Two Novel Migrated Pimarane-type Diterpenes, Neoorthosiphols A and B, from the Leaves of *Orthosiphon aristatus* (Lamiaceae)

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Two new migrated pimarane-type diterpenes called neoorthosiphols A and B have been isolated from a water decoction of the leaves of *Orthosiphon aristatus* (Lamiaceae) cultivated in Java, Indonesia. Their chemical structures have been elucidated on the basis of physicochemical evidence.

Key words *Orthosiphon aristatus*; Lamiaceae; neoorthosiphol; migrated pimarane-type diterpene; benzoate chirality

Orthosiphon aristatus (BL.) Miq. (Lamiaceae) is called kumis kucing in Javanese. The leaves of *Orthosiphon aristatus* are prescribed in Javanese traditional medicine (jamu)[1] for the treatment of hypertension and diabetes. We have conducted a chemical study of the water decoction of the leaves and isolated two novel migrated pimarane-type diterpenes designated neoorthosiphols A (**1**) and B (**2**).

Neoorthosiphols A (**1**) and B (**2**) were obtained from the chloroform-soluble portion of the water decoction by separation on normal phase adsorbents, in addition to four known isopimarane-type diterpenes, orthosiphols A (**3**) and B,[2] orthosiphonones A and B,[3] and three known benzochromenes.[3]

Neoorthosiphol A (**1**), colorless plates from ether, mp 148—149 °C, $[\alpha]_D$ −28.7° ($CHCl_3$), showed quasimolecular ion peaks at *m/z* 699 $[M+Li]^+$, $C_{38}H_{44}LiO_{12}$, in the FAB-MS, and the IR spectrum showed the presence of a hydroxyl (3420 cm^{-1}) group, a vinyl (3080, 712 cm^{-1}) group, and an ester (1720, 1267 cm^{-1}) group. The UV spectrum showed absorption bands at 230 nm (ε 24000) and 274 nm (ε 2000).

The ^{1}H-NMR spectrum[4] exhibited signals due to four *tertiary* methyls, two acetoxymethyls, four methine protons attached to an ester group, three olefinic protons, and ten aromatic protons. The ^{13}C-NMR[5] and DEPT spectra showed the presence of six methyl carbons, two methylene carbons, nine methine carbons, a ketonic carbonyl carbon, four ester carbonyl carbons, twelve aromatic carbons, and four *quaternary* carbons including two carbons bearing a hydroxyl function.

The ^{1}H–^{1}H COSY spectrum of **1** showed correlation peaks revealing the 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 analysis of the HMBC spectrum (Table 1), it has been deduced that **1** possesses a novel diterpene skeleton, which may be biosynthetically produced through a 1,2-shift of a C_2 unit into the C-12 from the C-13 position in a pimarane-type diterpene.

The ROESY spectrum showed correlation peaks between 19-H_3 and 20-H_3, 2-H and 19-H_3, 2-H and 20-H_3, 6β-H and 20-H_3, and 5-H and 9-H. This evidence and the coupling constants of protons on the A and B rings suggested that the conformation and configuration of A and B rings in **1** were the same as those in **3**.[2]

The orientations of 11-H and 17-CH_3 were both determined to be β-axial based on the coupling constant ($J_{9,11}$ = 11.0 Hz) and the correlations with 11-H and 17-H_3, and 11-H and 20-H_3 in the ROESY spectrum. The signals due to 20-H_3 and 17-H_3 were observed at lower fields than expected, which was assumed to be due to an anisotropic effect of 8β-axial-OH. This assumption is supported by the pyridine-induced solvent shifts[6] ($\delta_{CDCl_3}-\delta_{C_5D_5N}$) Δ = −0.43 ppm for 20-CH_3 and Δ = −0.30 ppm for 17-CH_3. Furthermore, the coupling constant $J_{11,12}$ (3.5 Hz) indicated that the vinyl group was oriented in the α-axial configuration.

In the HMBC experiment, **1** showed the presence of four characteristic cross-peaks between two hydroxymethine protons (1- and 11-H) and two benzoyl carbonyl carbons, and between two hydroxymethine protons (2- and 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 to C-14.

Neoorthosiphol B (**2**),[7] a colorless plate from ether, mp 194—195 °C, $[\alpha]_D$ +18.1° ($CHCl_3$), showed a quasimolecular ion peak at *m/z* 699 $[M+Li]^+$, $C_{38}H_{44}LiO_{12}$, in the FAB-MS. The IR, UV, and NMR spectra were very similar to those of **1** except for the chemical shifts for 2-H and 3-H in

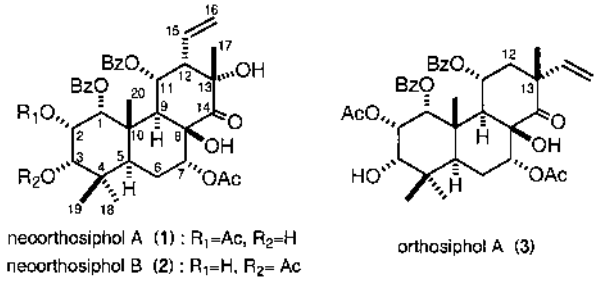

Fig. 1

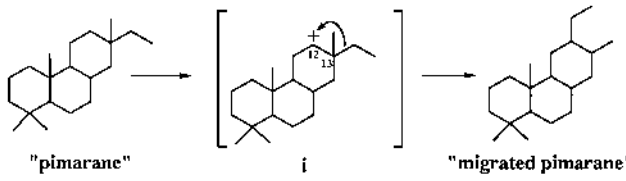

Fig. 2. Plausible Biosynthetic Route for "Migrated Pimarane" from "Pimarane"

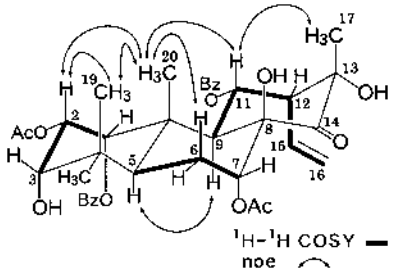

Fig. 3. ^{1}H–^{1}H COSY and ROESY Correlations of **1**

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Table 1. HMBC Correlations of Neoorthosiphol A (**1**)

^{1}H →	^{13}C
1-H	2-C, 5-C, 10-C, -COPh
2-H	1-C, -COCH$_3$
3-H	1-C, 2-C, 4-C, 5-C, 19-C
5-H	4-C, 7-C, 10-C, 18-C, 19-C, 20-C
6-Hα	7-C, 8-C, 10-C
6-Hβ	5-C
7-H	5-C, 8-C, 9-C, -COCH$_3$
9-H	1-C, 10-C, 11-C, 12-C, 20-C
11-H	9-C, 12-C, 13-C, 15-C, -COPh
12-H	9-C, 11-C, 13-C, 14-C, 15-C, 16-C, 17-C
15-H	9-C, 13-C
16-H$_2$	12-C
17-H$_3$	12-C, 13-C, 14-C
18-H$_3$	3-C, 4-C, 5-C, 19-C
19-H$_3$	3-C, 4-C, 5-C, 18-C
20-H$_3$	1-C, 5-C, 9-C, 10-C

the ^{1}H-NMR spectrum. A cross-peak between 3-H and 3-COCH$_3$ was observed in the HMBC, instead of that between 2-H and 2-COCH$_3$ in **1**.

Finally, the absolute configurations of **1** and **2** were established by application of the exciton chirality method.[8] Positive maxima (**1**: $[\theta]_{236}$ +63000, **2**: $[\theta]_{237}$ +47000) which were caused by two chromophoric benzoates at C-1 and C-11 were observed in those CD spectra. Consequently, it has been clarified that the absolute configurations of **1** and **2** are as shown.

In conclusion, we isolated two novel migrated pimarane-type diterpenoids, **1** and **2**, from the leaves of the lamiaceous plant *Orthosiphon aristatus*. It should be mentioned that **1** and **2** exhibit concentration-dependent suppressions of the contraction induced by K^+ and *l*-phenylephrine in the endothelium-denuded rat thoracic aorta. The IC_{50} values of **1** and **2** against the contraction by high K^+ were 10.5 μg/ml and 41.6 μg/ml, and against that by *l*-phenylephrine were 35.9 μg/ml and 42.6 μg/ml, respectively.

Acknowledgments The authors are grateful to Dr. Toshiyuki Matsubara, Toyama Prefectural Institute for Pharmaceutical Research, for bioassay examinations of the suppressive effect on the contraction of rat thoracic aorta.

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4) ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.02 (3H, s, 19-H$_3$), 1.08 (3H, s, 18-H$_3$), 1.39 (3H, s, 20-H$_3$), 1.66 (3H, s, 17-H$_3$), 1.87 (1H, br d, J=13.5 Hz, 6-Hα), 2.02 (3H, s, 2-COCH$_3$), 2.09 (1H, dd, J=13.5, 13.5 Hz, 6-Hβ), 2.18 (3H, s, 7-COCH$_3$), 2.61 (1H, d, J=13.5 Hz, 5-H), 2.94 (1H, dd, J=3.5, 9.8 Hz, 12-H), 2.98 (1H, d, J=11.0 Hz, 9-H), 3.52 (1H, d, J=3.1 Hz, 3-H), 4.45 (1H, d, J=9.8 Hz, 16-Ha), 4.81 (1H, d, J=16.8 Hz, 16-Hb), 5.07 (1H, ddd, J=9.8, 9.8, 16.8 Hz, 15-H), 5.32 (1H, dd, J=3.1, 3.1 Hz, 2-H), 5.40 (1H, br s, 7-H), 5.82 (1H, d, J=3.1 Hz, 1-H), 6.30 (1H, dd, J=3.5, 11.0 Hz, 11-H), 7.48 (2H, dd, J=7.9, 7.9 Hz), 7.61 (1H, dd, J=7.9, 7.9 Hz), 8.24 (2H, d, J=7.9 Hz) (11-COPh), 7.42 (2H, dd, J=7.9, 7.9 Hz), 7.57 (1H, dd, J=7.9, 7.9 Hz), 8.05 (2H, d, J=7.9 Hz) (1-COPh).
5) ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 15.5 (20-C), 20.9 (6-C, 2-CO<u>C</u>H$_3$), 21.2 (7-CO<u>C</u>H$_3$), 22.4 (19-C), 29.0 (17-C), 29.3 (18-C), 34.4 (5-C), 38.2 (4-C), 40.7 (9-C), 43.5 (10-C), 54.4 (12-C), 67.8 (2-C), 70.1 (7-C), 70.3 (11-C), 75.9 (1-C), 76.6 (13-C), 76.8 (8-C), 77.0 (3-C), 121.1 (16-C), 128.2, 129.3, 130.6, 133.3 (11-CO<u>Ph</u>), 128.5, 129.5, 130.5, 133.2 (1-CO<u>Ph</u>), 131.2 (15-C), 164.1 (1-<u>C</u>OPh), 166.9 (11-<u>C</u>OPh), 169.9 (7-<u>C</u>OCH$_3$), 170.0 (2-<u>C</u>OCH$_3$), 208.9 (14-C).
6) Demarco P. V., Farkas E., Doddrell D., Mylari B. L., Wenkert E., *J. Am. Chem. Soc.*, **90**, 5480—5486 (1968).
7) IR (KBr) cm^{-1}: 3410, 3080, 1720, 1250, 712. UV (MeOH) nm (ε): 230 (24000), 274 (2000). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.89 (3H, s, 18-H$_3$), 1.07 (3H, s, 19-H$_3$), 1.46 (3H, s, 20-H$_3$), 1.54 (3H, s, 3-COCH$_3$), 1.65 (3H, s, 17-H$_3$), 1.87 (1H, br d, J=13.7 Hz, 6-Hα), 2.08 (1H, dd, J=13.7, 13.7 Hz, 6-Hβ), 2.24 (3H, s, 7-COCH$_3$), 2.52 (1H, d, J=13.7 Hz, 5-H), 2.99 (1H, dd, J=3.9, 9.8 Hz, 12-H), 3.24 (1H, d, J=10.3 Hz, 9-H), 4.40 (1H, br s, 2-H), 4.47 (1H, d, J=11.6 Hz, 16-Ha), 4.87 (1H, d, J=16.8 Hz, 16-Hb), 5.03 (1H, d, J=2.7 Hz, 3-H), 5.28 (1H, ddd, J=9.8, 11.6, 16.8 Hz, 15-H), 5.43 (1H, br s, 7-H), 5.49 (1H, br s, 1-H), 6.27 (1H, dd, J=3.9, 10.3 Hz, 11-H), 7.42 (2H, dd, J=7.9, 7.9 Hz), 7.43 (2H, dd, J=7.9, 7.9 Hz), 7.59 (1H, dd, J=7.9, 7.9 Hz), 7.60 (1H, dd, J=7.9, 7.9 Hz), 7.98 (2H, d, J=7.9 Hz), 8.06 (2H, d, J=7.9 Hz) (1- and 11-CO<u>Ph</u>). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 16.0 (20-C), 20.5 (2-CO<u>C</u>H$_3$), 21.2 (7-CO<u>C</u>H$_3$), 21.3 (6-C), 22.2 (19-C), 28.0 (18-C), 28.2 (17-C), 36.3 (5-C), 37.2 (4-C), 41.4 (9-C), 44.4 (10-C), 54.0 (12-C), 66.0 (2-C), 70.2 (7-C), 70.5 (11-C), 76.1 (13-C), 76.7 (8-C), 78.5 (3-C), 80.0 (1-C), 121.0 (16-C), 128.4, 128.5, 129.3, 129.6, 130.1, 130.2, 133.5, 133.6 (1- and 11-CO<u>Ph</u>), 131.6 (15-C), 166.2 (11-<u>C</u>OPh), 167.3 (1-<u>C</u>OPh), 169.3 (7-<u>C</u>OCH$_3$), 170.7 (2-<u>C</u>OCH$_3$), 208.3 (14-C).
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