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

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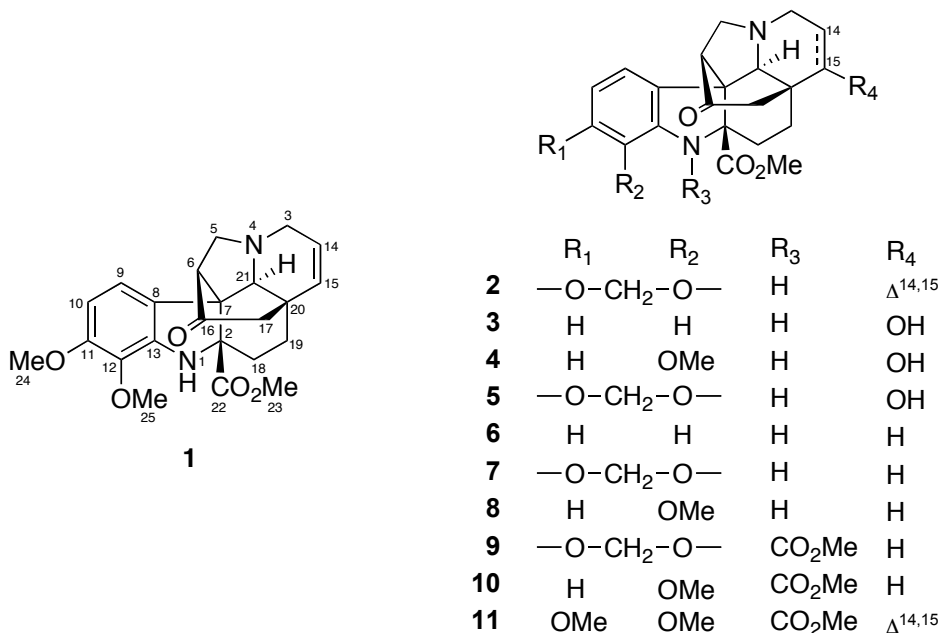
Abstract – A new alkaloid, kopreasin A (**1**) was isolated from the leaves of *Kopsia arborea* (Apocynaceae) together with ten known indole alkaloids (**2** - **11**), and the structure was elucidated by NMR spectral analysis using 2D techniques. These indole alkaloids showed a moderate vasorelaxant 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 Na₂CO₃, were extracted with CHCl₃. CHCl₃-soluble materials were subjected to a silica gel column (CHCl₃/MeOH/EtOAc 200:1:1 → 19:1:1 → 1:1:1) and the fractions eluted by CHCl₃/MeOH/EtOAc (200:1:1) were subjected to a silica gel column (Toluene/EtOAc 1:0 → 8:2 and then CHCl₃/MeOH 1:1 → 0:1). Toluene/EtOAc eluted fractions were purified by C₁₈ HPLC to afford kopreasin A (**1**, 0.002%) together with methyl 11,12-methylenedioxy-*N*-decarbomethoxy- $\Delta^{14,15}$ -chanofrucosinate (**2**, 0.003%),⁵ flavisiamines A (**3**, 0.001%),² C (**4**, 0.001%),² and D (**5**, 0.002%),² methyl *N*-decarbomethoxychanofrucosinate (**6**, 0.005 %),⁶ methyl 11,12-methylenedioxy-*N*-decarbomethoxy-

chanofrucosinate (**7**, 0.002 %),⁵ methyl 12-methoxy-*N*-decarbomethoxychanofrucosinate (**8**, 0.002 %),^{4a} methyl 11,12-methylenedioxychanofrucosinate (**9**, 0.002%),⁶ methyl 12-methoxychanofrucosinate (**10**, 0.001%),^{4a} and prunifoline B (**11**, 0.0007%).⁷



RESULTS AND DISCUSSION

Kopreasin A {**1**, [α]_D²⁵ +88 (c 1.0, MeOH)} showed the pseudomolecular ion peak at *m/z* 411 (M+H)⁺ in ESIMS, and the molecular formula, C₂₃H₂₆N₂O₅, 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 ¹³C 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 ¹³C 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), H₂-17 (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 H₂-17 to C-15 (δ_C 136.4) and C-20 (δ_C 36.9) and H₂-19 (δ_H 1.58 and 1.97) to C-2 (δ_H 75.1). 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 chanofrucosinate skeletal system with a double bond between C-14 and C-15 and methoxy groups at C-11 and C-12.

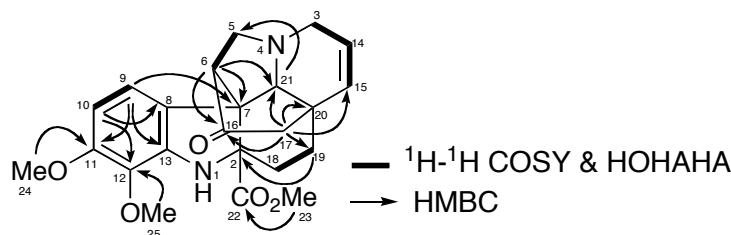


Figure 1. Selected 2D NMR correlations for kopreasin A (**1**)

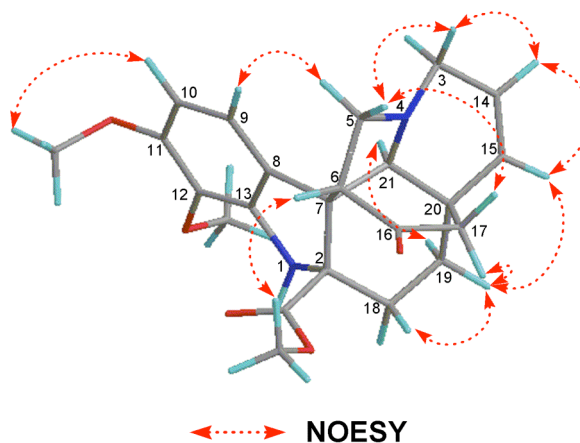


Figure 2. Selected NOESY correlations and relative stereochemistry for kopreasin A (**1**)

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 H₃-23. 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 chanofrucosinate 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*-decarbomethoxychanofrucosinate (**6**),

methyl 11,12-methylenedioxy- chanofrucosinate (**9**), and methyl 12-methoxychanofrucosinate (**10**) showed slow vasorelaxant actions. Among methyl chanofrucosinate type alkaloids without *N*-carbomethoxy function (**1** – **8**), methyl *N*-decarbomethoxychanofrucosinate (**6**) without any substituents at aromatic ring exhibited relatively potent vasorelaxant activity. Interestingly, methyl 11,12-methylenedioxychanofrucosinate (**9**) and methyl 12-methoxychanofrucosinate (**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 hydrophobicity 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. ^1H 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. ^1H 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 $^1J_{\text{CH}}=145$ Hz and HMBC experiments for $^nJ_{\text{CH}}=8$ Hz. 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 ($\text{CHCl}_3/\text{MeOH}/\text{EtOAc}$ 200:1:1 \rightarrow 19:1:1 \rightarrow 1:1:1) to give methyl 11,12-methylenedioxy-*N*-decarbomethoxy- $\Delta^{14,15}$ -chanofrucosinate (**2**, 0.003%).⁵ $\text{CHCl}_3/\text{MeOH}/\text{EtOAc}$ (19/1/1) eluted fractions were purified by C_{18} HPLC ($\text{MeCN}/\text{H}_2\text{O}/\text{CF}_3\text{CO}_2\text{H}$ 15/85/0.1) to give flavisiamines A (**3**, 0.001%), C (**4**, 0.001%), and D (**5**, 0.002%).² $\text{CHCl}_3/\text{MeOH}/\text{EtOAc}$ (200:1:1) eluted fractions were subjected to a silica gel column (toluene/ EtOAc 1:0 \rightarrow 8:2 and then $\text{CHCl}_3/\text{MeOH}$ 1/1 \rightarrow 0:1). Toluene/ EtOAc (85:15) eluted fractions were purified by C_{18} HPLC ($\text{MeCN}/\text{H}_2\text{O}/\text{CF}_3\text{CO}_2\text{H}$, 20:80:0.1) to afford kopreasin A (**1**, 0.002%) together with methyl *N*-decarbomethoxychanofrucosinate (**6**, 0.005%),⁶ methyl 11,12-methylenedioxy-*N*-decarbomethoxychanofrucosinate (**7**, 0.002%),⁵ methyl 12-methoxy-*N*-decarbomethoxychanofrucosinate (**8**, 0.002%),^{4a} and methyl 11,12-methylenedioxychanofrucosinate (**9**, 0.002%).⁶ Toluene/ EtOAc (8:2) eluted fractions were purified by C_{18} HPLC ($\text{MeCN}/\text{H}_2\text{O}/\text{CF}_3\text{CO}_2\text{H}$, 25:75:0.1) to afford methyl 12-methoxychanofrucosinate (**10**, 0.001%)^{4a} and prunifoline B (**11**, 0.0007%).⁷

Kopreasin A (1): colorless solid; $[\alpha]_{\text{D}}^{25} +88$ (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; ^1H and ^{13}C NMR data (Table 1); ESIMS m/z 411 ($\text{M}+\text{H}^+$); HRTOFMS m/z 411.1920 [$(\text{M}+\text{H})^+$, calcd for $\text{C}_{23}\text{H}_{27}\text{N}_2\text{O}_5$, 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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