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KOPREASIN A, A NEW INDOLE ALKALOID FROM *KOPSIA ARBOREA*

Kazumasa Zaima,[a] Yosuke Matsuno,[a] Yusuke Hirasawa,[a] Abdul Rahman,[b] Gunawan Indrayanto,[b] Noor Cholies Zaini,[b] and Hiroshi Morita [a,]*

[a] Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan; [b] Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

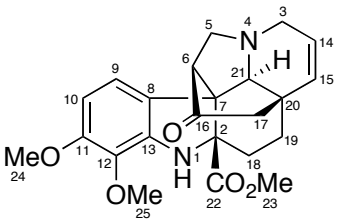
Abstract – A new alkaloid, kopreasin A (**1**) was isolated from the leaves of *Kopsia arborea* (Apocynaceae) together with ten known indole alkaloids (**2** - **11**), and the structure was elucidated by NMR spectral analysis using 2D techniques. These indole alkaloids showed a moderate vasorelaxant activity on isolated rat aorta ring.

INTRODUCTION

Indole alkaloids with unique skeletons often show some interesting biological activities.[1] In our research for structurally unique and biologically interesting indole alkaloids, we previously isolated new indole alkaloids, flavisiamines from leaves of *Kopsisa flavida* (Apocynaceae).[2] The genus *Kopsia* (Apocynaceae), which is widely distributed throughout tropical Asia, is noted for producing variety of indole alkaloids with useful biological activities.[3,4] Recent investigation of extracts from the leaves of *K. arborea* resulted in the isolation of a new indole alkaloid, kopreasin A (**1**) together with ten known indole alkaloids (**2** – **11**). In this paper, we report the isolation and structure elucidation of **1**, and vasorelaxant activity of isolated indole alkaloids (**1** – **11**).

The leaves of *K. arborea* were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH/EtOAc 200:1:1 → 19:1:1 → 1:1:1) and the fractions eluted by $CHCl_3$/MeOH/EtOAc (200:1:1) were subjected to a silica gel column (Toluene/EtOAc 1:0 → 8:2 and then $CHCl_3$/MeOH 1:1 → 0:1). Toluene/EtOAc eluted fractions were purified by C_{18} HPLC to afford kopreasin A (**1**, 0.002%) together with methyl 11,12-methylenedioxy-*N*-decarbomethoxy-$\Delta^{14,15}$-chanofruticosinate (**2**, 0.003%),[5] flavisiamines A (**3**, 0.001%),[2] C (**4**, 0.001%),[2] and D (**5**, 0.002%),[2] methyl *N*-decarbomethoxychanofruticosinate (**6**, 0.005 %),[6] methyl 11,12-methylenedioxy-*N*-decarbomethoxy-

chanofruticosinate (**7**, 0.002 %),[5] methyl 12-methoxy-*N*-decarbomethoxychanofruticosinate (**8**, 0.002 %),[4a] methyl 11,12-methylenedioxychanofruticosinate (**9**, 0.002%),[6] methyl 12-methoxy-chanofruticosinate (**10**, 0.001%),[4a] and prunifoline B (**11**, 0.0007%).[7]



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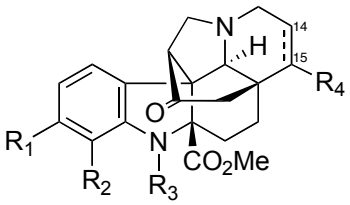

	R_1	R_2	R_3	R_4
2	—O–CH_2–O—		H	$\Delta^{14,15}$
3	H	H	H	OH
4	H	OMe	H	OH
5	—O–CH_2–O—		H	OH
6	H	H	H	H
7	—O–CH_2–O—		H	H
8	H	OMe	H	H
9	—O–CH_2–O—		CO_2Me	H
10	H	OMe	CO_2Me	H
11	OMe	OMe	CO_2Me	$\Delta^{14,15}$

RESULTS AND DISCUSSION

Kopreasin A {**1**, $[\alpha]_D^{25}$ +88 (*c* 1.0, MeOH)} showed the pseudomolecular ion peak at *m/z* 411 $(M+H)^+$ in ESIMS, and the molecular formula, $C_{23}H_{26}N_2O_5$, was established by HRESIMS [*m/z* 411.1920, $(M+H)^+$ Δ -1.8 mDa]. IR spectrum suggested the presence of NH (3420 cm^{-1}) and carbonyl (1720 cm^{-1}) groups. The ^{13}C NMR (Table 1) spectrum of **1** disclosed twenty-three carbon signals due to one carbonyl (δ_C 206.6), one ester carbonyl (δ_C 174.5), four sp^2 quaternary carbons (δ_C 152.8, 141.8, 134.5, and 126.4), three sp^3 quaternary carbons (δ_C 75.1, 58.2, and 36.9), four sp^2 methines (δ_C 136.4, 125.5, 118.6, and 104.1), two sp^3 methines (δ_C 66.4 and 54.5), five sp^3 methylenes (δ_C 59.8, 50.7, 46.8, 32.1, and 27.3), and three methyls (δ_C 60.2, 56.0, and 52.0) attached to an oxygen atom. ^1H and ^{13}C signals for **1** were assigned by detailed analysis of the HSQC spectrum. The ^1H-^1H COSY spectrum revealed connectivities of C-3 - C-15, C-5 to C-6, C-9 to C-10, and C-18 to C-19 (Figure 1). HMBC correlations of H-6 (δ_H 3.29) to C-16 (δ_C 206.6), C-21 (δ_C 66.4), and C-7 (δ_C 58.2), H_2-17 (δ_H 2.35 and 2.47) to C-16, C-21, and C-19 (δ_C 32.1), and H-21 (δ_H 2.74) to C-5 (δ_C 59.8) revealed the presence of a 6-aza-bicyclo[3.2.1]octan-2-one ring (C-5 to C-7, C-16 to C-17, C-20 to C-21, and N-4). The presence of an octahydroquinoline ring (C-7, C-2, C-18 to C-21, C-3, C-14 to C-15, and N-4) with a double bond between C-14 and C-15, fused to the 6-aza-bicyclo ring, was deduced from the HMBC correlations of H_2-17 to C-15 (δ_C 136.4) and C-20 (δ_C 36.9) and H_2-19 (δ_H 1.58 and 1.97) to C-2 (δ_H75.1). HMBC

correlations of H-9 (δ_H 6.88) to C-7, C-11 (δ_C 152.8), and C-13 (δ_C 141.8), H-10 (δ_H 6.36) to C-8 (δ_C 126.4) and C-12 (δ_C 134.5), and methyl signals at δ_H 3.82 and 3.85 to C-11 and C-12, respectively, revealed a dimethoxy dihydro indole ring (C-2, C-7 - C-13, and N-1). Thus, the structure of kopreasin A was elucidated to be **1** possessing methyl chanofruticosinate skeletal system with a double bond between C-14 and C-15 and methoxy groups at C-11 and C-12.

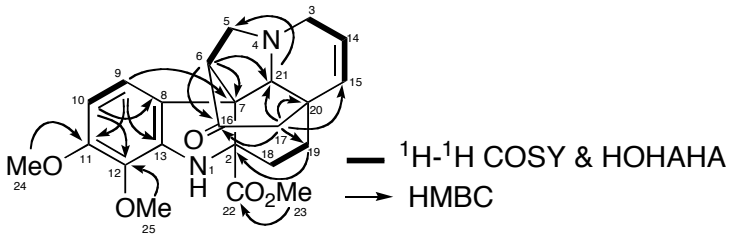

Figure 1. Selected 2D NMR correlations for kopreasin A (**1**)

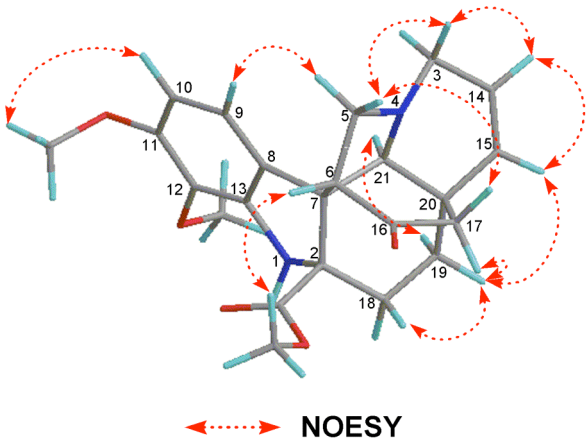

Figure 2. Selected NOESY correlations and relative stereochemistry for kopreasin A (**1**)

The relative stereochemistry of **1** was elucidated by NOESY correlations as shown in computer-generated 3D drawing (Figure 2). The presence of *trans* fused octahydroquinoline including stereochemistry at C-20 and C-21 was elucidated by NOESY correlations between H-15 and H-19b, between H-5b and H-17b, and between H-21 and H-19a. The β-configuration of a methoxy carbonyl group at C-2 was suggested by a NOESY correlation of H-6 to H_3-23. Thus, the relative stereochemistry of **1** was assigned as shown in Figure 2.

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. When phenylephrine (PE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, we added kopreasin A (**1**) and their related methyl chanofruticosinate type alkaloids (**2** – **11**). These indole alkaloids showed a moderate vasorelaxant activity on isolated rat aorta ring (**1**: 28%; **2**: 19%; **3**: 13%; **4**: 24%; **5**: 26%; **6**: 41%; **7**: 19%; **8**: 15%; **9**: 40%; **10**: 37%; **11**: 23% at 3×10^{-5} M, respectively). Methyl *N*-decarbomethoxychanofruticosinate (**6**),

methyl 11,12-methylenedioxy- chanofruticosinate (**9**), and methyl 12-methoxychanofruticosinate (**10**) showed slow vasorelaxant actions. Among methyl chanofruticosinate type alkaloids without *N*-carbomethoxy function (**1** – **8**), methyl *N*-decarbomethoxychanofruticosinate (**6**) without any substituents at aromatic ring exhibited relatively potent vasorelaxant activity. Interestingly, methyl 11,12-methylenedioxychanofruticosinate (**9**) and methyl 12-methoxychanofruticosinate (**10**) with *N*-carbomethoxy function showed a moderate vasorelaxant activity, although there are some substituents at aromatic ring. In addition, vasodilation may seem to be influenced by the hydrophobisity of whole molecule. The same relaxant actions were observed in the sample of aortic rings without endothelium. The mode of actions of these indole alkaloids on vasorelaxant and contractile activities are under investigation.

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

No.	δ_H	δ_c
2		75.1
3a	3.42 (d, 18.6)	50.7
3b	4.01 (d, 18.6)	
5a	4.23 (brs)	59.8
5b	2.88 (d, 11.2)	
6	3.29 (d, 5.6)	54.5
7		58.2
8		126.4
9	6.88 (d, 8.0)	118.6
10	6.36 (d, 8.0)	104.1
11		152.8
12		134.5
13		141.8
14	5.63 (d, 10.0)	125.5
15	5.99 (d, 10.0)	136.4
16		206.6
17a	2.35 (d, 18.0)	46.8
17b	2.47 (d, 18.0)	
18a	1.99 (m)	27.3
18b	1.87 (m)	
19a	1.97 (m)	32.1
19b	1.58 (m)	
20		36.9
21	2.74 (s)	66.4
11-OMe	3.82 (s)	56.0
12-OMe	3.85 (s)	60.2
22		174.5
23	3.62 (s)	52.0

EXPERIMENTAL

General Experimental Procedures. ^{1}H and 2D NMR spectra were recorded on a Bruker AV400 spectrometer and chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0) as internal standards. HSQC experiments were optimized for $^1J_{CH}$=145 Hz and HMBC experiments for $^nJ_{CH}$=8Hz. Mass spectra were recorded on a Micromass LCT spectrometer.

Plant Material. The leaves of *Kopsia arborea* were collected in Purwodadi Botanical Garden, East Java, Indonesia in 2006. A voucher specimen is deposited at the Purwodadi Botanical Garden, Indonesia.

Extraction and Isolation. The leaves of *K. arborea* (313 g) were extracted with MeOH, and the MeOH extract (22.8 g) was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated aqueous Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH/EtOAc 200:1:1 → 19:1:1 → 1:1:1) to give methyl 11,12-methylenedioxy-*N*-decarbomethoxy-$\Delta^{14,15}$-chanofruticosinate (**2**, 0.003%).[5] $CHCl_3$/MeOH /EtOAc (19/1/1) eluted fractions were purified by C_{18} HPLC (MeCN/H_2O/CF_3CO_2H 15/85/0.1) to give flavisiamines A (**3**, 0.001%), C (**4**, 0.001%), and D (**5**, 0.002%).[2] $CHCl_3$/MeOH/EtOAc (200:1:1) eluted fractions were subjected to a silica gel column (toluene/EtOAc 1:0 → 8:2 and then $CHCl_3$/MeOH 1/1 → 0:1). Toluene/EtOAc (85:15) eluted fractions were purified by C_{18} HPLC (MeCN/H_2O/CF_3CO_2H, 20:80:0.1) to afford kopreasin A (**1**, 0.002%) together with methyl *N*-decarbomethoxychanofruticosinate (**6**, 0.005%),[6] methyl 11,12-methylenedioxy-*N*-decarbomethoxychanofruticosinate (**7**, 0.002%),[5] methyl 12-methoxy-*N*-decarbomethoxychanofruticosinate (**8**, 0.002%),[4a] and methyl 11,12-methylenedioxy-chanofruticosinate (**9**, 0.002%).[6] Toluene/EtOAc (8:2) eluted fractions were purified by C_{18} HPLC (MeCN/H_2O/CF_3CO_2H, 25:75:0.1) to afford methyl 12-methoxychanofruticosinate (**10**, 0.001%)[4a] and prunifoline B (**11**, 0.0007%).[7]

Kopreasin A (1): colorless solid; $[\alpha]_D^{25}$ +88 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3420, 2940, 1720, 1240, and 1090 cm^{-1}; UV (MeOH) λ_{max} 290 (ε 14800) and 212 (ε 18500) nm; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 411 $(M+H)^+$; HRTOFMS *m/z* 411.1920 [$(M+H)^+$, calcd for $C_{23}H_{27}N_2O_5$, 411.1938].

Vasodilation Assay.[8] A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 5 mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a

force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.
After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M PE. The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (ACh), and aortic ring in which 80% relaxation occurred, were regarded as tissues with endothelium. When the PE-induced contraction reached a plateau, each sample was added.
These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

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