Total Synthesis of A Novel Tetracyclic Alkaloid, Cassiarin F from the Flowers of *Cassia siamea*

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ABSTRACT
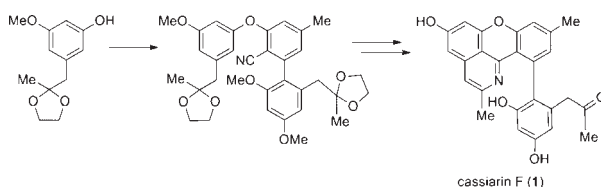
A novel alkaloid, cassiarin F (1), which showed potent antiplasmodial activity against *Plasmodium falciparum* in vitro, was isolated from the flowers of *Cassia siamea*, and its structure was elucidated on the basis of 2D NMR analyses. A total synthesis of 1 was also achieved by employing the Suzuki coupling constructing biaryl unit, nucleophilic aromatic substitution, and Houben−Hoesch type ring construction as key steps.

Cassia siamea Lam. (Leguminosae) has been used widely in traditional medicine, particularly for treatment of periodic fever and malaria in Indonesia.[1] Recently we isolated cassiarin A (**2**),[2] an alkaloid with an unprecedented tricyclic skeleton exhibiting potent antiplasmodial activity, and chrobisiamone A,[3] a bischromone, from the leaves of *C. siamea*.

Owing to the attractive biological activity and the unique structural feature of cassiarin A, which showed *in vivo* antimalarial[4] and vasorelaxant activities,[5] we investigated its total synthesis and succeeded in establishing an efficient synthetic strategy.[6] In 2009 we reported the isolation of new types of dimeric alkaloids, cassiarins D and E from the flowers of *C. siamea*.[7]

cassiarin F (1)   cassiarin A (2)

Further investigation on extracts from the flowers of *C. siamea* resulted in the isolation of a novel tetracyclic alkaloid, cassiarin F (**1**). In this paper, we would like to

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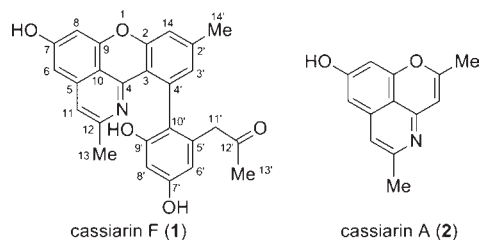[‡] Airlangga University.

[§] Tokushima Bunri University.

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report the structure elucidation based on spectroscopic analyses and a total synthesis of **1**.

Cassarin F (**1**)[8] was obtained as a yellow amorphous solid, and its molecular formula was established to be $C_{26}H_{21}NO_5$ by HRESITOFMS analysis. In addition to ^{1}H and ^{13}C NMR data (Table 1), 2D NMR correlations indicated that **1** had a cassiarin A type skeleton with a biaryl moiety, which is the first example of *Cassia* alkaloid (Figure 1). The biaryl moiety of **1** was considered to be racemic from its small optical rotation value and CD spectrum, in which no Cotton effect was observed. Cassiarin F (**1**) showed potent antiplasmodial activity against the chloroquine-sensitive *P. falciparum* strain 3D7 (IC_{50} 3.3 μM), while showing no cytotoxicity against human blood premyelocytic leukemia (HL-60, > 50 μM).

Figure 1. Selected 2D NMR correlations for cassiarin F (**1**) in pyridine-d_5.

Table 1. [1]H [δ_H (*J*, Hz)] and ^{13}C NMR Data [δ_C] of Cassiarin F (**1**) in Pyridine-d_5 at 300 K

Position		
2		155.7
3		119.1
4		148.3
5		139.9
6	6.91 (1H, d, 2.2)	101.2
7		161.8
8	6.98 (1H, d, 2.2)	100.1
9		154.1
10		111.6
11	6.89 (1H, s)	114.2
12		153.1
13	2.26 (3H, s)	24.6
14	7.01 (1H, d, 1.0)	117.0
2'		141.2
3'	7.09 (1H, d, 1.0)	130.5
4'		138.0
5'		134.0
6'	6.99 (1H, d, 2.4)	108.6
7'		158.7
8'	7.08 (1H, d, 2.4)	102.6
9'		157.2
10'		120.0
11'a	3.60 (1H, d, 13.3)	49.8
11'b	3.65 (1H, d, 13.3)	
12'		206.0
13'	1.97 (3H, s)	29.2
14'	2.20 (3H, s)	21.0

In order to confirm its unique tetracyclic structure, a total synthesis of cassiarin F (**1**) was undertaken. Our retrosynthetic analysis of **1** is outlined in Figure 2. In this approach, the two heterocyclic rings would be constructed at a late stage in the synthesis, because of their susceptibility to acidic and basic reaction conditions.

Making the two disconnections shown to **1** led to **3** as the key intermediate for the Houben–Hoesch type ring closure. Two further disconnections generated the three building blocks (phenol **4**, benzonitrile **5**, and boronic acid **6**) needed for the assembly of **3** (Figure 2). It was envisaged that a Suzuki coupling of **5** with **6** would lead to formation of the desired biphenyl unit.[9] Then selective nucleophilic aromatic substitution of the resulting halogenated biaryl derivative with **4** would give the key intermediate **3**.
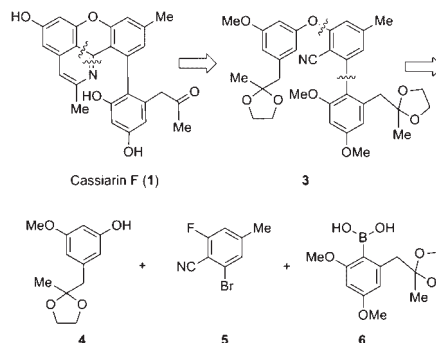
Figure 2. Retrosynthetic analysis for cassiarin F (**1**).

Our synthesis began with preparation of the boronic acid **6**, which was readily accessible from known propanone **7**.[10]

Ketalization of **7** with ethylene glycol and subsequent bromination with *N*-bromosuccinimide (NBS) gave bromide **8** in good yield. The boronic ester was obtained by halogen–metal interchange with *n*-butyllithium and trapping the generated compound with triisopropyl borate. The desired boronic acid **6** was obtained by hydrolysis of the ester with hydrochloric acid. Suzuki coupling of **6** with **5**[11] was carried out in the usual manner to provide the biaryl **9** in 84% yield (Scheme 1).

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(8) Cassiarin F (**1**): yellow amorphous solid; IR (KBr) ν_{max} 3430, 3270, 1710, and 1600 cm^{-1}; UV (MeOH) λ_{max} 202 (ε 13000), 245 (ε 3200) and 290 (ε 1400) nm; ^{1}H and ^{13}C NMR (Table 1); ESIMS (pos.) *m/z* 428 (M + H)$^+$; HRESITOFMS *m/z* 428.1473 (M + H)$^+$, calcd for $C_{26}H_{22}NO_5$ 428.1498.
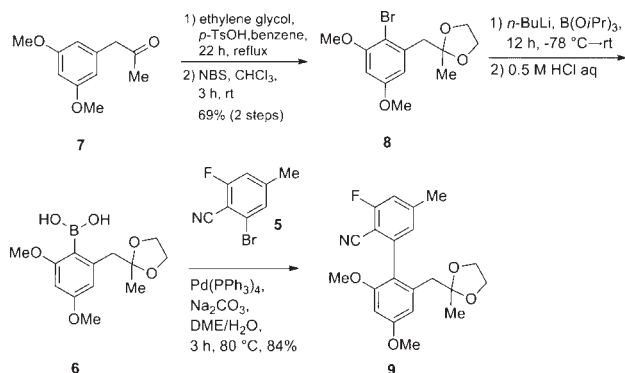
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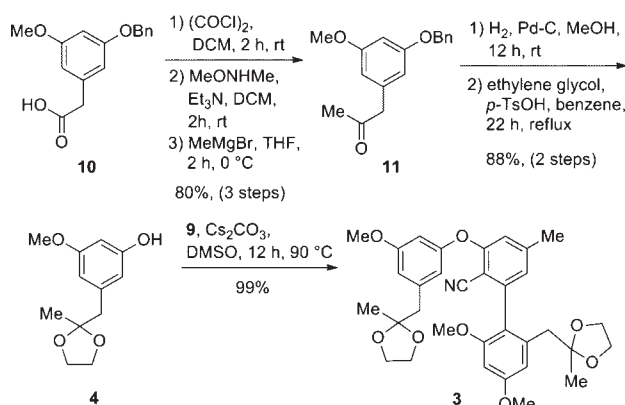
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Scheme 1. Synthesis of Key Biaryl Intermediate 9

Our first trial, starting from **3** in concentrated sulfuric acid, expectedly only led to extensive decomposition. Fortunately, treatment of **3** with sulfuric acid in acetic acid produced the tetracyclic core **12**. Trials with different combinations of acids or solvents are summarized (Scheme 3). The best result was obtained with the reaction conditions of entry 5. Thus a 30% yield of the tetracyclic core **12** with a side product **13**[14] was obtained using sulfuric acid and acetic acid in a ratio of 1:1.

Scheme 3. Construction of the Tetracyclic Core of Cassiarin F (**1**) via a Houben−Hoesch Type Ring Closure[a]

entry	acid or Lewis acid	solvent	ratio[a]	time (h)	yield (%)[b]	
					12	13
1	H_2SO_4	AcOH	1:4	12	12	0
2	H_2SO_4	CH_2Cl_2	1:1	1	12	0
3	H_2SO_4	AcOH	2:1	1	23	17
4	TfOH	AcOH	2:1	1	17	19
5	H_2SO_4	AcOH	1:1	1	30	10
6	H_2SO_4	AcOH	9:10	1	26	7
7	$BF_3 \cdot OEt_2$ (1 equiv)	CH_2Cl_2		3	0	0
8	$TiCl_4$ (1 equiv)	CH_2Cl_2		3	0	0

[a] Concentration; 1 mL for 10 mg of **3**. [b] Isolated yields.

The phenol **4** was readily accessed in a five-step process from the known carboxylic acid **10**.[12] Treatment of **10** with oxalyl chloride provided the corresponding acyl chloride, which was directly reacted with *N,O*-dimethylhydroxylamine in the presence of triethylamine to give the Weinreb amide. The resulting Weinreb amide was treated with MeMgBr to generate the desired methyl ketone **11** in 80% yield from **10**. Debenzylation of **11** via Pd-catalyzed hydrogenation, followed by ketalization of carbonyl group with ethylene glycol, gave the desired phenol **4**. Selective nucleophilic aromatic substitution of **9** with **4** in the presence of cesium carbonate in DMSO gave **3** in excellent yield (Scheme 2).

The tris-*O*-demethylation of aryl methyl ether **12** was achieved by treatment of iodocyclohexane in DMF.[15]

Spectroscopic data (^{1}H and ^{13}C NMR, MS, and IR) and TLC behavior of the synthesized compound were identical to those of the authentic sample of isolated compound.

In summary, we have isolated a novel alkaloid, cassiarin F (**1**), a hybrid compound consisting of cassiarin A and a biphenyl unit with an acetonyl moiety. The occurrence of a tetracyclic polysubstituted skeleton with a biaryl unit is very rare. Cassiarin F (**1**) might be biosynthetically produced by a transformation of a

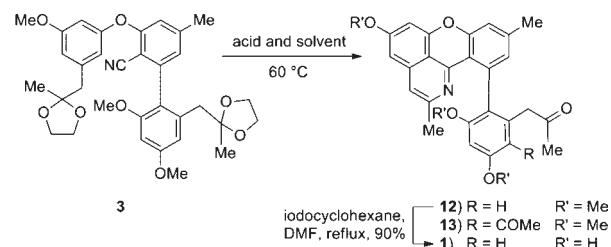**Scheme 2.** Synthesis of Key Intermediate **3** for Houben−Hoesch Type Ring Closure

Having the desired cyano compound in hand, our attention was focused on construction of the two heterocyclic rings as follows. It has been recognized that both a Brønsted acid and Lewis acid generate ketimine species from the corresponding nitriles in a Houben−Hoesch type cyclization.[13] Therefore, treatment of **3** with acid could lead to the formation of two heterocyclic rings, simultaneously.

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(14) **13** should be produced by the Friedel−Crafts type reaction with acetic acid. **13** was obtained in the way we predicted by the treatment of **12** with sulfuric acid in acetic acid. The position of methyl ketone of **13** was determined by NOESY correlations.

(15) (a) Zuo, L.; Yao, S.; Wang, W.; Duan, W. *Tetrahedron Lett.* **2008**, *49*, 4054–4056. (b) Since **12** has a labile functionality, methyl ketone, acidic conditions utilizing Lewis acids such as BBr₃ cannnot be applied for this purpose. The use of Lewis acids led to significant decomposition of the material.

bischromone, chrobisiamone A. We have also achieved the total synthesis of cassiarin F (**1**) (8 steps, 10% overall yield) by combination of the Suzuki coupling reaction, nucleophilic aromatic substitution, and a Houben−Hoesch type ring construction as key reactions. As such we have confirmed its structure; the strategy with a Houben−Hoesch type ring construction seems to be an innovative approach to the synthesis of the tetracyclic core of **1**.

Supporting Information Available. Experimental procedures for isolation of cassiarin F; preparation of compounds **1**, **3**, **4**, **6**, **8**, **9**, **11**, **12**, and **S1**; copies of ^{1}H, ^{13}C NMR, and 2D spectral data for natural cassiarin F; and ^{1}H and ^{13}C NMR spectral data for compounds **1**, **3**, **4**, **8**, **9**, **11**, **12**, **13**, and **S1**. This material is available free of charge via the Internet at http://pubs.acs.org.