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

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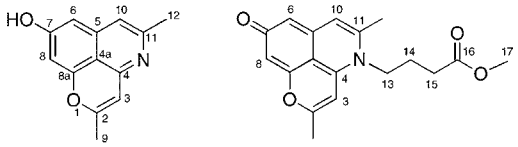
ABSTRACT

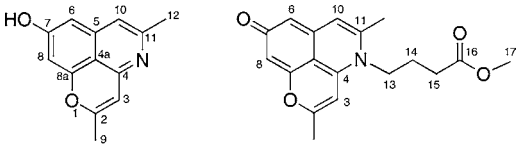


cassiarin A (**1**) cassiarin B (**2**)

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,

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(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

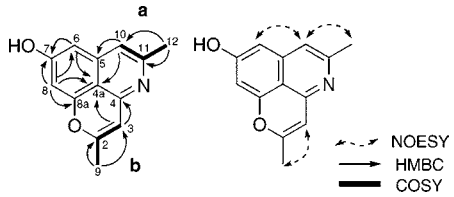


Figure 1. Selected 2D NMR correlations for cassiarin A (**1**).

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-

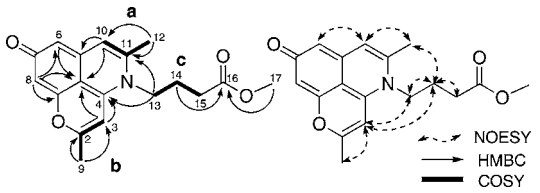


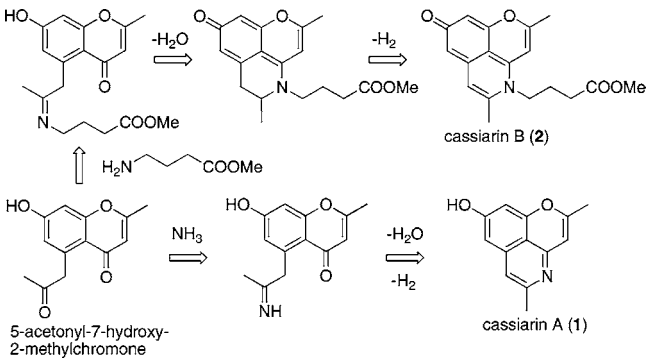
Figure 2. Selected 2D NMR correlations for cassiarin B (**2**).

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).

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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.

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