ORGANIC LETTERS

2007
Vol. 9, No. 18
3691−3693

Cassiarins A and B, Novel Antiplasmodial Alkaloids from *Cassia siamea*

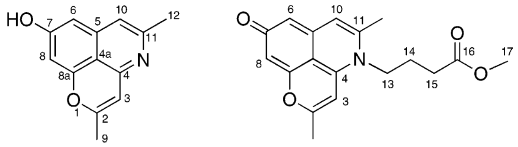
Hiroshi Morita,*,† Shiori Oshimi,† Yusuke Hirasawa,† Koichiro Koyama,† Toshio Honda,† Wiwied Ekasari,‡ Gunawan Indrayanto,‡ and Noor Cholies Zaini‡

Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa, Tokyo 142-8501, Japan, and Faculty of Pharmacy, Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

moritah@hoshi.ac.jp

Received July 11, 2007

ABSTRACT

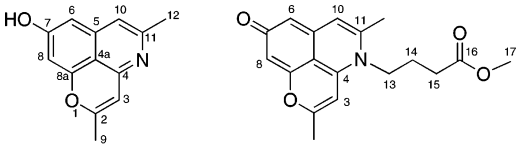


cassiarin A (**1**) cassiarin B (**2**)

Two novel alkaloids with an unprecedented tricyclic skeleton, cassiarins A (1) and B (2), have been isolated from the leaves of *Cassia siamea*, and the structures were elucidated on the basis of spectroscopic data. Cassiarin A (1) showed a potent antiplasmodial activity.

Malaria caused by parasites of the genus *Plasmodium* is one of the leading infectious diseases in many tropical and some temperate regions.[1] The emergence of widespread chloroquine-resistant and multiple-drug-resistant strains of malaria parasites leads to the need for the development of new therapeutic agents against malaria.[2]

During our studies on new lead substances against malaria from medicinal plants, cassiarins A (**1**) and B (**2**), novel aromatic alkaloids with an unprecedented tricyclic skeleton and potent antiplasmodial activity, have been isolated from the leaves of *Cassia siamea* (Leguminosae), which have been widely used in traditional medicine, particularly for the treatment of periodic fever and malaria.[3] This paper describes the isolation and structural elucidation of **1** and **2**.



cassiarin A (**1**) cassiarin B (**2**)

The leaves of *C. siamea* (300 g), which were collected at the Purwodadi Botanical Garden, Pasuruan, Indonesia (2005), were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted at pH 9 with saturated Na_2CO_3 and extracted with $CHCl_3$. $CHCl_3$-soluble alkaloidal materials were subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with $CHCl_3$/MeOH (4:1) was further purified on a silica gel column with $CHCl_3$/MeOH (9:1) to afford cassiarins A (**1**, 0.0008% yield) and B (**2**, 0.0017%) together with anhydrobarakol (0.0002%)[4] as reddish solids.

The ESIMS of cassiarin A (**1**)[5] showed a pseudomolecular ion peak at *m*/*z* 214 $(M + H)^+$, and the molecular formula $C_{13}H_{11}NO_2$ was established by HRESIMS [*m*/*z* 214.0890,

† Hoshi University.
‡ Airlangga University.

10.1021/ol701623n CCC: $37.00
Published on Web 08/09/2007

(1) Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 5274−5293.
(2) Gelb, M. H.; Hol, W. G. *Science* **2002**, *297*, 343−344. De Smet, P. A. G. N. *Drugs* **1997**, *54*, 801−840.
(3) (a) Mbatchi, S. F.; Mbatchi, B.; Banzouzi, J. T.; Bansimba, T.; Nsonde Ntandou, G. F.; Ouamba, J. M.; Berry, A.; Benoit-Vical, F. *J. Ethnopharmacol.* **2006**, *104*, 168−174. (b) Sanon, S.; Ollivier, E.; Azas, N.; Mahiou, V.; Gasquet, M.; Ouattara, C. T.; Nebie, I.; Traore, A. S.; Esposito, F.; Balansard, G.; Timon-David, P.; Fumoux, F. *J. Ethnopharmacol.* **2003**, *86*, 143−147.
(4) Bycroft, B. W.; Hassaniali-Walji, A.; Johnson, A. W.; King, T. J. *J. Chem. Soc. (C)* **1970**, *12*, 1686−1689.
(5) Cassiarin A (**1**): reddish solid; IR (KBr) ν_{max} 3420, 2940, 1660, 1620, 1395, 1370, and 1190 cm^{-1}; UV (MeOH) λ_{max} 215 nm (ϵ 19 000), 230 (sh, ϵ 14 000), 253 (ϵ 13 600), 315 (sh, ϵ 3600), 338 (ϵ 5000), and 370 (sh, ϵ 3200); ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 214 $(M + H)^+$; HRESIMS *m*/*z* 214.0890 (M + H; calcd for $C_{13}H_{12}NO_2$, 214.0868).

$(M + H)^+$, Δ +2.2 mmu]. IR absorptions implied the presence of OH and/or NH (3420 cm^{-1}) and ether (1660 and 1620 cm^{-1}) functionalities. ^1H and ^{13}C NMR data are presented in Table 1. The ^{13}C NMR spectrum revealed 13

Table 1. ^1H [δ_H (J, Hz)] and ^{13}C NMR Data (δ_C) of Cassiarins A (**1**) and B (**2**) in $CDCl_3/CD_3OD$ (1:1) at 300 K

	1		**2**	
2		161.5		168.0
3	6.03 (1H, s)	103.7	6.74 (1H, s)	97.3
4		150.6		148.6
4a		111.5		109.3
5		138.8		136.5
6	6.46 (1H, s)	102.9	6.48 (1H, s)	107.9
7		164.6		174.6
8	6.48 (1H, s)	100.7	6.60 (1H, s)	105.1
8a		156.4		156.7
9	2.20 (3H, s)	20.1	2.43 (3H, s)	21.0
10	6.70 (1H, s)	113.7	6.78 (1H, s)	117.1
11		149.5		141.4
12	2.34 (3H, s)	22.7	2.50 (3H, s)	20.2
13			4.10 (2H, t, J = 8.5)	48.0
14			1.98 (2H, m)	23.8
15			2.57 (2H, t, J = 6.3)	30.3
16				174.4
17			3.72 (3H, s)	52.3

carbon signals due to seven sp^2 quaternary carbons, four sp^2 methines, and two methyls. Among them, five quaternary carbons (δ_C 149.5, 150.6, 156.4, 161.5, and 164.6) were ascribed to those bearing a nitrogen or an oxygen atom.

Two partial structures, **a** (from C-10 to C-12) and **b** (from C-9 to C-2 and C-3), were deduced from $^1H-^1H$ COSY analysis of **1** in $CDCl_3-CD_3OD$ (1:1) (Figure 1). The

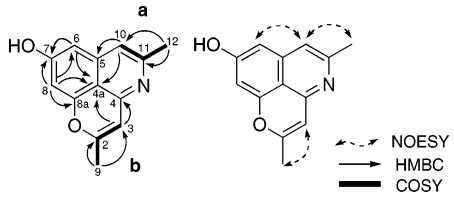


Figure 1. Selected 2D NMR correlations for cassiarin A (**1**).

presence of a tetrasubstituted benzene ring with a hydroxyl group was supported by HMBC correlations as shown in Figure 1. HMBC correlations for H-10 of C-4a (δ_C 111.5) and C-5 (δ_C 138.8) and for H-3 of C-4 (δ_C 150.6) and C-4a gave rise to the connectivity of partial structures **a** and **b** through a nitrogen and C-4 atoms. Connection between partial structure **a** and the benzene ring could be assigned by a NOESY correlation between H-6 and H-10. In addition, the presence of an ether linkage between C-2 (δ_C 161.5) and C-8a (δ_C 156.4) to form a pyran ring was also assigned as shown in Figure 1. Thus, cassiarin A (**1**) was concluded to be a unique tricyclic ring system consisting of a 3-methylisoquinolin-6-ol coupled with a 2-methyl-4*H*-pyran ring at C-4, C-4a, and C-8.

The ESIMS of cassiarin B (**2**)[6] showed a molecular ion peak at m/z 314 $(M + H)^+$, and the molecular formula was inferred as $C_{18}H_{19}NO_4$ by HRESIMS [m/z 314.1387 $(M + H)^+$, Δ −0.6 mmu]. The IR spectrum was indicative of the presence of conjugated ketone (1650 cm^{-1}) and ester (1730 cm^{-1}) functionalities. The ^{13}C NMR spectra of **2** at 300 K in $CDCl_3/CD_3OD$ (1:1) (Table 1) revealed 18 carbon signals due to two carbonyls, six sp^2 quaternary carbons, four sp^2 methines, three methylenes, and three methyls. Among them, six quaternary carbons (δ_C 109.3, 136.5, 141.4, 148.6, 156.7, and 168.0), one methylene (δ_C 48.0; δ_H 4.10), and one methyl (δ_C 52.3; δ_H 3.72) were ascribed to those bearing a nitrogen or an oxygen atom.

The $^1H-^1H$ COSY and HOHAHA spectra revealed connectivities of three partial structures, **a** (C-10 to C-12), **b** (C-9 to C-2 and C-3), and **c** (C-13 to C-15), as shown in Figure 2. The ^{13}C NMR data of **2** including DEPT experi-

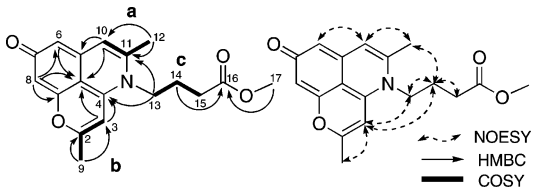


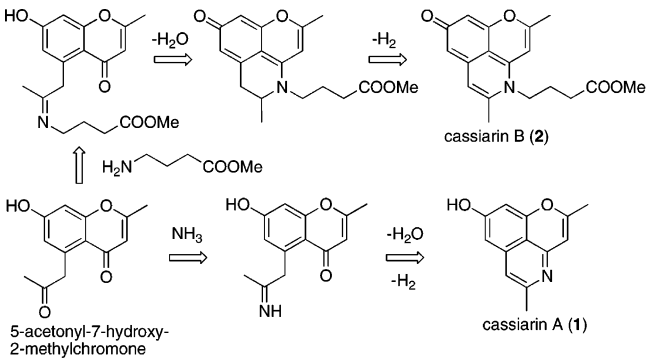
Figure 2. Selected 2D NMR correlations for cassiarin B (**2**).

ments revealed that the chemical shifts of C-3, C-4, and C-11 [δ_C 97.3, 148.6, and 141.4, respectively] were shifted to a higher field as compared with those [δ_C 103.7, 150.6, and 149.5, respectively] of **1**. Unit **c** showing HMBC correlations for H_2-14 and H_3-17 of C-16 indicated the presence of a methyl butanoate (C-13 to C-17). The connectivity from C-13 to C-4 and C-11 through a nitrogen atom was implied by long-range correlations for H_2-13 to C-4 and C-11 (Figure 2). In addition, the ^1H and ^{13}C signals at 6- and 8-positions in the cyclohexa-2,5-dienone functionality were observed at a lower field due to the deshielding effect (Table 1). 2D NMR data of **2** including the $^1H-^1H$ COSY, HSQC, and HMBC spectra corroborated well with those of the isoquinolin-6(2*H*)-one form of **1**. The structure with N-substituted methyl butanoate was well supported by NOESY correlations (Figure 2). Thus, cassiarin B was concluded to be **2**, consisting of a 3-methyl-6-oxoisoquinolin butanoate and a 2-methylpyran ring, whose skeleton was the same as that of cassiarin A (**1**).

A plausible biogenetic pathway for cassiarins A (**1**) and B (**2**) is proposed as shown in Scheme 1. Cassiarin A (**1**)

(6) Cassiarin B (**2**): reddish solid; IR (KBr) ν_{max} 2950, 1730, 1650, 1600, 1440, and 1170 cm^{-1}; UV (MeOH) λ_{max} 220 nm (ϵ 9400), 235 (ϵ 8800), 245 (ϵ 9400), 255 (ϵ 9700), 275 (ϵ 4400), 317 (ϵ 3800), and 372 (ϵ 2500); ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 314 $(M + H)^+$; HRESIMS m/z 314.1387 (M + H; calcd for $C_{18}H_{20}NO_4$, 314.1393).

Scheme 1. Plausible Biogenetic Path for Cassiarins A (**1**) and B (**2**)



might be derived through an imine intermediate of 5-acetonyl-7-hydroxy-2-methylchromone[7] followed by cyclization with the ketone of chromone as shown in Scheme 1, whereas cassiarin B (**2**) might be derived through intracyclization of the imine intermediate produced by 5-acetonyl-7-hydroxy-2-methylchromone and methyl 3-aminopropanoate.

Cassiarin A (**1**) showed promising in vitro antiplasmodial activity against *Plasmodium falciparum* (IC_{50} 0.005 μg/mL), whereas cassiarin B (**2**) showed a moderate activity (IC_{50} 6.9 μg/mL).[8] Cassiarin A (**1**) showed a good selectivity index with regard to the cytotoxicity on P388 cells (IC_{50} 35 μg/mL), and cassiarin B (**2**) was less cytotoxic (IC_{50} >100 μg/mL).

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and grants from Takeda Science Foundation and The Open Research Project. We also acknowledge the financial support provided by Assessment Service Unit, Faculty of Pharmacy, Airlangga University.

Supporting Information Available: 1D and 2D NMR spectra for cassiarins A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL701623N

(7) Ingkaninan, K.; IJzerman, A. P.; Verpoorte, R. *J. Nat. Prod.* **2000**, *63*, 315−317.

(8) Trager W.; Jensen, J. B. *Science* **1976**, *193*, 673−675.