Indonesian Medicinal Plants. XXIII.1) Chemical Structures of Two New Migrated Pimarane-type Diterpenes, Neoorthosiphol A and B, and Suppressive Effects on Rat Thoracic Aorta of Chemical Constituents Isolated from the Leaves of Orthosiphon aristatus (Lamiaceae)

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Two novel migrated pimarane-type diterpenes named neoorthosiphol A (1) and B (2) were isolated from the water decoction of the leaves of Orthosiphon aristatus (Lamiaceae), which has been prescribed in Javanese traditional medicine (jamu) for the treatment of hypertension, etc. The absolute chemical structures have been elucidated on the basis of physicochemical properties.

It has been found that two migrated pimarane-type diterpenes (1, 2), four isopimarane-type diterpenes (3, 4, 5, 6), three benzochromenes (7, 8, 9) and two flavones (12, 13) exhibit a suppressive effect on contractile responses in rat thoracic aorta, among thirteen chemical constituents (1—13) isolated from the leaves.

Key words Indonesian medicinal plant; Orthosiphon aristatus; Lamiaceae; migrated pimarane; neoorthosiphol; circular dichroism

A lamiaceous plant Orthosiphon aristatus (Bl.) Miq. is a popular medicinal plant in Southeast Asia and is well known by the name of kumis kucing. The leaves have been prescribed in Javanese traditional medicine (jamu)1) for the treatment of hypertension, etc. The water decoction of the leaves was partitioned into a mixture of chloroform and water. The chloroform-soluble portion of the water decoction shows an inhibitory effect on the contractile responses in rat thoracic aorta smooth muscle stimulated with K+", an activity which is thought to be closely related with antihypertensive activity.

In our previous papers, we reported the isolation of four isopimarane-type diterpenes [orthosiphol A (3)3) and B (4)3), orthosiphalones A (5) and B (6)]3) and three benzochromenes [methylripariochromene A (7),4) acetovanilloychromene (8),5) and orthochromene A (9)]3) from the chloroform-soluble portion of the water decoction. In a continuing study of the portion, we have isolated two novel migrated pimarane-type diterpenes designated neoorthosiphol A (1) and B (2), as well as four flavones [5-hydroxy-6,7,3',4'-tetramethoxyflavone (10), eupatorin (11), tetramethylscutellarein (12) and sinensetin (13)]. This paper describes the elucidation of the absolute chemical structures of 1 and 2, and the suppressive effects of the chemical constituents of the leaves on the contraction induced by K+ in rat thoracic aorta.8)

Neoorthosiphol A (1) Neoorthosiphol A (1) showed a quasi-molecular ion peak at m/z 693 [M+H]+, C38H45O12, in the FAB-MS, and the IR spectrum showed the presence of a hydroxyl (3420 cm−1) group, a vinyl (3080, 1720 cm−1) group and ester (1720, 1267 cm−1) group. The UV spectrum showed absorption bands at 230 nm (ε 24000) and 274 nm (ε...
The 1H-NMR spectrum showed signals due to four tertiary methyls, two acetoxyethyls, one hydroxymethine proton, four methine protons attached to an ester function, three olefinic protons and ten aromatic protons. The 13C-NMR and the distortionless enhancement by polarization transfer (DEPT) spectra revealed the presence of six methyl carbons, the distortionless enhancement by polarization transfer olefinic protons and ten aromatic protons. The 13C-NMR and four methine protons attached to an ester function, three aromatic carbons and four quaternary carbons, including two carbons bearing a hydroxyl function (δC 76.6, 8-C; δC 76.9, 13-C).

The 1H–1H correlated spectroscopy (COSY) spectrum of 1 showed correlation peaks revealing sequences from 1-C to 3-C via 2-C, from 5-C to 7-C via 6-C, and from 9-C to 16-C via 11-C, 12-C and 15-C (Fig. 3). From these findings and from analysis of the heteronuclear multiple bond correlation (HMBC) spectrum, it has been deduced that 1 possesses a novel diterpene skeleton, which may be biosynthetically produced through a 1,2-shift of a C2-unit into C-12 from the C-13 position in a pimarane-type diterpene.

The rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectrum of 1 showed correlation peaks between 19-H3 and 20-H3, 2-H and 19-H3, 2-H and 20-H3, 6β-H and 20-H3, and 5-H and 9-H, which suggested that the relative configuration and conformation of the A and B rings in 1 were the same as those in orthosiphol A (3). The orientations of 11-H and 17-H3 were both determined to be β-axial based on the coupling constants of J8,11 (11.0 Hz) and the correlations with 11-H and 17-H3, and 11-H and 20-H3 in the ROESY spectrum. The signals due to 20-H3 and 17-H3 were observed at a lower field than expected, which was assumed to be due to an anisotropic effect of the 8β-axial hydroxyl group. This assumption was supported by the pyridine-induced solvent shifts of δC(OCH3) − δC(D) = −0.43 ppm for 20-CH3 and δC = −0.30 ppm for 17-CH3. Furthermore, the coupling constant J11,12 (5.5 Hz) indicated that the vinyl group at C-12 was oriented to be an α-axial configuration.

In the HMBC experiment, 1 showed the presence of four characteristic cross-peaks between two hydroxymethine protons (11-H and 17-H3) and two benzoyl carbonyl carbons, and between two hydroxyethine protons (2-, 7-H) and two acetyl carbonyl carbons, respectively. In addition, the presence of cross-peaks between the protons at C-12 and C-17 and the ketonic carbonyl carbon, indicated that the ketonic function was oriented at C-14.

The absolute configuration of 1 was established by application of the exciton chirality method. Positive maximum (|θ|236 + 63000), which was caused by two chromophoric benzoates at C-1 and C-11, was observed in the circular dichroism (CD) spectrum. Consequently, it has been clarified that the absolute chemical structure of 1 is as shown.

**Neoorthosiphol B (2)**

Neoorthosiphol B (2) showed a quasi-molecular ion peak at m/z 693 [M+H]+, C38H45LiO12, in the FAB-MS.

The 1H- and 13C-NMR spectra of 2 were quite similar to those of neoorthosiphol A (1), except for the chemical shifts for 2-H and 3-H. Furthermore, a cross peak between 3-H and 3-COCH3 was observed in the HMBC spectrum, in stead of that between 2-H and 2-COCH3 in 1. From the evidence, the chemical structure of 2 was deduced as shown.

The absolute configuration was clarified by application of the exciton chirality method in the CD spectrum (|θ|237 +47000).

So far, thirteen chemical components (1–13) have been isolated from the chloroform-soluble portion in the water decoction of the leaves of Orthosiphon aristatus (kumis kucing). It should be mentioned that two migrated pimarane-type diterpenes (1, 2), the four isopimarane-type diterpenes (3, 4, 5, 6), the three benzochromenes (7, 8, 9) and the two flavones (12, 13) exhibit concentration-dependent suppression of contractions induced by K+ in endothelium-denuded rat thoracic aorta (Table 1).

### Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. FAB-MS were recorded on a JEOL SX-102A spectrometer. IR spectra were recorded on a Shimadzu FT-IR 8500 spectrometer. UV spectra were recorded on a Hitachi U-3500 spectrometer. CD
spectra were recorded on a JASCO J-500A spectrometer. 1H- and 13C-NMR spectra were obtained with a JEOL JNM-Lambda 500 spectrometer operating at 500 and 125 MHz for 1H and 13C nuclei, respectively; chemical shifts are reported in ppm relative to that of tetramethylsilane (δ = 0) as an internal standard, and coupling constants are given in hertz. HPLC was carried out with a Shimadzu LC-10A. Column chromatography was carried out on Silica gel 60 (70–230 mesh, Merck). Thin-layer chromatography on Silica gel 60F254 (Merck) was used to ascertain the purity of the compounds. The spots were visualized by spraying the plates with 1% Ce(SO4)2 in 10% aqueous sulfuric acid, followed by heating.

**Plant Material** Orthosiphon aristatus (Bl.) Miq. was collected in Yogyakarta, Java Island, Indonesia, in December, 1995, and identified at the Herbarium Bogoricense, Research and Development Centre for Biology-LPI, Indonesia.

**Extraction and Isolation Procedure** The leaves (800 g) of Orthosiphon aristatus (Bl.) Miq. (Lamiaceae) were extracted four times with boiled water. The combined solution was evaporated under reduced pressure to give the H2O extract (220 g, 27.5% from the leaves). The H2O extract (23 g) was fractionated into chloroform and water mixture (1:1). The chloroform phase (1.2 g) was sub-1:1-2:1 and a H2O extract (21.1 g, 25%). The CHCl3 extract (1.2 g) was subjected to silica gel column chromatography (SiO2 40 g, CDCl3) and the H2O extract (220 g, 27.5% from the leaves). The H2O extract (23 g) was fi-

**References**