Screening of Indonesian Medicinal Plant Extracts for Antibabesial Activity and Isolation of New Quassinoids from *Brucea javanica*

Subeki,[†] Hideyuki Matsuura,*[,‡] Kosaku Takahashi,[‡] Kensuke Nabeta,[‡] Masahiro Yamasaki,[§] Yoshimitsu Maede,[§] and Ken Katakura[†]

Laboratory of Parasitology, Department of Disease Control, and Laboratory of Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan, and Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

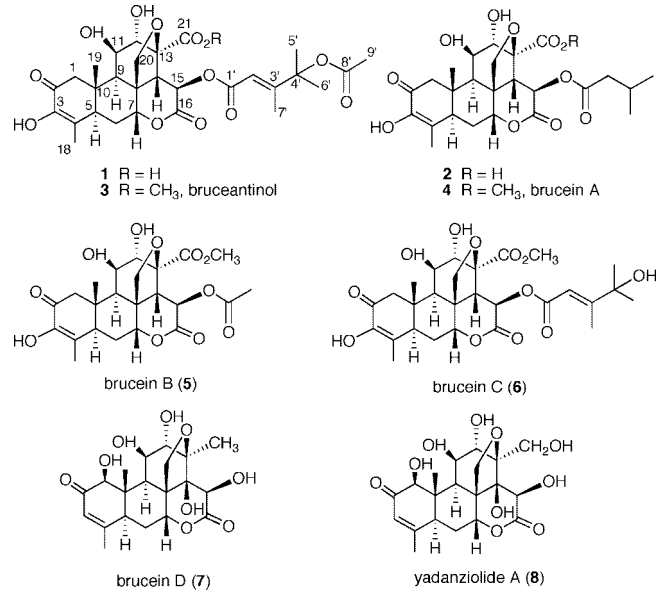
Received May 22, 2007

Boiled extracts derived from 28 Indonesian medicinal plants were screened for their antibabesial activity against *Babesia gibsoni* in vitro. Of these extracts, the fruit of *Brucea javanica* was the most active in inhibiting parasite growth at a concentration of 10 μg/mL. Bioassay-guided fractionation of the fruit extract of *Br. javanica* led to the isolation of two new quassinoids, bruceantinol B and bruceine J, and the structures of these compounds were elucidated on the basis of their spectroscopic data and by chemical transformation to known compounds. In addition, the known quassinoids bruceines A–D, bruceantinol, and yadanziolide A were isolated. Antibabesial activities were also examined in vitro, and bruceine A and bruceantinol were shown to be more potent than diminazene aceturate, a drug (IC_{50} = 103 ng/mL) used clinically against *B. gibsoni*, with IC_{50} values of 4 and 12 ng/mL, respectively.

Canine babesiosis is a tick-borne hemolytic disease in dogs caused by the intra-erythrocyte apicomplexan parasites, *Babesia gibsoni* and *Babesia canis*. The disease has been found to occur frequently in companion dogs and has become a serious problem clinically in various countries. Parasites of *Babesia* spp., as well as *Plasmodium* spp., invade red blood cells and induce severe clinical symptoms, such as fever, lethargy, hemolytic anemia, thrombocytopenia, lymphadenopathy, and splenomegaly.[1–3] Most dogs that recover from the acute stage of the disease become carriers and serve as potential sources of infection. These dogs are also at risk of recrudescent infection.[4] However, there is no successful chemotherapy for this disease, due to the limited number of useful drugs, their side effects, and other drawbacks of existing medication.[5,6] For this reason and to find new antibabesial drugs, we have screened several Indonesian medicinal plants for this purpose. Since promising antibabesial activity was found, further investigation of the boiled extract of the fruits of *Brucea javanica* (L.) Merr. (Simaroubaceae) was undertaken.

Brucea javanica, locally known as "buah makasar", is used in "Jamu" traditional folk medicine in Indonesia to treat malaria, dysentery, and cancer. The bitter principles of this plant are quassinoids, and some of them have been investigated extensively as antitumor agents.[7–9] These investigations have included clinical trials of the most potent of the *Br. javanica* quassinoids, bruceantin.[10,11] Bruceantin has been shown to possess high activity in vitro against *Entamoeba histolyca*.[12] Some quassinoids from this plant have also been found to exhibit antiplasmoidal activity against *Plasmodium falciparum*.[13,14] Despite the many phytochemical and pharmacological investigations, there are no reports on the antibabesial activity of this plant. We therefore investigated this plant and present herein data on two new quassinoids (bruceantinol B, **1**; bruceine J, **2**), along with six known related compounds (**3**–**8**) that possess antibabesial activity.

Boiling water extracts of the 23 selected Indonesian medicinal plants (Table S1, Supporting Information) were tested for in vitro antibabesial activity by assessing their ability to inhibit *B. gibsoni* growth. As shown in Table S2 (Supporting Information), all extracts exhibited, to different extents, dose-dependent inhibitory effects. At the lowest test concentration of 10 μg/mL, it was observed that the fruit extract of *Br. javanica* showed the most potent effect toward *B. gibsoni* growth (85.6% inhibition).



Silica gel column chromatography (MeOH–$CHCl_3$, 0:1, 3:97, 1:4, 7:3, 1:0) of the EtOAc-soluble portion of the boiled H_2O extract of *Br. javanica* fruit afforded five fractions. The MeOH–$CHCl_3$ (1:4) eluate gave, after silica gel column chromatography using hexane–EtOAc (1:1), 10 fractions. The fifth fraction gave bruceine A (**4**) on crystallization from MeOH, whereas the seventh, eighth, and ninth fractions afforded bruceantinol (**3**), bruceine B (**5**), and bruceine C (**6**), respectively,[15,16] on crystallization from hexane–EtOAc (9:1). The MeOH–$CHCl_3$ (7:3) eluate was subjected to silica gel column chromatography using MeOH–EtOAc (1:19) to give two new quassinoids (**1** and **2**), together with bruceine D (**7**) and yadanziolide A (**8**).[17,18] Identification of the known compounds was accomplished by comparing their spectroscopic data with those in the literature. The antibabesial activity of these compounds was evaluated against *B. gibsoni* quantitatively.

* To whom correspondence should be addressed. Tel: +81-11-706-2495. Fax: +81-11-706-2505. E-mail: matsuura@chem.agr.hokudai.ac.jp.
[†] Department of Disease Control.
[‡] Division of Applied Bioscience.
[§] Department of Veterinary Clinical Sciences.

10.1021/np070236h CCC: $37.00
Published on Web 09/27/2007

Table 1. ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) Assignments for **1** and **2**

	bruceantinol B (**1**)		bruceine J (**2**)	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	2.77 (d, 16.3)	50.2	2.76 (d, 16.2)	50.2
1b	2.46 (d, 16.0)		2.45 (d, 15.9)	
2		194.5		194.6
3		145.8		145.8
4		130.4		130.5
5	2.87 (brd, 12.8)	43.2	2.86 (brd, 12.8)	43.2
6a	2.20 (ddd, 14.5, 2.8, 2.8)	30.1	2.20 (ddd, 14.5, 2.8, 2.8)	30.1
6b	1.78 (ddd, 14.5, 14.5, 2.8)		1.78 (ddd, 14.5, 14.5, 3.0)	
7	4.76 (brs)	84.9	4.75 (brs)	85.1
8		46.3		46.0
9	2.14 (brd, 4.2)	42.8	2.10 (brd, 4.4)	42.8
10	42.2			42.1
11	4.10 (brd, 4.4)	71.1	4.09 (brd, 4.4)	70.9
12	3.85 (brs)	78.5	3.83 (brs)	78.5
13		84.0		83.4
14	3.68 (brs)	51.3	3.67 (brs)	51.6
15	6.40 (brs)	67.7	6.43 (brs)	67.4
16		169.5		169.3
18	1.73 (s)	13.4	1.73 (s)	13.4
19	1.27 (s)	16.0	1.25 (s)	16.1
20a	4.67 (d, 7.1)	74.4	4.67 (br)	74.5
	3.88 (d, 7.1)		4.01 (br)	
20b	1.73 (s)			
21		172.1		178.2
1′		166.9		173.5
2′	5.78 (s)	114.4	2.16 (d, 7.1)	43.5
3′		164.0	1.99 (m)	26.4
4′		84.0	0.90 (d, 6.2)	22.7
5′	1.48 (s)	26.5	0.89 (d, 6.4)	22.7
6′	1.47 (s)	26.7		
7′	2.01 (s)	14.9		
8′		171.8		
9′	1.93 (s)	21.7		

Compound **1** was isolated as a colorless amorphous solid. Fast atom bombardment mass spectrometry (FABMS) of **1** showed a pseudomolecular ion peak at *m/z* 591 [M − H]$^-$, indicating the molecular formula to be $C_{29}H_{35}O_{13}$. The IR spectrum displayed characteristic absorptions for hydroxyl (3407 cm^{-1}), δ-lactone and ester (1722 cm^{-1}), and α,β-unsaturated carbonyl (1685 and 1647 cm^{-1}) groups, respectively. The ^{1}H NMR spectrum showed resonances ascribable to three tertiary methyls (δ 1.48, 1.47, and 1.27), two olefinic methyls (δ 2.01, 1.73), one acetyl (δ 1.93), and one olefinic proton (δ 5.78) (Table 1). The ^{13}C NMR spectrum gave resonances of C-3 (δ 145.8), C-11 (δ 71.1), and C-12 (δ 78.5), indicating that hydroxyl groups are attached to these carbons (Table 1). Analysis of the ^{13}C NMR (δ 171.8, 166.9, 164.0, 114.4, 84.0, 26.7, 26.5, and 14.9), COSY, and HMBC spectra revealed the presence of a 4-acetoxy-3,4-dimethyl-2-pentenoyloxy group connected to C-15. Most quassinoids previously isolated from this plant possess a carbomethoxy group at C-13. However, these signals were not present in the spectra of **1**. Thus, the C-13 moiety of **1** was assigned as a free carboxylic acid. To confirm this carboxylic acid moiety, compound **1** was methylated with CH_2N_2 for 2.5 h. The usual workup was performed to give a colorless solid, which was purified by preparative thin layer chromatography (TLC) to afford **3**. Compound **3** was determined to be bruceantinol[15,16] by comparing its spectroscopic data with those reported. Therefore, compound **1** is a new quassinoid and has been named bruceantinol B.

Compound **2** was isolated as a colorless amorphous solid. FABMS of **2** showed a pseudomolecular ion peak at *m/z* 507 [M − H]$^-$, indicating the molecular formula to be $C_{25}H_{31}O_{11}$. The IR spectrum displayed characteristic absorptions for hydroxyl (3411 cm^{-1}), δ-lactone and ester (1724 cm^{-1}), and α,β-unsaturated carbonyl (1686 and 1649 cm^{-1}) groups. The ^{1}H NMR spectrum showed resonances ascribable to one tertiary methyl (δ 1.25), two secondary methyls (δ 0.90, 0.89), and one olefinic methyl (δ 1.73) (Table 1). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, with a free carboxylic acid group at C-13, except for the resonances ascribable to the ester side chain at C-15. This side chain was shown to be a 3-methylbutanoyloxy group, on the basis of analysis of the ^{13}C NMR (δ 173.5, 43.5, 26.4, 22.7, and 22.7), COSY, and HMBC spectra (Table 1). Methylation of **2** with CH_2N_2 gave bruceine A (**4**).[16] Accordingly, compound **2** is a new quassinoid and has been named bruceine J.

Table 2. Antibabesial Activity of Compounds **1–8** against *Babesia gibsoni* in Vitro[a]

compound	IC_{50} (ng/mL)
bruceantinol B (**1**)	978 ± 98
bruceine J (**2**)	742 ± 180
bruceantinol (**3**)	12 ± 3
bruceine A (**4**)	4 ± 1
bruceine B (**5**)	893 ± 223
bruceine C (**6**)	107 ± 51
bruceine D (**7**)	835 ± 100
yadanziolide A (**8**)	216 ± 46
diminazene aceturate[b]	103 ± 12

[a] Data are expressed as means ± standard deviation in triplicate analysis. [b] Positive control drug.

Although compounds **1** and **2** were isolated as naturally occurring constituents, it is possible that these compounds might be artifacts due to the use of boiling water for extraction. To investigate this, we carried out multiple reaction monitoring (MRM) using an ultra-performance liquid chromatography (UPLC)/tandem mass spectrometry (MS/MS) system with the negative-ion mode employed. Although the ionization of bruceantinol B (**1**) was not successful, the ionization of bruceine J (**2**) was accomplished. An authentic sample of **2** was subjected to the UPLC/MS/MS system, equipped with a photodiode array detector, run with the conditions described in the Experimental Section, and the peak of authentic **2** was observed at t_R = 2.45 min, together with the daughter ions *m/z* 100.4, 110.4, and 422.7 derived from *m/z* 508 in the MRM analysis. The sample that was prepared without using boiling water for extraction of raw material was subjected to the UPLC/MS/MS system, and the results of the MRM analysis are given in Figure S1 (Supporting Information). Since each corresponding daughter ion peak at *m/z* 100.4, 110.4, and 422.7 derived from bruceine J (**2**) (*m/z* 508) was observed at the same retention time (2.45 min), it was confirmed that **2** is a naturally occurring substance.

Compounds **1–8** were tested for their antibabesial activity against *B. gibsoni* in vitro.[19] *B. gibsoni* parasites treated with the test compounds (**1–8** and diminazene aceturate) demonstrated stagnation in the ring forms, including size reduction of the nucleus and disappearance of the cytoplasm. On the other hand, untreated parasites demonstrated typical petaloid forms after 3 days of incubation with clear cytoplasm in the parasite cells. Bruceine A (**4**) and bruceantinol (**3**) displayed promising activities (IC_{50} = 4 and 12 ng/mL, respectively) and exhibited more potent activity than the standard drug, diminazene aceturate (IC_{50} = 103 ng/mL) (Table 2). Bruceine C (**6**) showed almost the same antibabesial potency as diminazene aceturate, and the other isolated compounds (**1**, **2**, **5**, **7**, and **8**) exhibited IC_{50} values of <1 mg/ mL. A methyl ester carbonyl group at C-21 and a hydrophobic side chain attached at *O*-C-15 seem essential for potent activity among the quassinoids. These findings may provide the basis for the further understanding of *B. gibsoni* infections and contribute toward the development of new and effective treatments against this parasite.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 2000 series FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-500 FT-NMR spectrometer. FDMS, FABMS, HREIMS, and HRFABMS were obtained on a JEOL JMS-AX500 mass spectrometer. Column chromatography was performed on silica gel 60 (Spherical, 70–140 mesh, Kanto Chemical). Silica gel 60 F_{254} precoated plates (Merck) were used.

UPLC was performed on a Waters Acquity UPLC system, which was equipped with a binary solvent delivery system and a sample delivery system. MS was performed on a Waters Micromass Quattro Premier tandem quadrupole mass spectrometer. The LC/MS system control was achieved by using MassLynx 4.0.

UPLC Conditions. The UPLC separation was performed on a Waters Acquity ethylene-bridged (BEH) C_{18} column (1.7 μm, 2.1 × 100 mm) at 38 °C with a flow rate of 0.3 mL/min. The analytes were eluted from the column with a mixed solvent of 20% aqueous MeOH with 0.1% AcOH (solvent A) and MeOH with 0.1% AcOH (solvent B) using a linear gradient mode. From 0 s to 0.2 min, the combination of solvents A and B was 20:80, and from 0.2 min to 3.1 min, the combination of solvents A and B was linearly converted from 20:80 to 0:100. The column was finally eluted with solvent A:B (0:100) from 3.1 min to 4.0 min.

MS Conditions. All MS optimization experiments were performed in both the MS scan mode and the product scan mode. All quantifications were performed in the multiple reaction monitoring (MRM) mode. The tune page parameters and conditions for each of the MRM transitions were optimized by infusing the neat standard solution into the mass spectrometer at 10 μg/mL. To ensure that the tune page parameters were compatible with the UPLC flow during the tuning, an UPLC flow of 0.6 mL/min at solvent A:B (1:1) was introduced into the mass spectrometer at the same time by utilizing a T unit (Upchurch Scientific, Oak Harbor, WA). For MRM data collection during the LC experiments, the capillary voltage was 3.0 kV, the source temperature was 120 °C, the desolvation temperature was 350 °C, the desolvation gas flow was 800 L/h, and the cone gas flow was 50L/h. During each LC injection, the mass spectrometer was set to collect data in MRM mode using electrospray ionization (ESI) in the negative-ion mode. The MRM transitions for the analytes for bruceine J (**2**) in ESI^- were as follow: 506.90 → 100.4 using cone voltage 46.00 and collision energy 46.00, 506.90 → 110.4 using cone voltage 46.00 and collision energy 36.00, and 506.90 → 422.7 using cone voltage 46.00 and collision energy 28.00.

Plant Material. The plant materials were purchased from the Bandar Jaya traditional market, Lampung, Indonesia, in April 2005. The plants were identified by Aris Winarso at the Herbal Medicine Research and Education Centre of "Karya Tama", Lampung, Indonesia. Voucher specimens are deposited at the Laboratory of Bioorganic Chemistry, Graduate School of Agriculture, Hokkaido University, Japan. The names of these medicinal plants, their families, local name, parts used, and voucher specimen numbers for the experiments are listed in Table S1 (Supporting Information).

Plant Extract Preparation. For preliminary testing, 10 g samples of dried material of each plant part were boiled twice in 200 mL of H_2O for 30 min. The boiling solution was cooled, filtered, and freeze-dried to give a powder. The powdered extract was then tested for antibabesial activity against *B. gibsoni* in vitro.

Extraction and Isolation. Extraction and isolation of the active compounds were monitored by the assay of antibabesial activity. The dried fruits of *Br. javanica* (1 kg) were boiled twice in 5 L of H_2O for 30 min. The boiling solution was filtered and extracted with EtOAc to give aqueous and EtOAc layers. The EtOAc layer (60.6 g) was chromatographed on a silica gel column, eluted successively with $CHCl_3$ (2 L), MeOH–$CHCl_3$ (3:97, 2 L), MeOH–$CHCl_3$ (1:4, 2 L), MeOH–$CHCl_3$ (7:3, 2 L), and MeOH (2 L). The MeOH–$CHCl_3$ (1:4) eluate was evaporated to yield a residue (10.8 g), which was subjected to column chromatography on silica gel, eluted with hexane–EtOAc (1:1), to give 10 fractions (A–J). Fraction E was recrystallized from methanol to give bruceine A (**4**, 362 mg). Recrystallization of fractions G, H, and I from hexane–EtOAc (9:1) gave bruceantinol (**3**, 247 mg), bruceine B (**5**, 533 mg), and bruceine C (**6**, 362 mg), respectively. The MeOH–$CHCl_3$ (7:3) eluate was evaporated to yield a residue (4.9 g), which was subjected to column chromatography on silica gel, eluted with MeOH–EtOAc (1:19), to give six fractions (K–P). Fraction M was purified by preparative TLC, eluted with MeOH–EtOAc (1:19), to yield bruceine D (**7**, 159 mg). Fraction O was purified by preparative TLC using MeOH–EtOAc (1:9) to yield yadanziolide A (**8**, 44 mg). After removal of the solvent, fraction P (832 mg) was further subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (60:36:4) to give four subfractions (K–N). When rechromatograped on a silica gel column using $CHCl_3$–MeOH–H_2O (60:36:4), fractions L (67 mg) and M (48 mg) afforded bruceantinol B (**1**, 41 mg) and bruceine J (**2**, 36 mg), respectively.

Bruceantinol B (1): amorphous solid; $[\alpha]^{20}_D$ −41.0 (*c* 0.60, pyridine); IR (KBr) ν_{max} 3407 (OH), 1722 (δ-lactone and ester C=O), 1685, 1647 (α,β-unsaturated C=O) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; FABMS (negative) *m/z* 591 $[M - H]^-$; HRFABMS *m/z* 591.2073 $[M - H]^-$ (calcd for $C_{29}H_{35}O_{13}$, 591.2077).

Bruceine J (2): amorphous solid; $[\alpha]^{20}_D$ −73.0 (*c* 0.6, pyridine); IR (KBr) ν_{max} 3411 (OH), 1724 (δ-lactone and ester C=O), 1686, 1649 (α,β-unsaturated C=O) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; FABMS (negative) *m/z* 507 $[M - H]^-$; HRFABMS *m/z* 507.1864 $[M - H]^-$ (calcd for $C_{25}H_{31}O_{11}$, 507.1866).

Methylation of 1 and 2. An excess of an Et_2O solution of CH_2N_2, which was prepared from *N*-methyl-*N*-nitrosourea, was added to a MeOH (1 mL) solution of **1** (10 mg, 0.0169 mmol) at 0 °C. After the reaction mixture was stirred at 0 °C for 2.5 h, the solvent was evaporated to give a crude product. Purification of the crude product by preparative TLC (hexane–EtOAc, 1:4) afforded **3** (8.4 mg, amorphous solid, $[\alpha]^{20}_D$ −7.3 (*c* 1.2, pyridine). Methylation of **2** (10 mg, 0.0197 mmol) was carried out in the same manner as described above and afforded **4** (8.0 mg, amorphous solid, $[\alpha]^{20}_D$ −80.3 (*c* 0.8, pyridine). The physical and spectroscopic data of **3** and **4** were identical with those reported for bruceantinol and bruceine A, respectively.[15,16]

Extraction for MRM Experiment. The dried fruits of *Br. javanica* (0.1 kg) were extracted with 70% aqueous MeOH. The 70% aqueous MeOH layer was filtered to give a dark blown solution, which was evaporated and extracted with EtOAc to give aqueous and EtOAc layers. The volatile components of the EtOAc layer were removed under reduced pressure, and the residue was dissolved with MeOH (2 mL) and placed on a Bond Elut DEA cartridge column. The column was successively washed with MeOH (2 mL × 2) and 1 mol/L AcOH–MeOH (2 mL × 3). The volatile components of the AcOH–MeOH eluents were removed, and a portion of the residue was subjected to the UPLC/MS/MS experiment.

Antibabesial Assay. The in vitro assay against *B. gibsoni* was described in detail in a previous paper.[19] In this study, diminazene aceturate (Ganaseg) was used as control.

Acknowledgment. The authors are grateful to Mr. Kenji Watanabe and Dr. Eri Fukushi for measuring the NMR and mass spectra. Financial support and a postdoctoral fellowship (to Subeki) from the Japan Society for the Promotion of Science and financial support provided by grants-in-aid for scientific research (grant 17-05213 to Ken Katakura) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan are very much appreciated.

Supporting Information Available: Tables listing plants collected and preliminary screening data and figure showing UPLC/photodiode array and MS/MS MRM chromatograms for bruceine J. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Farwell, C. E.; LeGrand, E. K.; Cobb, C. C. *J. Am. Vet. Med. Assoc.* **1982**, *180*, 507–511.
(2) Groves, M. G.; Dennis, G. L. *Exp. Parasitol.* **1972**, *31*, 153–159.
(3) Taboada, J.; Merchant, S. R. *Vet. Clin. North Am. Small Anim. Pract.* **1991**, *21*, 103–123.
(4) Conrad, P.; Thomford, J.; Yamane, I.; Whiting, J.; Bosma, L.; Uno, T.; Holshuh, H. J.; Shelly, S. *J. Am. Vet. Med. Assoc.* **1991**, *199*, 601–605.
(5) Ruff, M. D.; Fowler, J. L.; Fernau, R. C.; Matsuda, K. *Am. J. Vet. Res.* **1973**, *34*, 641–645.
(6) Breitschwerd, E. B. In *Babesiosis: Infectious Diseases of Dog and Cat*; Greene, C. E., Ed.; B.W. Saunders: Philadelphia, 1990; pp 796–803.
(7) Lee, K. H.; Hayashi, N.; Okano, M.; Nozaki, H.; Ju-Ichi, M. *J. Nat. Prod.* **1984**, *47*, 550–551.
(8) Fukamiya, N.; Okano, M.; Miyamoto, M.; Tagahara, K.; Lee, K. H. *J. Nat. Prod.* **1992**, *55*, 468–475.
(9) Rahman, S.; Fukamiya, N.; Tokuda, H.; Nishino, H.; Tagahara, K.; Lee, K. H.; Okano, M. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 751–756.
(10) Bedikian, A. Y.; Valdivieso, M.; Bodey, G. P.; Murphy, W. K.; Freireich, E. J. *Cancer Treat. Rep.* **1979**, *63*, 1843–1847.

(11) Liesmann, J.; Belt, R. J.; Haas, C. D.; Hoogstraten, B. *Cancer Treat. Rep.* **1981**, *65*, 883–885.
(12) Wright, C. W.; O'Neill, M. J.; Phillipson, J. D.; Warhurst, D. C. *Antimicrob. Agents Chemother.* **1988**, *32*, 1725–1729.
(13) Guru, P. Y.; Warhurst, D. C.; Harris, A.; Phillipson, J. D. *Ann. Trop. Med. Parasitol.* **1983**, *77*, 433–435.
(14) O'Neill, M. J.; Bray, D. H.; Boardman, P.; Chan, K. L.; Phillipson, J. D.; Warhurst, D. C.; Peters, W. *J. Nat. Prod.* **1987**, *50*, 41–48.
(15) Polonsky, J.; Varenne, J.; Prange, T.; Pascard, C. *Tetrahedron Lett.* **1980**, *21*, 1853–1856.
(16) Phillipson, J. D.; Darwish, F. A. *Planta Med.* **1981**, *41*, 209–220.
(17) Lee, K. H.; Imakura, Y.; Sumida, Y.; Wu, R. Y.; Hall, I. H.; Huang, H. C. *J. Org. Chem.* **1979**, *44*, 2180–2185.
(18) Yoshimura, S.; Sakaki, T.; Ishibashi, M.; Tsuyuki, T.; Takahashi, T.; Honda, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2673–2679.
(19) Subeki; Matsuura, H.; Yamasaki, M.; Yamato, O.; Maede, Y.; Katakura, K.; Suzuki, M.; Trimurningsih, C.; Yoshihara, T. *J. Vet. Med. Sci.* **2004**, *66*, 871–874.

NP070236H