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Search for pharmacochemical leads from tropical rainforest plants*

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Abstract: Three Indonesian medicinal plants were investigated to elucidate their biologically active constituents. From the leaves of *Scurrula fusca* (Loranthaceae), a parasitic plant, was isolated a mixture (presumably a complex) of perseitol (a heptitol) and potassium ion which exhibited inhibitory activity on the incorporation of ^{3}H-leucine to the Ehrlich ascites tumor cells in rat. Three benzochromenes and four isopimarane-type diterpenes isolated from the leaves of *Orthosiphon aristatus* (Lamiaceae) were shown to exhibit inhibitory effects on smooth muscle contractions caused by several stimulants. Furthermore, it was found that the leaves of *Monocarpia marginalis* (Annonaceae), collected in August, contained two juvenile hormones, JH III and methyl (*E*,*E*)-farnesoate, in high content, while these hormones were not detected in the leaves collected in February next year.

Indonesia comprises the second largest area of tropical rainforest next to Brazil in the world. It cover about 10% of the world's rainforest which corresponds to 40–50% of the rainforest in Asia. In countrysides in Indonesia witch doctors called 'dukun' treat patients by use of various kinds of medicinal plants. And Javanese people have been using their traditional medicines called 'Jamu' for preventing and/or treating diseases.

Since 1985, we have been carrying out a series of scientific expeditions on naturally occurring drug materials in Indonesia in collaboration with Research and Development Centre for Biology, Indonesia Institute of Science (LIPI), the major purpose being to make the list of medicinal plants mainly in Indonesian tropical rainforest [1]. This paper deals with some recent pharmacochemical investigations on the plant materials collected in our expeditions.

SCURRULA FUSCA (LORANTHACEAE)

Scurrula fusca (Bl.) G. Don. is parasitic on *Ficus riedelii* (Moraceae) and water decoction of the leave has been used for treatment of cancer in South Sumatra (local name: benalu) and Central Sulawesi (local name: benalu alus). The air-dried leaves were extracted with hot methanol. Upon cooling, the extra yielded precipitates (named BAP, 5.0% from the dried leaves), while the filtrate provided, after chromatography, two flavonoid-glycosides, quercetin 3-*O*-α-L-rhamnopyranoside (0.052%) and quercetin 3-*O*-β-D-xylopyranoside (0.038%).

The precipitates (BAP) were insoluble in lipophilic solvents but slightly soluble in DMSO and water. The ^{1}H and ^{13}C-NMR spectra (in DMSO) of BAP suggested that the organic part of BAP might be a heptitol, and X-ray crystallographic analysis of the heptaacetate, $[\alpha]_D$ −14.7° ($CHCl_3$), revealed that the organic part was perseitol which was hitherto isolated from the seeds of avocado, *Persea gratissima* [2]. As for the inorganic part of BAP, fluorescent X-ray analysis disclosed to be potassium ion.

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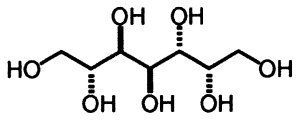
BAP was further purified by treatment with pure water and subsequent filtration to give BAP-W which was analyzed by atomic absorption analysis to determine the ratio of perseitol and potassium ion was 27:1. Finally, treatment of BAP with acidic resin (Dowex 50W × 8, H^+ form) afforded BAP-DX which was found identical to perseitol.

BAP-W (perseitol + K^+ ion) and BAP-DX (perseitol) were examined their effects on several enzyme activities (lactate dehydrogenase, creatine phosphokinase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, and amylase) in the supernatant of the Ehrlich ascites tumor cells in rat. Among those enzymes, lactate dehydrogenese and creatine phosphokinase were shown to be activated by BAP-DW (1×10^{-6} M), while BAP-W showed no effect. However, the activity of glutamate oxaloacetate transaminase was increased by adding mixtures of perseitol and K^+ ion (used KSCN) (1:1, 2:1, and 3:1) at 1×10^{-6}, 1×10^{-7}, and 1×10^{-8} M.

It was also shown that BAP-W (1×10^{-6}, 1×10^{-7}, 1×10^{-8} M) exhibited an inhibitory effect on the incorporation of ^{3}H-leucine to the Ehrlich ascites tumor cells, which presented an interesting subject of study for verifying medicinal use of the leaves. Recently, it was reported by Itokawa and his group that dulcitol (galactitol, *meso*) isolated from the stem bark of *Maytenus ebenifolia* (Celastraceae) showed anti-tumor activity on Sarcoma 180 ascites in mice [3].



perseitol

Scheme 1

ORTHOSIPHON ARISTATUS (LAMIACEAE)

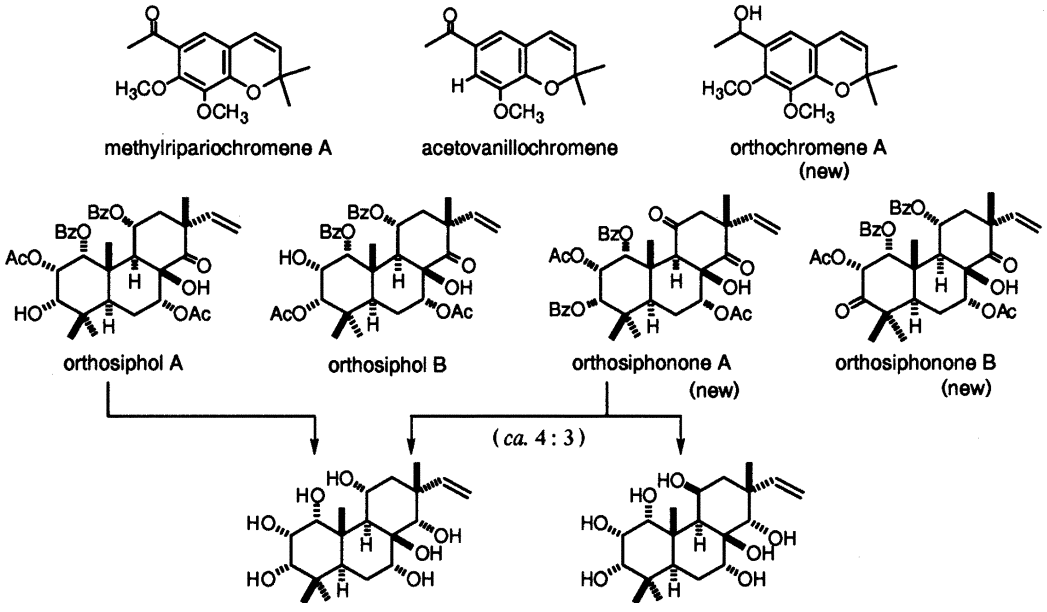
A lamiaceaous plant *Orthosiphon aristatus* (Bl.) Miq. is a popular medicinal herb in South-east Asia. It was originated in Java Island and is well known by the name of 'kumis kucing'. Javanese people prescribe the leaves in their Jamu traditional medicine [4], mainly for treatment of hypertension.

Water decoction of the air-dried leaves was partitioned into a mixture of chloroform and water. The chloroform-soluble portion showed an inhibitory effect on the contractive responses in the rat thoracic aorta smooth muscle stimulated with KCl beforehand, while the aqueous portion did not. The chloroform-soluble portion was then separated by silica gel column chromatography and normal phase HPLC to provide three benzochromenes: methyripariochromene A (major, 0.063% from the dried leaves [5]) acetovanillochromene (0.03%)[6], orthochromene A (new, 0.024%), and four isopimarane-type diterpenes: orthosiphol A (0.039%)[7], orthosiphol B (0.035%) [7], orthosiphonone A (new, 0.023%), and orthosiphonone B (new, 0.003%).

Orthochromene A showed absorption bands due to hydroxyl and aromatic groups in the IR and UV spectra. The ^{1}H- and ^{13}C-NMR spectra indicated that orthochromene A was a benzochromene derivative. The structure was finally elucidated by NOESY and HMBC spectra, as well as by $NaBH_4$ reduction of methylripariochromene A giving orthochromene A. Thus, orthochromene A was revealed to be a 1:1 mixture of 13*S* and 13*R* isomers, which were further supported by chiral HPLC analysis.

The chemical structures of two new isopimarane-type diterpenes were determined from following reactions. Treatment of orthosiphonone A with $LiAlH_4$ afforded a heptaol (11α OH: 11β OH ≈ 4:3), of which the 11α-OH isomer was obtained as a sole product by the same reduction of orthosiphol A, the absolute stereostructure being elucidated as shown by exiton-chirality method (Scheme 2) [7,8]. The structure of orthosiphonone B was revealed by conversion from orthosiphol A through treatment with pyridinium chlorochromate. Seven compounds isolated from the leaves of *Orthosiphon aristatus* exhibited inhibitory activities on contractive responses in the rat thoracic aorta smooth muscle with KCl, the results as shown in Table 1. Isopimarane-type diterpenes showed about 10 times stronger activities than benzochromene-type compounds.

Upon changing the concentration of Ca^{2+} ion (from 0.3 mM to 30 mM), methyripariochromene A (the major constituent of the leaves) exhibited concentration-dependent inhibition on the thoracic aorta



Scheme 2

Table 1 Effects on contractive responses in rat thoracic aorta stimulated with KCl

	IC_{50} (μg/mL)		IC_{50} (μg/mL)
methylripariochromene A	23.8	orthosiphol A	4.99
acetovanillochromene	23.5	orthosiphol B	2.40
orthochromene A	34.7	orthosiphonone A	2.32
		orthosiphonone B	2.54

contraction stimulated with KCl (Fig. 1). However, when using $PGF_{2\alpha}$ as a stimulant and upon changing Ca^{2+} concentration, no effect was observed. It was presumed therefore that benzochromenes such as methylripariochromene A may cause vasodilation through the inhibition of Ca^{2+} ion influx like channel blockers.

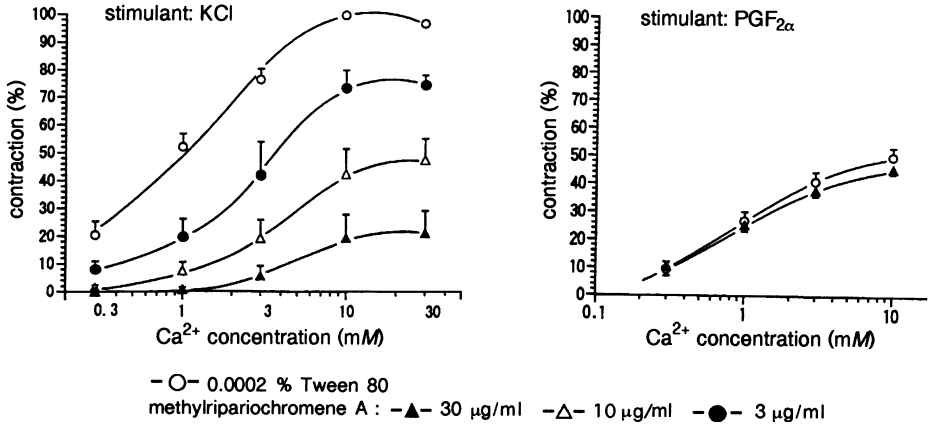


Fig. 1 Effects of methlyripariochromene A in rat thoracic aorta stimulated with KCl and $PGF_{2\alpha}$.

On the other hand, orthosiphol A and orthosiphonone A exhibited an inhibitory effect on the rat thoracic aorta contraction caused by KCl, while they did not exhibit any effect on the same contraction caused by *l*-phenylepherine and $PGF_{2\alpha}$ as well (Table 2). These findings suggested that the activity of isopimarane-type diterpenes may concern with depolarization.

Table 2 Effects on contractive responses of orthosiphol A and orthosiphonone A in rat thoracic aorta

		IC_{50} (μg/mL)	
Stimulants	Conc. (mM)	orthosiphol A	orthosiphonone A
KCl	60	4.99	2.32
l-phenylephrine	3×10^{-4}	>100	>100
$PGF_{2\alpha}$	1×10^{-2}	>100	>100

Furthermore, it seems worth mentioning that the major chemical constituent methylripariochromene A exhibited moderate relaxant activity on guinea-pig trachea smooth muscle without stimulants (Fig. 2) and with stimulants (histamine, acetylcholine, and U46619). (Table 3) These findings have led us to presume that the leaves of *Orthosiphon aristatus* may be effective against trachea troubles like cough.

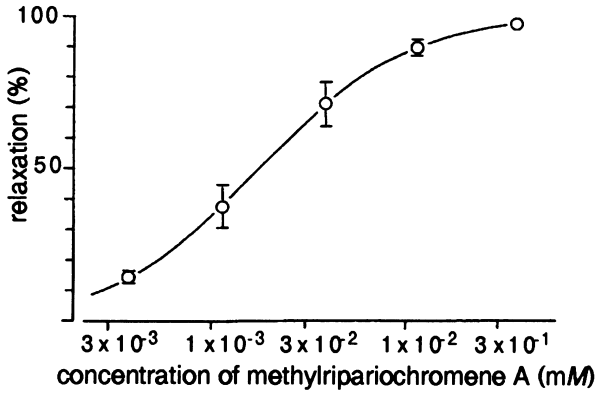


Fig. 2 Activity of methylripariochrornene A on guinea-pig trachea smooth muscle.

Table 3 Effects of methylripariochromene A on contraction of guinea-pig trachea smooth muscle

		(%)		
Stimulants	Conc. (mM)	10	30	100 (μg/mL)
KCl	60	4 ± 3	50 ± 5	112 ± 12
Histamine	1×10^{-2}	22 ± 9	79 ± 12	130 ± 16
Acethylcholine	1×10^{-5}	10	40 ± 14	123 ± 15
U46619*	1×10^{-3}	26 ± 2	106 ± 12	

* Thromboxane A_2 receptor stimulant.

MONOCARPIA MARGINALIS (ANNONACEAE)

Monocarpia marginalis (Scheff.) Sinclair (Annonaceae) is a medium sized tree (10–20 m) growing in primary rainforest, and is called 'senahe' in West Kalimantan, Borneo Island. Water decoction of the leaves has been traditionally used as a remedy for eye inflammation in the area.

The air-dried leaves of 'senahe' collected in August (during dry season in West Kalimantan), 1992, were extracted with acetone. The extract was separated by silica gel column chromatography and normal phase HPLC to afford two juvenile hormones (10*R*)-(+)-juvenile hormone III (JH III, 6.0% from the dried leaves) and methyl (*E,E*)-famesoate (0.6%), together with several sesquiterpenes and phytol. The isolation of those juvenile hormones in such high content appears to be the first example from plant.

Interestingly, those juvenile hormones were not detected in the leaves collected in February (during rainy season in West Kalimantan), 1993, which seems to be very interesting also from a viewpoint of ecochemical system in tropical rainforest (Scheme 3).

(10*R*)-(+)-juvenile hormone III methyl (*E*,*E*)-farnesoate

Scheme 3

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