

# Cassane- and Norcassane-Type Diterpenes from *Caesalpinia crista* of Indonesia and Their Antimalarial Activity against the Growth of *Plasmodium falciparum*

Thein Zaw Linn,<sup>†</sup> Suresh Awale,<sup>†</sup> Yasuhiro Tezuka,<sup>†</sup> Arjun H. Banskota,<sup>†</sup> Surya K. Kalauni,<sup>†</sup> Faisal Attamimi,<sup>‡</sup> Jun-ya Ueda,<sup>†</sup> Puji Budi Setia Asih,<sup>§</sup> Din Syafruddin,<sup>§</sup> Ken Tanaka,<sup>⊥</sup> and Shigetoshi Kadota<sup>\*,†</sup>

*Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia, Eijkman Institute for Molecular Biology, Jalan Diponegoro 69, Jakarta 10430, Indonesia, and National Police Agency, 2-1-2 Kasumigaseki, Chiyoda-Ku, Tokyo 100-8974, Japan*

Received August 16, 2004

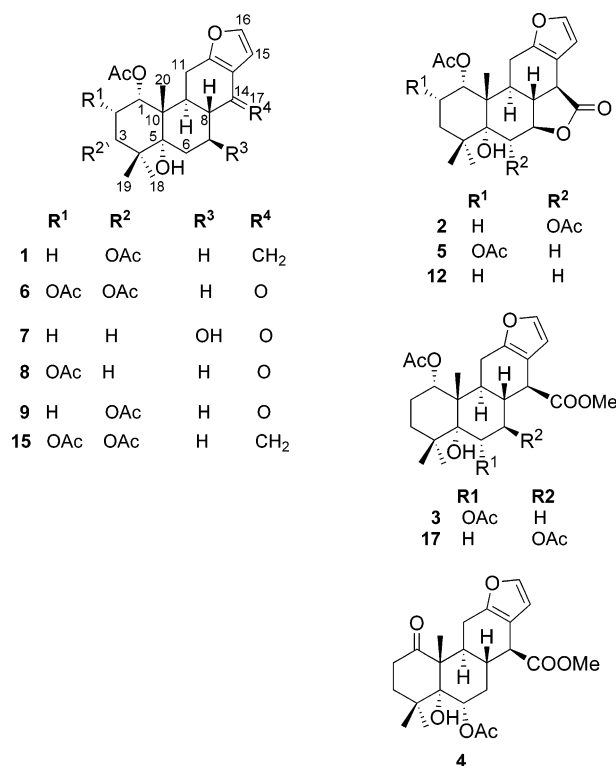
The CH<sub>2</sub>Cl<sub>2</sub> extract of the seed kernels of *Caesalpinia crista*, which exhibited promising antimalarial activity against *Plasmodium berghei*-infected mice in vivo, was examined and resulted in the isolation of seven new furanocassane-type diterpenes [caesalpinins C–G (1–5) and norcaesalpinins D and E (6, 7)] together with norcaesalpinins A–C (8–10) and 11 known compounds (norcaesalpinins A–C, 2-acetoxy-3-deacetoxycaesaldekarin e, caesalmin B, caesaldekarin e, caesalpin F, 14(17)-dehydrocaesalpin F, 2-acetoxycaesaldekarin e, 7-acetoxybonducellpin C, and caesalmin G). Their structures were determined on the basis of spectroscopic analysis. The isolated diterpenes showed significant dose-dependent inhibitory effects on *Plasmodium falciparum* FCR-3/A2 growth in vitro. Their IC<sub>50</sub> values ranged from 90 nM to 6.5 μM, and norcaesalpinin E (7) showed the most potent inhibitory activity (IC<sub>50</sub>, 90 nM).

Malaria is a parasitic disease affecting 200–300 million people in the tropical and subtropical regions of the world and claims the lives of approximately three million people each year.<sup>1</sup> The appearance of drug-resistant *Plasmodium falciparum* since 1960 has made the treatment of malaria increasingly problematic, and apparently the battle against malaria has not been successful. From the historical discovery of quinine from the *Cinchona* tree and the recent discovery of artemisinin from *Artemisia annua* L. (Asteraceae), there is anticipation that new leads may emerge from other tropical plant sources.

*Caesalpinia crista* Linn. (Fabaceae) is a popular traditional medicinal plant and is widely distributed throughout the tropical and subtropical regions of Southeast Asia. In Indonesia, it is commonly known as “Bagore”, and a decoction of the roots has been used as a tonic and for the treatment of rheumatism and backache.<sup>2</sup> Its seed kernels have been used as an antimalarial and anthelmintic.<sup>2</sup> As a part of our exploration of medicinal plant resources from Southeast Asia, we observed that the CH<sub>2</sub>Cl<sub>2</sub> extract of the seed kernels of *C. crista* possessed significant antimalarial activity in mice infected with *Plasmodium berghei*.<sup>3</sup> Further separation of the CH<sub>2</sub>Cl<sub>2</sub> extract led to the isolation of seven new diterpenes, named caesalpinins C–G (1–5) and norcaesalpinins D and E (6, 7). In this paper, we report the isolation and structure elucidation of these new diterpenes together with antimalarial activity of the isolated compounds against *Plasmodium falciparum* FCR-3/A2 growth in vitro.

## Results and Discussion

Air-dried seed kernels of *C. crista* Linn. were extracted with CH<sub>2</sub>Cl<sub>2</sub> by overnight percolation at room temperature. In a test for antimalarial activity in vivo, the CH<sub>2</sub>Cl<sub>2</sub> extract showed significant inhibition of parasitemia level



(98.6%) at a dose of 10 mg/kg in mice infected with *P. berghei*. The CH<sub>2</sub>Cl<sub>2</sub> extract was fractionated by silica gel column chromatography with a benzene/EtOAc gradient system to give nine fractions. Fractions 2–5 were further subjected to repeated silica gel column chromatography followed by normal- and reversed-phase preparative TLC to afford five new cassane-type diterpenes, caesalpinins C–G (1–5), and two additional new norcassane-type diterpenes, norcaesalpinins D and E (6, 7), together with 11 known compounds, norcaesalpinins A–C (8–10), 2-acetoxy-3-deacetoxycaesaldekarin e (11),<sup>4</sup> caesalmin B (12),<sup>5</sup> caesaldekarin e (13),<sup>6</sup> caesalpin F (14),<sup>7</sup> 14(17)-dehydrocae-

\* To whom correspondence should be addressed. Tel: 81-76-434-7625. Fax: 81-76-434-5059. E-mail: kadota@ms.toyama-mpu.ac.jp.

<sup>†</sup> Toyama Medical and Pharmaceutical University.

<sup>‡</sup> Hasanuddin University.

<sup>§</sup> Eijkman Institute for Molecular Biology.

<sup>⊥</sup> National Police Agency, Tokyo.

**Table 1.**  $^1\text{H}$  NMR (400 MHz) Data ( $\delta$ ) for Compounds **1–7** in  $\text{CDCl}_3$  ( $J$  values in parentheses)

position	1	2	3	4	5	6	7
1	4.88 t (3.1)	4.90 br s	4.84 br s		5.26 d (2.9)	5.29 d (3.5)	4.94 t (2.9)
2	2.37 m, 2.15	2.03 m, 1.17 m	1.94 m, 1.75 m	1.91 m, 1.77 m	5.35 ddd (13.1, 4.9, 2.9)	5.54 t (3.5)	1.99 m, 1.77 m
3	4.95 t (3.1)	1.81 m 1.21 m	1.27 m 1.07 m	2.57 m 2.48 m	1.96 m 1.46 dd (13.1, 4.9)	5.18 d (3.5)	1.74 m 1.20 m
6	1.93 m 1.78 m	5.61 d (9.4)	5.36 dd (11, 5.5)	5.3 dd (11.5, 5.2)	2.38 dd (12.3, 5.0) 1.72 td (12.3, 2.7)	1.66 m 1.85 m	2.15 dd (11.1, 5.8) 1.67 td (11.1, 2.1)
7	1.21 m 2.06 m	4.77 dd (11.3, 9.4)	1.90 m 1.79 m	1.94 m 1.61 dd (23.2, 11.5)	4.67 td (11.0, 5.0)	2.20 m 1.80 m	4.38 td (11.1, 5.8)
8	2.36 m	2.09 m	2.17 m	2.13 m	2.05 m	2.38 td (12.3, 5.0)	2.39 dd (12.4, 11.1)
9	2.87 td (11.2, 5.6)	2.82 td (13.5, 8.8)	2.58 td (11.5, 6.0)	2.62 m	2.75 dt (13.3, 8.5)	3.20 td (12.3, 5.0)	3.08 td (12.4, 5.1)
11	2.54 dd (16.1, 11.2) 2.30 dd (16.1, 5.6)	2.53 m	2.42 ddd (16.6, 11.5, 2.7) 2.26 dd (16.6, 6.0)	2.38 m 3.35 dd (16.4, 4.9)	2.60 dd (17.9, 8.5) 2.49 dd (17.9, 8.5)	2.66 dd (17.0, 12.3) 2.52 dd (16.0, 5.0)	2.74 dd (17, 5.1) 2.53 dd (17, 12.0)
14		3.30 d (13.5)	3.30 d (9.5)	3.30 d (9.5)	3.23 d (13.3)		
15	6.45 d (2.5)	6.59 d (1.9)	6.14 d (2.0)	6.10 d (1.9)	6.60 d (1.7)	6.64 d (1.9)	6.65 d (1.9)
16	7.23 d (2.5)	7.30 d (1.9)	7.25 d (2.0)	7.20d (1.9)	7.30 d (1.7)	7.30 d (1.9)	7.33 d (1.9)
17	4.92 d (2.7) 5.12 d (2.7)						
18	1.11 s	1.17 s	1.13 s	1.10 s	1.15 s	1.13 s	1.07 s
19	1.15 s	1.18 s	1.17 s	1.20 s	1.21 s	1.25 s	1.11 s
20	1.16 s	1.22 s	1.19 s	1.40 s	1.26 s	1.32 s	1.24 s
1-OAc	2.04 s	2.16 s	2.11 s		2.18 s	1.99 s	2.10 s
2-OAc					1.99 s	2.11 s	
3-OAc	2.03 s					2.13 s	
6-OAc		2.16 s	2.09 s	2.09 s			
5-OH	3.27 br s	3.16 s	3.10 s		3.08 d (2.7)	3.30 d (3.0)	2.81 d (2.1)
7-OH							4.70 s
17-OCH <sub>3</sub>			3.75	3.74			

salpin F (**15**),<sup>7</sup> 2-acetoxycasaldekarin e (**16**),<sup>7</sup> 7-acetoxycasaldekarin C (**17**),<sup>8</sup> and casalmin G (**18**)<sup>9</sup> (Figure S1).

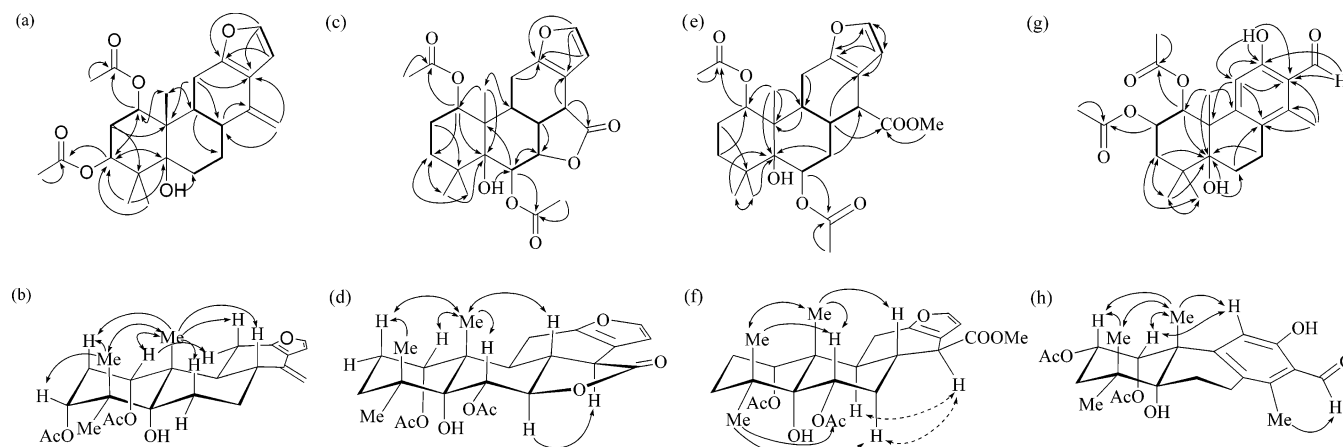
Caesalpinin C (**1**) was isolated as colorless amorphous solid with  $[\alpha]_{\text{D}}^{25} + 30.2^\circ$  ( $\text{CHCl}_3$ ), whose molecular formula was determined to be  $\text{C}_{24}\text{H}_{32}\text{O}_6$  by HRFABMS. IR absorptions at 3575 and 1735  $\text{cm}^{-1}$  indicated the presence of hydroxyl and carbonyl groups, respectively. The  $^1\text{H}$  NMR spectrum (Table 1) displayed signals corresponding to three tertiary methyls, two oxygen-substituted methines, two aliphatic methines together with two protons of a 1,2-disubstituted furan ring ( $\delta$  7.23, 6.45), and two acetyl methyls. Moreover, the  $^{13}\text{C}$  NMR spectrum (Table 2) showed six olefinic carbons ( $\delta$  151.3, 142.2, 141.5, 119.1, 106.3, 104.3) and three oxygen-substituted carbons ( $\delta$  73.8, 76.9, 76.7) together with two ester carbonyl carbons. These  $^1\text{H}$  and  $^{13}\text{C}$  NMR data were similar to those of 14(17)-dehydrocaesalpin F (**15**),<sup>7</sup> except for the number of acetyl groups. Thus, **1** was considered to be a derivative of **15** having only one acetyl group instead of two in **15**. This was confirmed by analysis of the COSY, HMQC, and HMBC spectra. The locations of the acetyl groups were determined to be C-1 and C-3, on the basis of the long-range correlations of the ester carbonyl carbon at  $\delta$  169.4 (1-OCO) with the protons at  $\delta$  2.04 (1-OCOCH<sub>3</sub>) and 4.88 (H-1) and of the ester carbonyl carbon at  $\delta$  169.3 (3-OCO) with the protons at  $\delta$  2.03 (3-OCOCH<sub>3</sub>) and 4.95 (H-3). The relative configuration was assigned on the basis of NOEs between H<sub>3</sub>-20 and H-1, H-2, H-6<sub>ax</sub>, H-8, H-11, and H<sub>3</sub>-19, and the coupling constants for H-1 ( $J = 3.1$  Hz), H-3 ( $J = 3.1$  Hz), and H-9 ( $J_{9,11\text{ax}} = 11.2$  Hz). Thus caesalpinin C was **1**.

Caesalpinins D (**2**) and G (**5**) both were colorless amorphous solids with  $[\alpha]_{\text{D}}^{25} + 63.2^\circ$  ( $\text{CHCl}_3$ ) and  $[\alpha]_{\text{D}}^{25} + 58.2^\circ$

**Table 2.**  $^{13}\text{C}$  NMR (100 MHz) Data ( $\delta$ ) for Compounds **1–7** in  $\text{CDCl}_3$ 

position	1	2	3	4	5	6	7
1	73.8	74.9	75.8	212.6	73.8	73.9	75.2
2	26.5	22.4	21.8	38.1	66.5	66.2	22.7
3	76.9	32.6	32.5	35.3	35.2	77.4	29.9
4	41.6	39.4	38.5	38.5	40.9	43.3	38.4
5	76.7	83.7	78.2	80.7	81.6	76.7	77.9
6	23.4	72.6	72.0	72.7	30.1	26.5	33.6
7	22.3	82.3	32.5	32.2	80.2	20.5	67.2
8	35.1	44.4	34.0	34.0	29.8	44.0	51.0
9	38.7	32.6	36.6	37.9	33.4	40.2	38.6
10	43.8	47.6	45.2	56.1	45.9	45.6	43.6
11	26.5	21.6	21.8	23.4	21.4	23.2	22.8
12	151.3	151.3	150.0	151.5	151.3	165.8	166.6
13	119.1	113.9	113.9	112.5	113.8	120.2	119.8
14	142.2	41.5	48.0	48.1	41.1	195.5	198.4
15	106.3	107.8	108.7	108.5	107.8	106.8	106.3
16	141.5	142.0	141.4	141.0	141.8	143.3	143.4
17	104.3	173.2	173.7	173.9	174.4		
18	23.1	29.7	24.9	28.8	28.1	23.4	27.9
19	25.4	24.3	30.9	26.8	25.4	25.5	24.9
20	18.0	17.0	16.6	15.1	17.3	18.8	18.1
1-OCOCH <sub>3</sub>	21.4	21.3	21.4		20.9	21.2	21.5
1-OCOCH <sub>3</sub>	169.4	168.6	169		168.6	169.9	169.2
2-OCOCH <sub>3</sub>					21.0	21.4	
2-OCOCH <sub>3</sub>					170.3	170.1	
3-OCOCH <sub>3</sub>	21.1					20.9	
3-OCOCH <sub>3</sub>	169.3					169.6	
6-OCOCH <sub>3</sub>		21.3	22.2	21.7			
6-OCOCH <sub>3</sub>		169.7	170.1	169.2			
17-OCH <sub>3</sub>							

( $\text{CHCl}_3$ ), respectively. Their IR spectra indicated the presence of hydroxyl,  $\gamma$ -lactone, and ester groups, while the molecular formulas were identical,  $\text{C}_{24}\text{H}_{30}\text{O}_8$ , by HR-



**Figure 1.** (a, c, e, g) Connectivities (bold lines) deduced by the COSY spectrum and key HMBC correlations (arrows) and (b, d, f, h) selected NOE (arrows) and ROESY (dashed arrows) correlations observed for **1**, **2**, **3**, and **8**.

FABMS. Their  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were also similar and resembled those of caesalmin B (**12**),<sup>5</sup> except for the presence of one more acetyl group. The locations of the additional acetoxy groups were C-6 and C-2, respectively, on the basis of the deshielding of H-6 ( $\delta$  5.61) and H-2 ( $\delta$  5.35), respectively, compared with those of **12** (H-6,  $\delta$  1.84; H-2,  $\delta$  1.92, 1.65). This was confirmed by HMBC correlations of the acetyl carbonyl carbons with the protons of acetyl methyl and of the protons of acetoxy-bearing carbons: the carbonyl carbon at  $\delta$  169.7 and protons at  $\delta$  1.99 and 5.29 in **2** and carbonyl carbon at  $\delta$  170.3 and protons at  $\delta$  1.99 and 5.35 in **5**. The relative configurations were determined on the basis of coupling constants and the results of difference NOE experiments. Thus, the structures of caesalpinins D and G were **2** and **5**, respectively.

Caesalpinin E (**3**) was isolated as a colorless amorphous solid, and its molecular formula was determined to be  $\text{C}_{25}\text{H}_{34}\text{O}_8$  by HRFABMS. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **3** displayed signals corresponding to three tertiary methyls, two oxymethines, two aliphatic methines, a 1,2-disubstituted furan ring, two acetyl methyls, and a sharp singlet due to a carbomethoxy group. These data were similar to those of 7-acetoxybonducellpin C (**17**),<sup>8</sup> suggesting that **3** was an isomer of **17** with respect to the location of the acetoxy group. Analysis of the COSY, HMQC, and HMBC spectra indicated that H-6 ( $\delta$  5.36) was deshielded and H-7 ( $\delta$  1.90, 1.79) shielded compared with H-6 at  $\delta$  2.17 and H-7 at  $\delta$  5.22 of **17**. Hence the acetoxy group of caesalpinin E (**3**) was at C-6, not at C-7, which was confirmed by the HMBC correlations (Figure 1e). The orientation at C-6 was determined to be  $\alpha$ -OAc from the coupling constants of H-6 (dd,  $J = 11, 5.5$  Hz), while that at C-14 was  $\beta$ -COOMe from the ROESY correlations of H-14 with H-7 and H-9 and the large  $J$  value (9.5 Hz) between H-14 and H-8. The relative configuration was confirmed by the results of difference NOE and ROESY experiments (Figure 1f). Thus, the structure of caesalpinin E was **3**.

Caesalpinin F (**4**) was isolated as a colorless amorphous solid whose molecular formula was  $\text{C}_{23}\text{H}_{30}\text{O}_7$  by HRFABMS. The  $^1\text{H}$  (Table 1) and  $^{13}\text{C}$  NMR (Table 2) spectra were similar to those of caesalpinin E (**3**), except for the replacement of the oxymethine signal by that of a ketone carbonyl at  $\delta_{\text{C}}$  212.6, whose location was deduced to be on C-1 on the basis of the HMBC correlations of H<sub>2</sub>-2, H<sub>2</sub>-3, H-9, and H<sub>3</sub>-20 with the ketone carbonyl carbon. The relative configuration was the same as that of **3** by difference NOE experiments. Thus, caesalpinin F (**4**) was the 1-keto derivative of **3**.

**Table 3.** Antimalarial Activity of the Isolated Compounds

compound	IC <sub>50</sub> ( $\mu\text{M}$ )
<b>1</b>	0.76
<b>2</b>	0.80
<b>3</b>	6.50
<b>4</b>	0.65
<b>6</b>	2.0
<b>7</b>	0.09
<b>8</b>	0.80
<b>9</b>	0.26
<b>10</b>	5.0
<b>11</b>	0.098
<b>12</b>	0.80
<b>13</b>	4.0
<b>15</b>	0.20
<b>16</b>	6.50
<b>17</b>	0.60
<b>18</b>	> 10

Norcaesalpinin D (**6**) was isolated as a colorless amorphous solid and had an IR spectrum similar to those of norcaesalpinins A (**8**) and B (**9**). Its molecular formula  $\text{C}_{25}\text{H}_{32}\text{O}_9$  was determined by HRFABMS. The  $^1\text{H}$  (Table 3) and  $^{13}\text{C}$  NMR (Table 2) spectra of **6** were also similar to those of **8** and **9**, but showed the presence of one more acetyl group ( $\delta_{\text{C}}$  77.4,  $\delta_{\text{H}}$  5.18) and one more oxymethine accompanied by the disappearance of signals due to one of three methylenes in **8** and **9**. The *O*-acetyl groups were attached to C-1, C-2, and C-3 by analysis of the COSY and HMQC spectra, which was further confirmed by HMBC analysis. NOE difference spectra showed that the relative configuration of **6** was the same as those of **8** and **9**. Thus, norcaesalpinin D (**6**) was 3-*O*-acetylnorcaesalpinin A.

The molecular formula of norcaesalpinin E (**7**) was  $\text{C}_{21}\text{H}_{28}\text{O}_6$  by HRFABMS. The  $^1\text{H}$  (Table 3) and  $^{13}\text{C}$  NMR (Table 2) spectra were similar to those of bonducellpin C,<sup>8</sup> except for the disappearance of signals due to one of two carbomethoxy groups, while the  $^{13}\text{C}$  NMR spectrum indicated the presence of 19 carbons including a ketone carbonyl carbon. Thus, **7** was also a norditerpene. HMBC analysis indicated that the ketone carbon was at C-14; that is, **7** was a 17-norcasane-type diterpene. NOE difference spectra showed that the relative configuration of **7** was the same as that of norcaesalpinins A (**8**) and B (**9**). Thus, norcaesalpinin E (**7**) was 17-norbonducellpin C.

Of the new diterpenes, **6** and **7** are 17-norcasane-type diterpenes, which may be biosynthesized through oxidative decarboxylation of C-17 of cassane-type diterpenes such as 14(17)-dehydrocaesalpin F (**13**).<sup>7</sup> Among the isolated compounds, 2-acetoxy-3-deacetoxycaesaldekarnin e (**11**),<sup>4</sup> 14(17)-



dehydrocaesalpin F (**15**),<sup>7</sup> 2-acetoxycaesaldekarin e (**16**),<sup>7</sup> and 7-acetybonducellpin C (**17**)<sup>8</sup> were obtained from natural sources for the first time.

The isolated compounds, except **5** and **14**,<sup>12</sup> were tested for their inhibitory activities against *P. falciparum* FCR-3/A2 growth in vitro.<sup>13</sup> All of them displayed significant dose-dependent inhibition, norcaesalpinin E (**7**) exhibiting the most potent activity with an IC<sub>50</sub> value of 90 nM (Table 3). The IC<sub>50</sub> values of **7**, **9**, **11**, and **15** were less than that reported for a well-known antimalarial drug, chloroquine (IC<sub>50</sub>, 283–291 nM).<sup>14,15</sup>

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in CHCl<sub>3</sub> solutions. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in  $\delta$  values. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as matrix. Column chromatography was performed with BW-802MH silica gel (Fuji Silysia, Aichi, Japan). Analytical and preparative TLC were carried out on precoated silica gel 60F<sub>254</sub> and RP-18F<sub>254</sub> plates (Merck, 0.25 or 0.50 mm thickness).

**Plant Material.** Seed kernels of *Caesalpinia crista* Linn. were collected at Polewali Mamasa, South Sulawesi Province, Indonesia, in September 2001 by one of the authors (F.A.). A voucher specimen (TMPW 21499) is preserved in the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

**Extraction and Isolation.** A powder of air-dried seed kernels of *C. crista* (1.0 kg) was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 L  $\times$  2) at room temperature, overnight. The CH<sub>2</sub>Cl<sub>2</sub> extract (151 g) was separated by silica gel column chromatography (8.5  $\times$  45.0 cm) with a benzene–EtOAc gradient system to give nine fractions. Fraction 1 (4.5 g) was fatty substances, as indicated by the NMR spectrum.

Fraction 2 (3.4 g) was rechromatographed (2.5  $\times$  40.0 cm) with a hexane–EtOAc gradient system to afford three subfractions (fraction 2-1, 55 mg; fraction 2-2, 109 mg; fraction 2-3, 117 mg). Subfraction 2-1 was subjected to preparative TLC with hexane–EtOAc (4:6) to give 2-acetoxy-3-deacetoxycaesaldekarin e<sup>4</sup> (**11**, 8 mg). Subfractions 2-2 and 2-3 were also separated by preparative TLC with hexane–EtOAc (4:6) to give norcaesalpinin A (**8**, 32.9 mg) and norcaesalpinin C (**10**, 5.9 mg), respectively.

Fraction 3 (1.81 g) was rechromatographed (3.5  $\times$  17.7 cm) with a hexane–EtOAc gradient system to give four subfractions. Subfractions 3-1 and 3-2 were caesalmin B<sup>6</sup> (**12**, 3.5 mg) and caesaldekarin e<sup>6</sup> (**13**, 20.3 mg), respectively. Subfractions 3-3 (28.7 mg) and 3-4 (52.7 mg) were separately subjected to preparative TLC with 1% MeOH–CHCl<sub>3</sub>; the former gave norcaesalpinin B (**9**, 19.7 mg), and the latter gave caesalpinin C (**1**, 19.7 mg) and caesalpin F<sup>7</sup> (**14**, 3.4 mg).

Fraction 4 (0.73 g) was rechromatographed (3.5  $\times$  14.8 cm) with a hexane–EtOAc gradient system to afford two subfractions (fraction 4-1, 235 mg; fraction 4-2, 490 mg). Subfraction 4-1 (235 mg) was subjected to preparative TLC with 0.5% acetone–CHCl<sub>3</sub> to give **13** (4.4 mg), 14(17)-dehydrocaesalpin F<sup>7</sup> (**15**, 7.7 mg), 2-acetoxycaesaldekarin e<sup>7</sup> (**16**, 6.0 mg), 7-acetybonducellpin C<sup>8</sup> (**17**, 4.4 mg), caesalpinin D (**2**, 0.8 mg), and norcaesalpinin D (**6**, 9.3 mg). Subfraction 4-2 (490 mg) was rechromatographed (1.5  $\times$  21.0 cm) over alumina with 1% acetone–CHCl<sub>3</sub> to afford two fractions (fraction 4-2-1, 53 mg; fraction 4-2-2, 35 mg). Each fraction was separated by preparative TLC with 0.5% acetone–CHCl<sub>3</sub>, and **2** (5.9 mg), **6** (5.5 mg), **16** (4.9 mg), and **17** (10.2 mg) were obtained from fraction 4-2-1 and caesalpinin F (**4**, 5.4 mg), **9** (2.9 mg), norcaesalpinin E (**7**, 1.4 mg), and **17** (4.6 mg) were from fraction 4-2-2.

Fraction 5 (0.45 g) was rechromatographed (3.5  $\times$  15.8 cm) with a hexane–EtOAc gradient system to afford two subfractions. Subfraction 5-1 (241 mg) was separated by preparative TLC with 1% acetone–CHCl<sub>3</sub> to give **7** (7.6 mg) and three subfractions. Subfraction 5-1-1 was purified by reversed-phase preparative TLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (2:1:1) to give caesalpinin G (**5**, 1.5 mg) and **16** (2.8 mg). Subfractions 5-1-2 and 5-1-3 were also purified by reversed-phase preparative TLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (2:1:1) to give **2** (3.8 mg) and caesalmin G<sup>9</sup> (**18**, 1.9 mg), respectively.

**Caesalpinin C (1):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +30.2° (c 0.11, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1735 cm<sup>-1</sup>; HRFABMS *m/z* 417.2314 [calcd for C<sub>24</sub>H<sub>33</sub>O<sub>6</sub> (M + H)<sup>+</sup>, 417.2277]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Caesalpinin D (2):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +63.2° (c 0.057, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1750, 1735 cm<sup>-1</sup>; HRFABMS *m/z* 447.2025 [calcd for C<sub>24</sub>H<sub>31</sub>O<sub>8</sub> (M + H)<sup>+</sup>, 447.2031]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Caesalpinin E (3):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +126.0° (c 0.02, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1735 cm<sup>-1</sup>; HRFABMS *m/z* 463.2317 [calcd for C<sub>25</sub>H<sub>35</sub>O<sub>8</sub> (M + H)<sup>+</sup>, 463.2332]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Caesalpinin F (4):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +47.0° (c 0.081, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1730, 1710 cm<sup>-1</sup>; HRFABMS *m/z* 419.2051 [calcd for C<sub>23</sub>H<sub>31</sub>O<sub>7</sub> (M + H)<sup>+</sup>, 419.2027]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Caesalpinin G (5):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +58.2° (c 0.063, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1750, 1735 cm<sup>-1</sup>; HRFABMS *m/z* 447.2009 [calcd for C<sub>24</sub>H<sub>31</sub>O<sub>8</sub> (M + H)<sup>+</sup>, 447.2019]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Norcaesalpinin D (6):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +3.3° (c 0.09, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1740, 1715 cm<sup>-1</sup>; HRFABMS *m/z* 477.2106 [calcd for C<sub>25</sub>H<sub>33</sub>O<sub>9</sub> (M + H)<sup>+</sup>, 477.2125]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Norcaesalpinin E (7):** colorless amorphous solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +84.7° (c 0.01, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3575, 1735, 1715 cm<sup>-1</sup>; HRFABMS *m/z* 377.1946 [calcd for C<sub>21</sub>H<sub>29</sub>O<sub>6</sub> (M + H)<sup>+</sup>, 377.1964]; <sup>1</sup>H and <sup>13</sup>C NMR, see Tables 1 and 2.

**Antimalarial Activity.** To determine the antimalarial activity of each isolated compound, a malaria parasite, *P. falciparum* FCR-3/A2 clone, was propagated in a 24-well culture plate in vitro in the presence of a wide concentration range of each compound, following the procedure described previously (Budimulja et al., 1997). Each compound was separately dissolved in DMSO to obtain a 10<sup>-2</sup> M stock and kept at -20 °C until used. The parasite growth was monitored by making a blood smear every day. The concentration response parasite growth data were analyzed by a linear regression function using the Sigma-plot 2000 computer program to determine the 50% inhibitory concentration (IC<sub>50</sub>). The IC<sub>50</sub> value is defined as that concentration of compound producing 50% growth inhibition relative to untreated control.

**Acknowledgment.** A part of this work was supported by a Grant-in-Aid for International Scientific Research (No. 16406002) from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

**Supporting Information Available:** Figures S1 and S2 showing the structures of the known compounds isolated from *C. crista* and possible biogenesis of norcaesalpinins C and D are available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) Omar, S.; Zhang, J.; MacKinnon, S.; Leaman, D.; Durst, T.; Philogene, B. J. R.; Arnason, J. T.; Sanchez-Vindas, P. E.; Poveda, L.; Tamez, P. A.; Pezzuto, J. M. *Curr. Top. Med. Chem.* **2003**, *3*, 133–139.
- (2) P. T. Eisai Indonesia. *Medicinal Herb Index in Indonesia*, 1st ed.; 1986; p 140.
- (3) A part of this work was reported as a preliminary communication. Banskota, A. H.; Attamimi, F.; Linn, T. Z.; Usia, T.; Tezuka, Y.; Kalauni, S. K.; Kadota, S. *Tetrahedron Lett.* **2003**, *44*, 6879–6882.
- (4) Balmain, A.; Bjamer, K.; Connolly, J. D.; Ferguson, G. *Tetrahedron Lett.* **1967**, *49*, 5027–5031.
- (5) Jiang, R.-W.; But, P. P.-H.; Ma, S.-C.; Mak, T. C. W. *Phytochemistry* **2001**, *57*, 517–521.

- (6) Kitagawa, I.; Simanjuntak, P.; Mahmud, T.; Kobayashi, M.; Fujii, S.; Uji, T.; Shibuya, H. *Chem. Pharm. Bull.* **1996**, *44*, 1157–1161.
- (7) Pascoe, K. O.; Burke, B. A.; Chan, W. R. *J. Nat. Prod.* **1986**, *49*, 913–915.
- (8) Peter, S. R.; Tinto, W. F.; Mclean, S.; Reynolds, W. F.; Yu, M. *J. Nat. Prod.* **1997**, *60*, 1219–1221.
- (9) Jiang, R.-W.; Ma, S.-C.; But, P. P.-H.; Mak, T. C. W. *J. Nat. Prod.* **2001**, *64*, 1266–1272.
- (10) Barry, J.; Kagan, H.-B.; Snatzke, G. *Tetrahedron* **1971**, *27*, 4737–4748.
- (11) Snatzke, G. *Tetrahedron* **1965**, *21*, 413–419.
- (12) The activity of **5** and **14** could not be measured due to the small amount of sample isolated.
- (13) Budimulja, A. S.; Syafruddin, D.; Tapchaisri, P.; Wilariat, P.; Marzuki, S. *Mol. Biochem. Parasitol.* **1997**, *84*, 137–141.
- (14) Ringwald, P.; Eboumbou, E. C. M.; Bickii, J.; Basco, L. K. *Antimicrob. Agents Chemother.* **1999**, *43*, 1525–1527.
- (15) Pradines, B.; Mamfoumbi, M.; Parzy, D.; Medang, O.; Lebeau, C.; Mbina, J. R. M.; Doury, J. C.; Kombila M. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 105–108.

NP0401720