (s)	1.46 (s)	1.28 (s)	1.41 (s)	1.29 (s)
OAc	2.11 (s)					

At 270 MHz, in CD_3OD, δ.

Table 2. ^{13}C-NMR Data for Samaderines X (**1**), Y (**2**), Z (**3**), and E (**4**), Indaquassin X (**5**), and Indaquassin C (**6**)

Carbon	1	2	3	4	5	6
1	83.8	83.9	83.9	83.7	83.9	83.8
2	200.8	200.7	200.7	200.6	200.3	200.3
3	126.0	126.0	126.0	125.9	127.9	127.9
4	166.3	166.3	166.3	166.3	168.8	168.7
5	45.5	44.5	45.6	45.4	50.6[a]	50.4
6	29.8	29.6	30.0	29.8	69.4	69.7
7	86.2	86.3	85.7	82.4	85.8	86.1
8	47.9	45.8	43.8[a]	45.9[b]	51.8	65.1
9	44.2	43.2	44.5	47.5	45.7	46.2
10	49.7	48.1[a]	47.5	47.5[b]	52.3	52.3
11	76.4	76.7	76.7	76.3	76.1	76.2
12	81.0	81.6	81.8	82.2	82.5	82.6
13	82.6	82.3	83.1	84.9	85.4	84.6
14	53.9	51.3	57.3	83.7	83.1	82.1
15	70.2	30.3	68.0	38.0	71.6	38.4
16	171.0	173.9	176.2	174.0	176.1	172.9
17	73.7	74.4	73.7	71.7	70.5	71.0
18	23.4	23.4	23.4	23.3	27.9	27.9
19	12.3	12.3	12.3	12.3	13.3	13.1
20	24.2	22.9	24.8	17.9	19.1	17.8
OAc	172.2					
	21.5					

At 67.8 MHz, in CD_3OD, δ_C. *a*) Observed by measuring in pyridine-d_5 at 67.8 MHz. *b*) Observed by measuring in DMSO-d_6 at 67.8 MHz.

by comparing its CD spectra with those of known compounds.[11] Thus, **1** showed two positive maxima at 315 and 220 nm and one negative maximum at 248 nm.[11] Consequently, the chemical structure of samaderine X (**1**) has been determined to be as shown in Chart 1.

Samaderine Z (3) Samaderine Z (**3**) was obtained as a white amorphous solid. The IR spectrum of **3** showed absorption bands ascribable to hydroxyl (3413 cm^{-1}), δ-lactone (1725 cm^{-1}) and enone (1661 cm^{-1}) moieties. The FAB-MS of **3** showed the *quasi*-molecular $(M+Na)^+$ ion peak at *m/z* 417, while high-resolution MS analysis revealed the molecular formula as $C_{20}H_{26}O_8$.

The ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of samaderine X (**1**) except that the signals of the acetoxyl group at C-15 in **1** were lacking and instead the signal due to a 15-carbinyl proton was observed at δ 5.18 (1H, d, J=12.5 Hz) in **3**, being upfield-shifted as compared to that observed (δ 6.28, br d, J=*ca.* 13 Hz) in **1**. These findings have led us to presume that **3** is a 15-deacetyl derivative of **1**, and this was further supported by the MS, IR and 2D-NMR analyses. Finally, treatment of samaderine X (**1**) with potassium carbonate in methanol provided samaderine Z (**3**) as a sole product. Consequently, the absolute stereostructure of samaderine Z (**3**) has been determined to be as shown in Chart 1.

Samaderine Y (2) This quassinoid was obtained as colorless prisms of mp 182—183 °C from *n*-hexane and $CHCl_3$, and the molecular formula $C_{20}H_{26}O_7$ was determined from the molecular ion peak observed in EI-MS and by high-resolution MS measurement. The IR and UV spectra of **2** showed similar absorption patterns to those of samaderine Z (**3**).

The ^{1}H- and ^{13}C-NMR spectra of **2** also showed very similar resonance patterns to those of samaderine Z (**3**), except for signals assignable to C-14 methine [δ 2.17 (m); δ_C 51.3] and C-15 methylene [δ 2.58 (dd, J=5.5, 19 Hz), 3.43 (dd, J=14, 19 Hz); δ_C 30.3] moieties. These signals have led us to presume that samaderine Y (**2**) is a 15-dehydroxyl analog of samaderine Z (**3**) (Tables 1 and 2). In the NOESY experiment on **2**, spatial correlations were found between the 19-methyl protons and the 17a-proton and between the 7β-proton and the 17b-proton and 14-proton, indicating that the C-14 methine proton has a β-configuration. The NOESY experiment on **2** also disclosed several other spatial correlations, which were very much like those observed in samaderine X (**1**).

Based on the above evidence, as well as a detailed comparison of the coupling constants (J values) in the ^{1}H-NMR data for **2** with those in **1** and **3**, samaderine Y (**2**) has been concluded to be a 15-dehydroxyl derivative of samaderine Z (**3**) as shown in Chart 1.

Indaquassin X (5) Indaquassin X (**5**) was isolated from the *n*-BuOH extract and its IR and UV spectra showed absorption bands and maxima indicative of the presence

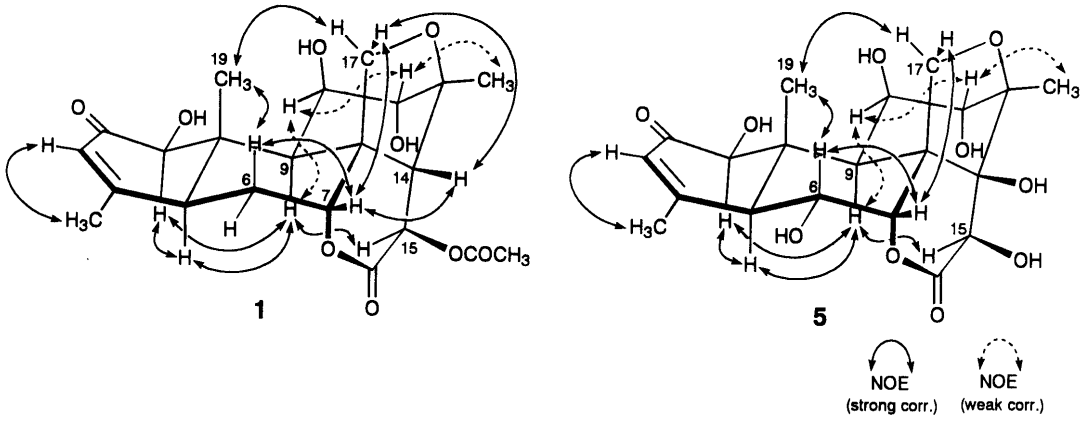
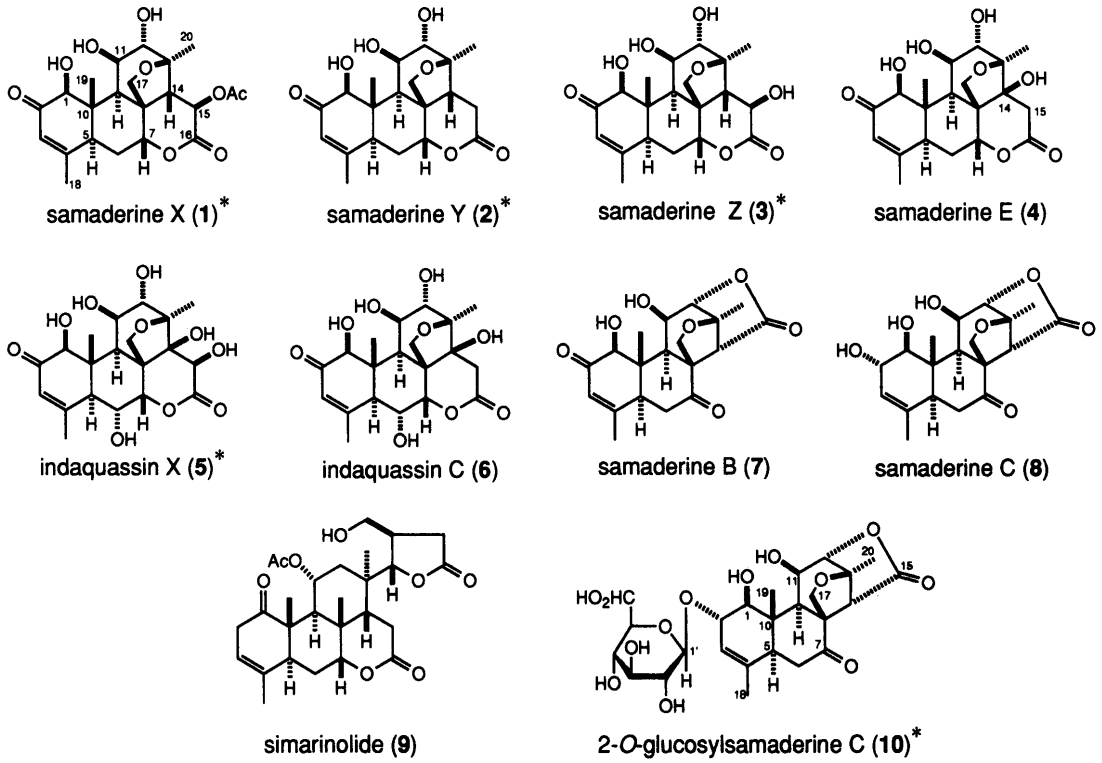


Fig. 1. NOESY Data for Samaderine X (**1**) and Indaquassin X (**5**)



* New quassinoids described in the present paper.

Chart 1

of hydroxyl, δ-lactone and enone moieties, which were similar to those in samaderine Z (**3**). From the high-resolution EI-MS of **5**, the molecular formula was determined to be $C_{20}H_{26}O_{10}$, suggesting that **5** has two more hydroxyl residues as compared to **3**.

The ^{13}C-NMR spectrum of indaquassin X (**5**) showed signals of one more quaternary carbon and one less methylene carbon than those of samaderine Z (**3**). A more precise comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** and **5** has revealed that the C-6 methylene (δ_C 30.0) and C-14 methine (δ_C 57.3) moieties in **3** are substituted with a hydroxyl residue resulting in hydroxymethine (δ_C 69.4) and hydroxyquaternary (δ_C 83.1) carbons in **5**, respectively (Table 2). From the coupling constants observed for the 5-proton, 6-proton, and 7-proton ($J_{5,6}$ = *ca.* 11 Hz and $J_{6,7}$ = 3 Hz) (Table 1), it has been presumed that **5** has a 6α-equatorial hydroxyl residue, and this was confirmed by the NOE correlation observed between the 19β-methyl protons and 6β-carbinyl proton. Furthermore, the 15β-hydroxyl configuration was confirmed by the significant NOE correlation observed between the 9α-proton and the 15α-proton (Fig. 1).

Consequently, the chemical structure of indaquassin X (**5**) has been determined to be as shown in Chart 1.

2-*O*-Glucosylsamaderine C (10) 2-*O*-Glucosylsamaderine C (**10**) was obtained as a white amorphous solid. The IR spectrum of **10** suggested the presence of hydroxyl (3370 cm^{-1}), γ-lactone (1773 cm^{-1}) and ketone (1705 cm^{-1}) moieties, while there was no indication of the existence of an α,β-unsaturated carbonyl moiety in its IR and UV spectra. These findings were reminiscent of a known C_{19} quassinoid, samaderine C (**8**), which was isolated simultaneously from the ethyl acetate-soluble portion of the stem and which lacks an α,β-unsaturated

Table 3. ^{1}H-NMR Data for Samaderines B (7), C (8), and 2-*O*-Glucosylsamaderine C (10)

Proton (s)	7	8	10
1	4.30 (d, 2.5)	4.05 (d, 7.5)	4.00 (d, 8)
2		4.56[a]	4.53 (br d, *ca.* 8)
3	6.12 (br s)	5.72 (br s)	5.71 (br s)
5	2.93 (m)	2.54 (m)	2.40 (m)
6α	2.92 (m)	2.76 (d-like, *ca.* 13)	2.69 (d-like, *ca.* 13)
6β	2.66 (dd, 13.5, 14)	2.52 (m)	2.42 (m)
9	2.55 (br d, *ca.* 3.5)	2.41 (d, 4)	2.29 (d, 5)
11	5.33 (m)	5.39 (m)	5.28 (m)
12	4.55 (br d, *ca.* 3)	4.55[a]	4.47 (d, 3.5)
14	4.00 (d, 1)	3.97 (d, 1)	3.96 (s)
17a	5.18 (d, 8)	5.31 (br d, *ca.* 8)	5.26 (d, 8)
17b	3.91 (dd, 1, 8)	3.95 (br d, *ca.* 8)	3.92 (d, 8)
18	1.71 (s)	1.61 (s)	1.35 (s)
19	1.49 (s)	1.49 (s)	1.53 (s)
20	1.53 (s)	1.49 (s)	1.47 (s)
11-OH	5.70 (d, 6.5)	5.72 (br d, *ca.* 4)	5.56 (d, 6)
Glc			
1′			5.21 (d, 8)
2′			4.13 (m)
3′			4.25 (m)
4′			4.23 (m)
5′			4.01 (m)
6′			4.44 (dd, 5.5, 12)
			4.59 (d, 12)

At 270 MHz, in pyridine-d_5, δ. a) These signals overlapped.

Table 4. ^{13}C-NMR Data for Samaderines B (7), C (8), and 2-*O*-Glucosylsamaderine C (10)

Carbon	7	8	10
1	82.1	81.5	79.9
2	197.8	72.8	84.0
3	125.3	127.0	124.6
4	160.6	133.0	133.8
5	47.7	47.9	47.3
6	39.1	39.6	39.1
7	204.9	206.1	205.9
8	61.5	61.6	61.4
9	50.2	50.5	50.0
10	47.6	43.9	43.5
11	70.8	71.2	70.8
12	84.9	85.1	84.8
13	87.9	87.8	87.6
14	56.7	57.1	56.7
15	172.8	172.8	172.9
17	75.6	76.3	76.0
18	21.5	20.7	19.9
19	10.4	11.1	10.7
20	20.7	20.2	20.5
Glc			
1′			106.4
2′			75.6
3′			78.2
4′			71.2
5′			78.4
6′			62.4

At 67.8 MHz, in pyridine-d_5, δ.

carbonyl group in its structure. The FAB-MS of **10** showed the *quasi*-molecular $(M+Na)^+$ ion peak at *m/z* 549, the composition of which was defined as $C_{25}H_{34}NaO_{12}$ by high-resolution FAB-MS analysis.

The ^{1}H-NMR spectrum of 2-*O*-glucosylsamaderine C (**10**) exhibited signals attributable to its samaderine C (**8**) moiety and one anomeric proton signal at δ 5.21 (1H, d, $J=8$ Hz), which was assigned to a β-glucosidic linkage (Table 3). In the ^{13}C-NMR spectrum of **10**, signals ascribable to a monosaccharide moiety as well as signals assignable to the aglycone moiety, being almost superimposable on those of **8**, were observed. Thus, **10** was subjected to enzymatic hydrolysis with β-glucosidase to liberate D-(+)-glucose ($[\alpha]_D$ +46.5° in H_2O) and an aglycone, which was found to be identical with samaderine C (**8**) by NMR, IR, TLC and $[\alpha]_D$ comparisons. As for the location of the glucosidic linkage in **10**, the glycosylation shifts[12] observed in the ^{13}C-NMR of **10** were informative, *i.e.* +1.6 ppm for C-1, −11.2 ppm for C-2, and +2.4 ppm for C-3.

Consequently, the sugar moiety is attached to the C-2 hydroxyl group of **10**, and thus the structure of 2-*O*-glucosylsamaderine C (**10**) can be expressed as 2-*O*-glucopyranosylsamaderine C, as shown in Chart 1.

In conclusion, we have isolated, from the stems of an Indonesian simaroubaceous plant, *Quassia indica*, four new quassinoids named samaderines X (**1**), Y (**2**), and Z (**3**) and indaquassin X (**5**), and a new C_{19} quassinoid glycoside designated 2-*O*-glucosylsamaderine C (**10**), and elucidated their structures. We also characterized five known quassinoids, samaderines B (**7**), C (**8**), and E (**4**), indaquassin C (**6**), and simarinolide (**9**), from the stems.

Among the ten quassinoids described in this paper, we have so far submitted samaderines X (**1**), Z (**3**), E (**4**), and B (**7**), and simarinolide (**9**) to an *in vitro* antimalarial test.[13] It was found that **1**, **3**, **4**, and **7** exhibit significant inhibitory activities (**1**: IC_{50} 0.014 μM, IC_{90} 0.069 μM; **3**: IC_{50} 0.071 μM, IC_{90} 0.19 μM; **4**: IC_{50} 0.056 μM, IC_{90} 0.093 μM; **7**: IC_{50} 0.21 μM, IC_{90} 0.69 μM) against the cultured parasite *Plasmodium falciparum* (a chloroquine-resistant K1 strain in human erythrocytes), as shown in Fig. 2. Furthermore, samaderines X (**1**), E (**4**), and B (**7**) were found to exhibit potent cytotoxic activities as listed in Table 5.

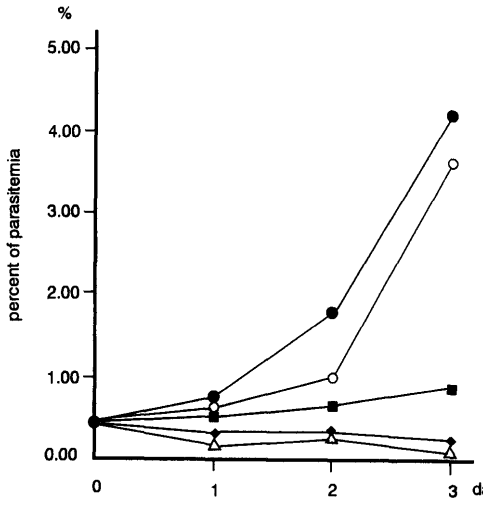


Fig. 2. Inhibition of the Growth of the Chloroquine-Resistant Strain *Plasmodium falciparum* K1, at 0.1 μM Concentration of Samples

Samaderine X (**1**) (△), samaderine Z (**3**) (■), samaderine E (**4**) (◆), samaderine B (**7**) (○), and control (●). Each point is the mean of triplicate cultures (standard deviation was always less than 10% of the mean).

Samaderines X (**1**), B (**7**), and C (**8**), and indaquassin

Table 5. *In Vitro* Cytotoxic Activities of **1**—**10** against KB Cells

Sample	IC_{50} (μg/ml)
Samaderine X (**1**)[a]	0.02
Samaderine Y (**2**)[a]	0.10
Samaderine Z (**3**)[a]	0.20
Samaderine E (**4**)	0.04
Indaquassin X (**5**)[a]	0.60
Indaquassin C (**6**)	1.00
Samaderine B (**7**)	0.07
Samaderine C (**8**)	0.40
Simarinolide (**9**)	>10
2-*O*-Glucosylsamaderine C (**10**)[a]	>10

a) Denote new quassinoids reported in the present paper.

X (**5**) showed at least 40% inhibition of neutrophil adhesion to tumor necrosis factor-α (5 JRU/ml)-stimulated endothelial cells at 1 μg/ml concentration. Furthermore, samaderines X (**1**) and B (**7**) were found to exhibit anti-inflammatory activity in Sprague-Dawley rats. Thus, the exudate volume and the number of leukocyte in the pleural cavity were measured 4 h after carrageenin injection. Samaderines X (**1**) and B (**7**), administered 0 and 60 min after carrageenin injection at a dose of 1 mg/kg, inhibited the exudate volume by 79 and 78%, respectively, and the number of leukocytes by 95 and 94%, respectively.[14]

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[4a]

Plant Materials The stems of *Quassia indica* (Simaroubaceae) were collected in the Poso area of Central Sulawesi, Indonesia, in August 1992. The plant was identified at Herbarium Bogoriense, Research and Development Centre for Biology-LIPI, Indonesia. Voucher specimens have been deposited at the Herbarium Bogoriense and the Faculty of Pharmaceutical Sciences, Fukuyama University.

Isolation of Samaderines X (1), Y (2), Z (3), Indaquassin X (5), 2-*O*-Glucosylsamaderine C (10) and Five Other Known Quassinoids The air-dried stems (2 kg) of *Quassia indica* (Simaroubaceae) were extracted with MeOH under reflux, and the solvent was evaporated off under reduced pressure to yield the MeOH extract (65 g, 3.2% from the air-dried stems). The MeOH extract was partitioned into an ethyl acetate–water (1 : 1) mixture. The ethyl acetate phase was taken and concentrated *in vacuo* to yield the ethyl acetate extract (21 g, 1.0%), while the water phase was further partitioned with *n*-BuOH. The solvent was evaporated off from each phase under reduced pressure to yield the *n*-BuOH extract (16 g, 0.8%) and the water extract (28 g, 1.4%). In a preliminary bioassay of cytotoxicity against KB cells, the ethyl acetate and *n*-BuOH extracts both showed significant activities. The ethyl acetate extract was then subjected to silica gel column chromatography (SiO_2 300 g, eluted successively with *n*-hexane : AcOEt = 2 : 1—1 : 9, AcOEt, and MeOH) to provide fr. I (1.97 g), fr. II (1.04 g), fr. III (0.24 g), fr IV (0.89 g), fr. V (1.62 g), fr. VI (2.76 g), fr. VII (1.74 g), fr. VIII (3.72 g) and fr. IX (5.43 g). During collection and evaporation of the above fractions, some precipitates were produced in fr. V and fr. VI. These precipitates from fr. V and fr. VI were separated and washed with MeOH to afford pure fine crystals, which were finally characterized as samaderine B (**7**, 447 mg, 0.022% from the air-dried stems) and samaderine C (**8**, 340 mg, 0.017%), respectively. After separation of **7**, the mother liquor of fr. V (1.17 g) was subjected to silica gel column chromatography (SiO_2 58 g, *n*-hexane : AcOEt = 2 : 7, MeOH) to give fr. V-1 (696 mg), fr. V-2 (20 mg), and fr. V-3 (259 mg). The fr. V-3 was further purified over Sephadex LH-20 (elution with MeOH) to give simarinolide (**9**, 200 mg, 0.01%). The mother liquor of fr. VI (2.42 g), after separating **8**, was also subjected to silica gel column chromatography [SiO_2 120 g, $CHCl_3$: MeOH : H_2O = 50 : 3 : 1 (lower phase)] to give fr. VI-1 (70 mg), fr. VI-2 (520 mg), fr. VI-3 (1040 mg), fr. VI-4 (600 mg), and fr. VI-5 (90 mg). The fr. VI-2 (500 mg) was further purified by silica gel column chromatography (SiO_2 25 g, *n*-hexane : AcOEt = 2 : 7) and subsequently by HPLC (Capcell Pak C_{18} SG 120 250 mm × 10 mm i.d., MeOH : H_2O = 65 : 35) to afford samaderine X (**1**, 80 mg, 0.004%) and samaderine Y (**2**, 8 mg, 0.0004%). On the other hand, fr. VII (1.70 g) was repeatedly chromatographed over silica gel [(SiO_2 90 g, $CHCl_3$: MeOH : H_2O = 40 : 3 : 1 (lower phase)] and Sephadex LH-20 (MeOH) columns, and then purified by HPLC (Cosmosil 5C_{18} AR 250 mm × 10 mm i.d., CH_3CN : H_2O = 1 : 9) to afford samaderine Z (**3**, 12 mg, 0.0006%) and samaderine E (**4**, 20 mg, 0.001%).

The *n*-BuOH extract (16 g) was also subjected to silica gel column chromatography (SiO_2 250 g, $CHCl_3$: MeOH : H_2O = 40 : 3 : 1—6 : 4 : 1, MeOH) to give fr. B-1 (0.093 g), fr. B-2 (0.250 g), fr. B-3 (0.090 g), fr. B-4 (0.170 g), fr. B-5 (0.490 g), fr. B-6 (0.520 g), fr. B-7 (0.300 g), fr. B-8 (1.370 g), and frs. B-9—B-12 (12.5 g). The fr. B-6 (100 mg) was then purified by column chromatography with Sephadex LH-20 (MeOH) and subsequently by HPLC (Capcell Pak C_{18} SG 120 250 mm × 10 mm i.d., MeOH : H_2O = 20 : 80) to afford indaquassin C (**6**, 46 mg, 0.012% from the air-dried stems). In the same manner, fr. B-8 (500 mg) gave indaquassin X (**5**, 73 mg, 0.010%) and 2-*O*-glucosylsamaderine C (**10**, 80 mg, 0.011%).

Samaderine X (**1**): Colorless prisms, mp 171—172 °C (*n*-hexane–$CHCl_3$), $[\alpha]_D$ −5.1° (c = 0.7, pyridine, 24 °C). IR ν_{max}^{KBr} cm^{-1}: 3420, 2972, 1743, 1668. UV λ_{max}^{MeOH} nm (ε): 237 (8800). ^{1}H-NMR: as given in Table 1. ^{13}C-NMR: as given in Table 2. FAB-MS *m/z*: 437 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{22}H_{29}O_9$ (M+H): 437.1802. Found: 437.1819. CD ($c = 12.0 \times 10^{-3}$, MeOH, 20 °C): $[\theta]_{315}$ +7500 (pos. max.), $[\theta]_{275}$ 0, $[\theta]_{248}$ −31500 (neg. max.), $[\theta]_{232}$ 0, $[\theta]_{220}$ +30000 (pos. max.).

Samaderine Y (**2**): Colorless prisms, mp 182—183 °C (*n*-hexane–$CHCl_3$), $[\alpha]_D$ −29.8° (c = 0.5, pyridine, 26 °C). IR ν_{max}^{KBr} cm^{-1}: 3395, 2924, 1718, 1662. UV λ_{max}^{MeOH} nm (ε): 239 (9200). ^{1}H-NMR: as given in Table 1 and NOESY correlations: between 5-H and 1-H, 9-H; between 7-H and 14-H, 17b-H; between 9-H and 1-H, 11-H, 15α-H; between 14-H and 15β-H; between 19-H_3 and 6β-H, 17a-H. ^{13}C-NMR: as given in Table 2. FAB-MS *m/z*: 379 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{20}H_{27}O_6$ (M+H): 379.1747. Found: 379.1763. CD ($c = 8.5 \times 10^{-4}$, MeOH, 20 °C): $[\theta]_{322}$ +4300 (pos. max.), $[\theta]_{293}$ 0, $[\theta]_{246}$ −18000 (neg. max.), $[\theta]_{233}$ 0, $[\theta]_{218}$ +22000 (pos. max.).

Samaderine Z (**3**): A white amorphous solid, $[\alpha]_D$ +46.1° (c = 0.3, MeOH, 25 °C). IR ν_{max}^{KBr} cm^{-1}: 3413, 2928, 1725, 1661. UV λ_{max}^{MeOH} nm (ε): 240 (8700). ^{1}H-NMR: as given in Table 1. ^{13}C-NMR: as given in Table 2. FAB-MS *m/z*: 417 $(M+Na)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{20}H_{26}NaO_8$ (M+Na): 417.1542. Found : 417.1513.

Indaquassin X (**5**): A white amorphous solid, $[\alpha]_D$ +65.8° (c = 0.9, pyridine, 20 °C). IR ν_{max}^{KBr} cm^{-1}: 3383, 2961, 2899, 1736, 1661. UV λ_{max}^{MeOH} nm (ε): 244 (8900). ^{1}H-NMR: as given in Table 1. ^{13}C-NMR: as given in Table 2. EI-MS *m/z*: 426 (M^+, 3), 408 (30), 393 (10), 111 (100). High-resolution EI-MS *m/z*: Calcd for $C_{20}H_{26}O_{10}$: 426.1523. Found: 426.1513. CD ($c = 21 \times 10^{-3}$, MeOH): $[\theta]_{354}$ −1200 (neg. max.), $[\theta]_{331}$ 0, $[\theta]_{312}$ +1600 (pos. max.), $[\theta]_{291}$ 0, $[\theta]_{280}$ −600 (neg. max.), $[\theta]_{266}$ 0, $[\theta]_{218}$ +28500 (pos. max.).

2-*O*-Glucosylsamaderine C (**10**): A white amorphous solid, $[\alpha]_D$ +49.0° (c = 0.8, pyridine, 20 °C). IR ν_{max}^{KBr} cm^{-1}: 3370, 2885, 1792, 1773, 1705. ^{1}H-NMR: as given in Table 3. ^{13}C-NMR: as given in Table 4. FAB-MS *m/z*: 549 $(M+Na)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{25}H_{34}NaO_{12}$ (M+Na): 549.1940. Found: 549.1958.

Alkaline Hydrolysis of Samaderine X (1) A solution of **1** (3 mg) in 0.1% K_2CO_3–MeOH (1.5 ml) was stirred at room temperature under an N_2 atmosphere for 1 h and then the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form). After removal of the resin by filtration, the solvent was evaporated *in vacuo*. Purification of the product by preparative TLC ($CHCl_3$: MeOH : H_2O = 30 : 3 : 1, lower phase) afforded samaderine Z (**3**) (2 mg) (identified by ^{1}H-NMR and IR comparisons).

Enzymatic Hydrolysis of 2-*O*-Glucosylsamaderine C (10) A solution of **10** (17 mg) in an acetate buffer (pH = 5.6, 1 ml) was treated with β-glucosidase (Sigma, No. G-0395, from almonds, *ca.* 10 mg) at 37 °C for 5 h. The whole reaction mixture was extracted with AcOEt, and the solvent was evaporated from the AcOEt phase under reduced pressure to afford samaderine C (**8**, 11 mg), which was identified by ^{1}H-NMR, IR, MS, and $[\alpha]_D$ comparisons. The aqueous phase, after separation of the AcOEt phase, was passed through a silica gel column (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to afford D-(+)-glucose ($[\alpha]_D$ +46.5°, c = 0.4, measured at 24 h after dissolving in H_2O).

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References and Notes

1) Part XVI: Kitagawa I., Nam I. B., Yokokawa Y., Yoshikawa M., Ohashi K., Shibuya H., *Chem. Pharm. Bull.*, **44**, 1693—1699 (1996).
2) Present address: *Faculty of Pharmaceutical Sciences, Kinki University, 3–4–1, Kowakae, Higashiosaka, Osaka 577, Japan.*
3) *a*) On taxol: Kitagawa I., Mahmud T., Kobayashi M., Roemantyo, Shibuya H., *Chem. Pharm. Bull.*, **43**, 365—367 (1995); *b*) Recent review: Kitagawa I., Shibuya H., "Phytochemistry of Plants Used in Traditional Medicine," ed. by Hostettman K., Marston A., Maillard M., Hamburger M., Claredon Press, Oxford Univ. Press, New York, 1995, pp. 335—358.
4) On simaroubaceous plants: *a*) Kitagawa I., Mahmud T., Simanjuntak P., Hori K., Uji T., Shibuya H., *Chem. Pharm. Bull.*, **42**, 1416—1421 (1994); *b*) Yoshikawa M., Harada E., Aoki S., Yamahara J., Murakami N., Shibuya H., Kitagawa I., *ibid.*, **41**, 2101—2105 (1993).
5) *a*) Anderson M. M., O'Neill M. J., Phillipson J. D., Warhurst D. C., *Planta Med.*, **57**, 62—64 (1991); *b*) Hall I. H., Liou Y. F., Lee K. H., Chaney S. G., Willingham W., Jr., *J. Pharm. Sci.*, **72**, 626—630 (1983).
6) Kitagawa I., Mahmud T., Yokota K., Kobayashi M., Shibuya H., Abstract of Papers, the 38th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, held at Niigata, Japan, Nov. 1994, p. 331. Recently, we became aware of the isolation of **2** from *Ailanthus malabarica* by Ohmoto T., *et al.*[7])
7) Aono H., Koike K., Kanako J., Ohmoto T., *Phytochemistry*, **37**, 579—584 (1994).
8) Zylber J., Polonsky J., *Bull. Soc. Chim. Fr.*, **1964**, 2016—2019.
9) Wani M. C., Taylor H. L., Wall M. E., McPhail A. T., Onan K. D., *J. Chem. Soc., Chem. Commun.*, **1977**, 295—297.
10) Polonsky J., Varon Z., Prange T., Pascard C., Moretti C., *Tetrahedron Lett.*, **22**, 3605—3608 (1981).
11) Li X., Wu L. J., Konda Y., Iguchi M., Takahasi H., Harigaya Y., Onda M., *J. Hetero. Chem.*, **26**, 493—501 (1989).
12) Tanaka O., *Yakugaku Zasshi*, **105**, 323—351 (1985).
13) Tanabe K., Izumo A., Kato M., Miki A., Doi S., *J. Protozool.*, **36**, 139—143 (1989).
14) The details will be reported in due course.