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ANTI-MALARIAL ACTIVITIES OF ACYLATED BRUCEOLIDE DERIVATIVES

Nobutoshi Murakami,[a] Takashi Umezome,[a] Taifo Mahmud,[a] Masanori Sugimoto,[a] Motomasa Kobayashi,*,[a] Yusuke Wataya,[b] and Hye-Sook Kim[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1-6 Yamada-oka, Suita, Osaka 565, Japan and Faculty of Pharmaceutical Sciences, Okayama University,[b] Tsushima-naka 1-1-1, Okayama 700, Japan.

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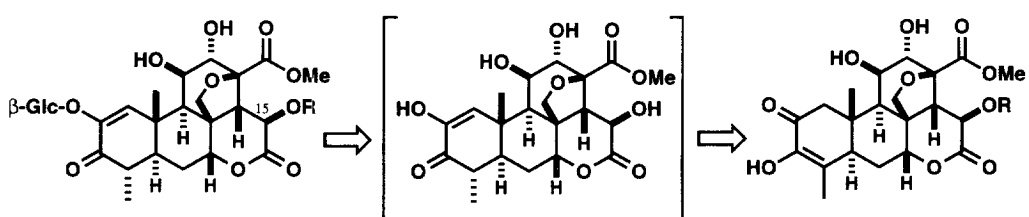
Abstract : Several *O*-acylated derivatives of bruceolide (**2**) were synthesized and their anti-malarial activities together with selective toxicities were examined. It was found that 3,15-di-*O*-acetyl- (**3c**), 3,15-di-*O*-propionyl- (**3d**) and 15-*O*-propionylbruceolide (**3b**), as well as bruceine B (**3a**), exhibited potent anti-malarial activities with high selective toxicities.

Resistance of the human malaria parasite *Plasmodium falciparum* to chloroquine and to other common anti-malarial drugs has stimulated considerable effort towards the characterization of new and mechanically-novel anti-malarial agents.[1] A certain species of the Simaroubaceae family has been used in traditional medicine in order to combat malaria.[2] This anti-malarial activity has been considered to be attributed to quassinoid constituents. Thus, several naturally occurring quassinoids were shown to exhibit significant inhibitory activity against chloroquine-resistant strains of *P. falciparum*.[3] However, the anti-malarial activities of only limited quassinoid constituents were evaluated and there has been little investigation of the selective toxicities between the malaria parasite and cells of host animals.

On the other hand, the fruits of *Brucea javanica* (Simaroubaceae) which are readily available in Chinese traditional natural medicine called "Ya-tan-tzu" contain quassinoid glycosides such as bruceoside



yadanziosides
(R= acyl residues)
deacetylyadanzioside F (**1** : R= H)
β-Glc = β-D-glucopyranosyl

bruceolide (**2** : R= H)
bruceine B (**3a** : R= Ac)

Chart 1

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A[4] and yadanziosides[5] as major constituents. Since these glycosides have a common structure except for acyl residues attached to the hydroxyl group on C-15, the quassinoid glycoside fraction obtained from *B. javanica* was treated with NaOMe-MeOH to afford deacetylyadanzioside F (**1**)[6] in good yield. Furthermore, the deglucosylation of **1** concomitant with tautomerizm of the α-hydroxy enone moiety furnished bruceolide (**2**), which corresponds to the common structure of several naturally occurring anti-malarial quassinoids. This circumstance stimulated us to search for new anti-malarial pharmaceuticals utilizing bruceolide (**2**) as the starting material. This paper deals with anti-malarial activities of acylated derivatives of bruceolide (**2**), of which the practical preparation procedure was herein established.

After defatting "Ya-tan-tzu" (the dried fruits of *B. javanica*) with *n*-hexane extraction, the residue was extracted with MeOH under reflux. The MeOH extract was partitioned into *n*-hexane and MeOH, then the MeOH soluble portion was subjected to Diaion HP-20 column chromatography eluting with H_2O and MeOH successively. Treatment of the MeOH eluate with 5% NaOMe-MeOH and followed by SiO_2 and ODS column chromatography gave deacetylyadanzioside F (**1**) in 0.21% yield from "Ya-tan-tzu". Enzymatic hydrolysis of **1** using cellulase from *Aspergillus niger* in acetate buffer (pH 5.0) gave bruceolide (**2**) in 86% yield.

Acylation of **2** was carried out using both carboxylic acid and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI·HCl) in the presence of 4-dimethylaminopyridine (DMAP) to afford the corresponding 3,15-di-*O*-acylderivatives (**3c-3k**),[7] while a propionyl group was introduced under the condition of propionic anhydride in pyridine, and a palmitoyl group by treatment with palmitoyl chloride in pyridine. 15-*O*-Acylderivatives (**3a, 3b**) were prepared after protection of the C-3 hydroxyl group. For example, the triethylsilyl chloride (TESCl) treatment of **2** in pyridine gave 3-*O*-TES-bruceolide (**4**), which was subjected to ordinary acetylation by Ac_2O in pyridine and subsequent removal of the TES group using HF·pyridine to furnish bruceine B (**3a**) in 63% yield from **2**.

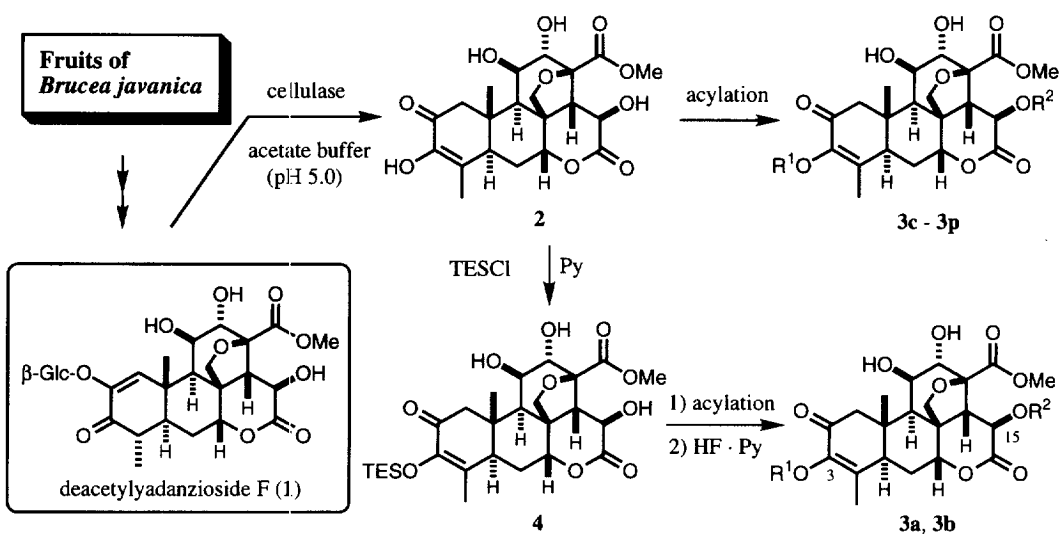

Chart 2

Table 1. Anti-malarial activities of *O*-acylated bruceolide derivatives

	Compound		*P. falciparum*	FM3A cell	Selective
	R^1	R^2	A: EC_{50} (M)	B: EC_{50} (M)	toxicity(B/A)
2	H	H	1.7×10^{-6}	$>2.1 \times 10^{-5}$(100%)[a]	>12.3
3a	H	Ac	2.4×10^{-8}	8.0×10^{-6}	333
3b	H	CH_3CH_2CO	2.9×10^{-8}	7.0×10^{-6}	241
3c	Ac	Ac	3.9×10^{-8}	1.6×10^{-5}	410
3d	CH_3CH_2CO	CH_3CH_2CO	3.5×10^{-8}	6.1×10^{-6}	174
3e	$CH_3(CH_2)_4CO$	$CH_3(CH_2)_4CO$	9.8×10^{-8}	$<1.7 \times 10^{-7}$(30%)	
3f	$CH_3(CH_2)_8CO$	$CH_3(CH_2)_8CO$	3.9×10^{-7}	$<1.6 \times 10^{-7}$(9.4%)	
3g[b]	$CH_3(CH_2)_{14}CO$	$CH_3(CH_2)_{14}CO$	$>1.6 \times 10^{-5}$(89.9%)		
3h	PHCH=CHCO(*E*)	PHCH=CHCO(*E*)	8.2×10^{-8}	$<1.5 \times 10^{-7}$(9.8%)	
3i	CH_3CH=$CHCH_3CO$ (*E*)	CH_3CH=$CHCH_3CO$ (*E*)	7.0×10^{-7}	6.0×10^{-6}	9
3j	CH_3CH=$CHCH_3CO$ (*Z*)	CH_3CH=$CHCH_3CO$ (*Z*)	$>1.0 \times 10^{-6}$(100%)	$>1.6 \times 10^{-5}$(97%)	
3k	PhCO	PhCO	$>1.0 \times 10^{-6}$	4.0×10^{-7}	
3l	$CH_3(CH_2)_4CO$	H	1.0×10^{-6}	1.0×10^{-6}	
3m	$CH_3(CH_2)_8CO$	H	$>1.0 \times 10^{-6}$	4.0×10^{-7}	
3n	CH_3CHNH_2CO	CH_3CHNH_2CO	1.0×10^{-6}	$>1.6 \times 10^{-5}$(100%)	>16
3o	$(CH_3)_2CHCHNH_2CO$	$(CH_3)_2CHCHNH_2CO$	5.7×10^{-7}	$>1.5 \times 10^{-5}$(88.9%)	>26
3p	$PhCH_2CHNH_2CO$	$PhCH_2CHNH_2CO$	2.8×10^{-6}	$>1.5 \times 10^{-5}$(100%)	>5.4

a) Values in parentheses are the viability percentage of FM3A cells.
b) Cytotoxicity was not examined because of weak anti-malarial activity.

Furthermore, 3-*O*-acylderivatives (**3l**, **3m**) were synthesized by condensation between bruceolide (**2**) and a corresponding carboxylic acid in the presence of EDCI·HCl and DMAP on the basis of differences in reactivity of the four hydroxyl groups in **2**. Amino acid conjugated derivatives (**3n**-**3p**) were prepared as hydrochloride salts as follows. After *N*-Boc amino acid was coupled with **2** using EDCI·HCl and DMAP, the Boc group was removed by dry HCl-MeOH treatment to furnish **3n**-**3p**.

Anti-malarial activities and selective toxicities between *P. falciparum* and cells of host-animals, FM3A cells derived from a mammary tumor in mice, are summarized in Table 1. Among the compounds tested, 3,15-di-*O*-acetyl- (**3c**), 3,15-di-*O*-propionyl- (**3d**), and 15-*O*-propionylbruceolide (**3b**) as well as bruceine B (**3a**)[8] strongly inhibited the growth of *P. falciparum*. These four compounds also showed high enough selective toxicities such that they could be subjected to the in vivo anti-malarial test. In addition to the four derivatives, 3,15-di-*O*-cinnamoyl- (**3h**) and 3,15-di-*O*-hexanoylbruceolide (**3f**) also exhibited potent anti-malarial activities, whereas they showed little selective toxicities. With respect to the congeners containing straight-chain acyl residues, prolongation of the carbon chain tended to bring about the decrease of activity.

In the case of derivatives (**3n**-**3p**) conjugated with amino acids, hydrophilic acyl donors, neither potent anti-malarial activities nor good selective toxicities were observed. Respective comparison of activities between the diacyl and monoacylderivatives possessing the same acyl residue indicated the following participation of the acyl group in anti-malarial activity. Namely, the acetyl or propionyl residues attached to the hydroxyl group on C-15 are important for the growth inhibition of *P. falciparum*, while these residues to the 3-hydroxyl group are related to selective toxicity.

As a result of syntheses of the *O*-acylated bruceolide derivatives (**3a**-**3p**) and evaluation of their anti-malarial activities, 3,15-di-*O*-acetyl- (**3c**), 3,15-di-*O*-propionyl- (**3d**), and 15-*O*-propionylbruceolide (**3b**) were newly found to inhibit the proliferation of *P. falciparum* with high selective toxicities. It is noteworthy that 3,15-di-*O*-acetylbruceolide (**3c**) exhibited higher selective toxicity than brucein B (**3a**), since **3c** was prepared from bruceolide (**2**) in a higher yield and through fewer reaction steps than **3a**. It should be also noted that our present method afforded more than fifty times of bruceine B (**3a**) having various biological activities[9] along with anti-malarial activity, compared with conventional usual separation from *B. javanica*. A program aiming at bruceolide (**2**) derivatives with higher selective toxicity and/or more potent activity than **3c** and investigation of the structure-activity relationships are in progress.

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7. All new compounds were spectrally characterized, and full preparative details and characteristics will be presented in a full paper on this subject. Each derivative (**3b**-**3p**) was prepared from **2** in the following yield: **3b** (55 %), **3c** (92 %), **3d** (90 %), **3e** (83 %), **3f** (52 %), **3g** (68 %), **3h** (75 %), **3i** (32 %), **3j** (29 %), **3k** (97 %), **3l** (35 %), **3m** (37 %), **3n** (63 %), **3o** (84 %), **3p** (69 %).
8. Although bruceine B (**3a**) was reported to show inhibitory activity against chloroquine-resistant strains of *P. falciparum*, selective toxicity of **3a** has not been examined. Ref. Kirby, G. C.; O'Neill, M. J.; Phillipson, J. D.; Warhurst, D. C. *Biochem. Pharmacol.* **1989**, *38*, 4367-4374.
9. Recently, we found bruceine B (**3a**) inhibits the endothelial cell-neutrophil leukocyte adhesion *in vitro* and exhibits anti-inflammatory activity *in vivo*.[10]
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