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

Kazuyoshi OHASHI,[a] Takako BOHGAKI,[a] Toshiyuki MATSUBARA,[b] and Hirotaka SHIBUYA*,[a]

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,[a] Sanzo, 1 Gakuen-cho, Fukuyama, Hiroshima 729–0292, Japan and Toyama Prefectural Institute for Pharmaceutical Research,[b] 17–1, Nakataikouyama, Kosugi-machi, Imizu-gun, Toyama 939–0363, Japan. Received September 27, 1999; accepted November 23, 1999

Two novel migrated pimarane-type diterpenes named neoorthosiphols 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 lamiaceaous 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)[2] 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 [orthosiphols A (**3**)[3] and B (**4**),[3] orthosiphonones A (**5**) and B (**6**)[1]] and three benzochromenes [methylripariochromene A (**7**),[4] acetovanillochromene (**8**),[5] and orthochromene A (**9**)[1]] 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 neoorthosiphols A (**1**) and B (**2**),[6] as well as four flavones [5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**10**), eupatorin (**11**), tetramethylscutellarein (**12**) and sinensetin (**13**)].[7] 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 $[M+H]^+$, $C_{38}H_{45}O_{12}$, 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

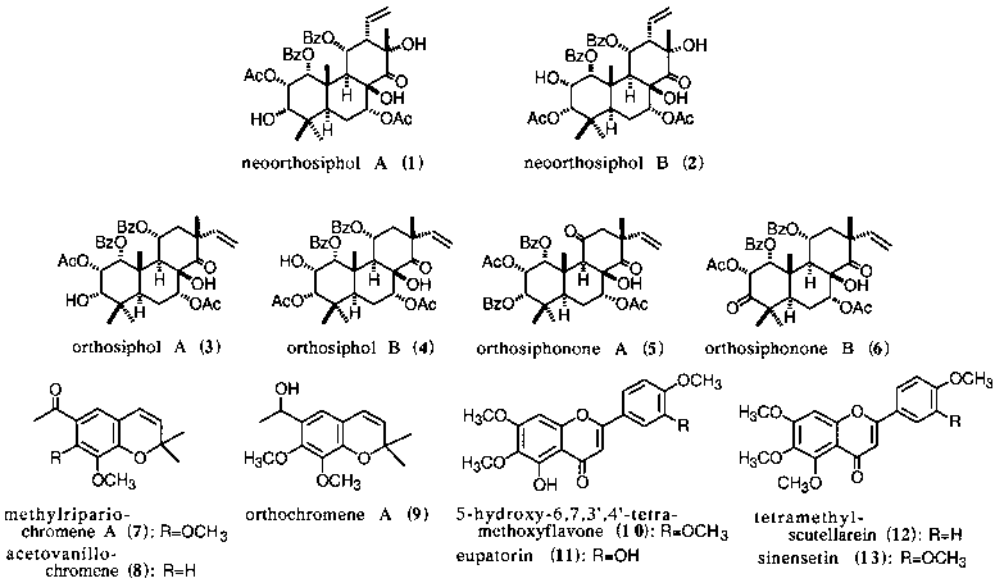

Fig. 1

* To whom correspondence should be addressed.

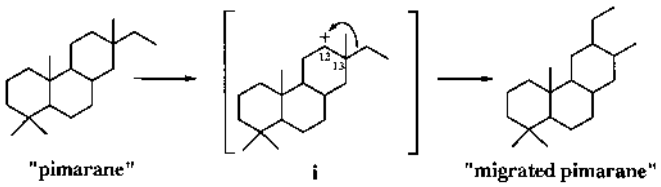

Fig. 2. Plausible Biosynthetic Route for "Migrated Pimarane" from "Pimarane"

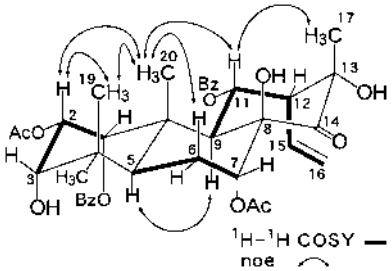

Fig. 3. ^{1}H–^{1}H COSY and ROESY Correlations of **1**

2000).

The ^{1}H-NMR spectrum showed signals due to four tertiary methyls, two acetoxymethyls, one hydroxymethine proton, four methine protons attached to an ester function, three olefinic protons and ten aromatic protons. The ^{13}C-NMR and the distortionless enhancement by polarization transfer (DEPT) spectra revealed the presence of six methyl carbons, two methylene carbons, nine methine carbons, one ketonic carbonyl carbon, four ester carbonyl carbons, twelve aromatic carbons and four quaternary carbons, including two carbons bearing a hydroxyl function (δ_C 76.6, 8-C; δ_C 76.9, 13-C).

The ^{1}H–^{1}H 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-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, 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**).[3] The orientations of 11-H and 17-H_3 were both determined to be β-axial based on the coupling constants of $J_{9,11}$ (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 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[9] ($\delta_{CDCl_3} - \delta_{C_5D_5N}$)$\Delta = -0.43$ ppm for 20-CH_3 and $\Delta = -0.30$ ppm for 17-CH_3. Furthermore, the coupling constant $J_{11,12}$ (3.5 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 (1-, 11-H) and two benzoyl carbonyl carbons, and between two hydroxymethine 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.

Table 1. Suppressive Effects on the Contraction Induced by K^+ in Endothelium-Denuded Rat Thoracic Aorta

Compound	IC_{50} (μmol/ml)
Neoorthosiphol A (**1**)	1.52×10^{-2}
Neoorthosiphol B (**2**)	6.01×10^{-2}
Orthosiphol A (**3**)	4.93×10^{-3}
Orthosiphol B (**4**)	4.23×10^{-3}
Orthosiphonone A (**5**)	3.69×10^{-3}
Orthosiphonone B (**6**)	3.89×10^{-3}
Methylriparіochromene A (**7**)	8.32×10^{-2}
Acetovanillochromene (**8**)	1.01×10^{-1}
Orthochromene A (**9**)	1.31×10^{-1}
5-Hydroxy-6,7,3′,4′-tetramethoxyflavone (**10**)	No suppression
Eupatorin (**11**)	No suppression
Tetramethylscutellarein (**12**)	8.08×10^{-3}
Sinensetin (**13**)	4.92×10^{-2}
Nifedipine[a]	1.79×10^{-5}

a) Positive control.

The absolute configuration of **1** was established by application of the exciton chirality method.[10] Positive maximum ($[\theta]_{236}$ +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 $[M+H]^+$, $C_{38}H_{45}LiO_{12}$, in the FAB-MS.

The ^{1}H- and ^{13}C-NMR spectra of **2**, including ^{1}H–^{1}H COSY, ROESY and HMBC correlations, 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-$\underline{C}OCH_3$ was observed in the HMBC spectrum, instead of that between 2-H and 2-$\underline{C}OCH_3$ 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[10] in the CD spectrum ($[\theta]_{237}$ +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).[8] This finding may be related to the use of kumis kucing for the treatment of hypertension in Javanese traditional medicine (jamu).

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. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-Lambda 500 spectrometer operating at 500 and 125 MHz for ^{1}H and ^{13}C 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 60F_{254} (Merck) was used to ascertain the purity of the compounds. The spots were visualized by spraying the plates with 1% $Ce(SO_4)_2$ in 10% aqueous sulfuric acid, followed by heating.

Plant Material *Orthosiphon ariatatus* (Bl.) Miq. was collected in Yogyakarta, Java Island, Indonesia, in December, 1995, and identified at the Herbarium Bogoriense, Research and Development Centre for Biology-LIPI, 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 H_2O extract (220 g, 27.5% from the leaves). The H_2O extract (23 g) was partitioned into a chloroform and water mixture (1:1). The chloroform phase was concentrated under reduced pressure to give a $CHCl_3$ extract (1.9 g, 2.3%) and a H_2O extract (21.1 g, 25%). The $CHCl_3$ extract (1.2 g) was subjected to silica gel column chromatography (SiO_2 40 g, *n*-hexane : EtOAc=2 : 1→EtOAc→$CHCl_3$: MeOH=10 : 1→MeOH) to give fraction 1 (820 mg, 1.5%), fraction 2 (80 mg, 0.15%), fraction 3 (110 mg, 0.21%), fraction 4 (130 mg, 0.25%). Fraction 1 (600 mg) was again purified by HPLC (YMC-Pack SIL, 250×20 mm; *n*-hexane : EtOAc=2 : 1) to afford neoorthosiphol A (**1**, 15 mg, 0.039%), neoorthosiphol B (**2**, 1.9 mg, 0.005%), orthosiphol A (**3**, 15 mg, 0.039%), orthosiphol B (**4**, 14 mg, 0.035%), orthosiphonone A (**5**, 8.9 mg, 0.023%) and orthosiphonone B (**6**, 1.2 mg, 0.003%), methylripariochromene A (**7**, 244 mg, 0.63%), acetovanillochromene (**8**, 11 mg, 0.030%), orthochromene A (**9**, 12 mg, 0.024%), 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**10**, 4.6 mg, 0.012%), and eupatorin (**11**, 2.7 mg, 0.007%). Fraction 2 (40 mg) was purified by HPLC (YMC-Pack SIL, 250×10 mm; *n*-hexane : EtOAc=2 : 3) tetramethylscutellarein (**12**, 4.1 mg, 0.011%) and sinensetin (**13**, 3.2 mg, 0.009%).

Neoorthosiphol A (**1**): Colorless plates from ether, mp 148—149 °C, $[\alpha]_D$ −28.7° ($CHCl_3$, at 20 °C). IR (KBr) cm^{-1}: 3420, 3080, 1720, 1267, 712. UV (MeOH) nm (ε): 230 (24000), 274 (2000). CD (MeOH) nm ([θ]): 204 (+60000), 235 (+63000). ^{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). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 15.5 (20-C), 20.9 (2C, 6-C, 2-$CO\underline{C}H_3$), 21.2 (7-$CO\underline{C}H_3$), 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$\underline{Ph}$), 128.5, 129.5, 130.5, 133.2 (1-CO$\underline{Ph}$), 131.2 (15-C), 164.1 (1-$\underline{C}$OPh), 166.9 (11-$\underline{C}$OPh), 169.9 (7-$\underline{C}OCH_3$), 170.0 (2-$\underline{C}OCH_3$), 208.9 (14-C). FAB-MS *m/z*: 693 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{38}H_{45}O_{12}$: 693.2911. Found: 693.2924 $(M+H)^+$.

Neoorthosiphol B (**2**): Colorless plates from ether, mp 194—195 °C, $[\alpha]_D$ +18.1° ($CHCl_3$, at 21 °C). IR (KBr) cm^{-1}: 3410, 3080, 1720, 1250, 712. UV (MeOH) nm (ε): 230 (24000), 274 (2000). CD (MeOH) nm ([θ]): 203 (+65000), 237 (+47000). ^{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-, 11-COPh). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 16.0 (20-C), 20.5 (2-$CO\underline{C}H_3$), 21.2 (7-$CO\underline{C}H_3$), 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-, 11-CO$\underline{Ph}$), 131.6 (15-C), 166.2 (11-$\underline{C}$OPh), 167.3 (1-$\underline{C}$OPh), 169.3 (7-$\underline{C}OCH_3$), 170.7 (2-$\underline{C}OCH_3$), 208.3 (14-C). FAB-MS *m/z*: 693 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{38}H_{45}O_{12}$: 693.2911. Found: 693.2894 $(M+H)^+$.

Effects of Thirteen Constituents from the Leaves of *Orthosiphon aristatus* on Contractile Responses Induced by K^+ in Endothelium-Denuded Rat Thoracic Aorta Male Wistar rats were sacrificed and their thoracic aorta were isolated. Each aortaspiral strip (approx. 2.5 mm×20 mm) was prepared following a removement of connective tissues, and suspended in a 30 ml organ bath filled with Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.55 mM $CaCl_2\cdot 2H_2O$, 1.18 mM $MgSO_4\cdot 7H_2O$, 1.18 mM KH_2PO_4, 11.1 mM $NaHCO_3$, 11.1 mM glucose) maintained at 37 °C. The solution was continuously aerated with a gas mixture of 95% O_2 and 5% CO_2. A resting tension of 1.0 g was initially applied to each preparation, and it was then allowed to equilibrate for 90 min before the start of the experiment. The endothelium was removed by gently rubbing the internal surface of the aorta with filter paper, and the functional loss of endothelial cell was confirmed pharmacologically by the absence of a 10^{-6} M ACh-induced relaxing response in *l*-phenylephrine-induced contractions. The muscle responses were measured isometrically by means of a force displacement transducer (TB-652T, Nihon Kohden, Tokyo) coupled to an amplifer (EF-601G, Nihon Kohden), and were described on a pen recorder. After the contractions by 60 mM K^+ reached a plateau, a sample was applied cumulatively. The relaxing response was expressed in terms of a percentage of the maximal relaxation developed by 10^{-4} M papaverine. IC_{50} values were determined from the dose–response curves using computer software (Delta Graph TM, Polaroid Computing, Tokyo).

Acknowledgments This work was supported by a Grant-in-Aid (No. 0641071, 08041186) for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

1) Part XXII: Shibuya H., Bohgaki T., Matsubara T., Watarai M., Ohashi K., Kitagawa I., *Chem. Pharm. Bull*., **47**, 695—698 (1999).
2) Riswan S., Sangat H. M., "Jamu as a Javanese Traditional Medicine in Indonesia," The Bioresources-Diversity, Ethnobiology Development and Sustainability International Centenary Conference, Sydney, July 1991.
3) Masuda T., Masuda K., Shiragami S., Jitoe A., Nakatani N., *Tetrahedron*, **48**, 6787—6793 (1992).
4) Guerin J. C., Reveillere H. P., Ducrey P., Toupet L., *J. Nat. Prod.*, **52**, 171—175 (1989).
5) Tailor D. R., Wright J. A., *Phytochemistry*, **10**, 1665—1669 (1971).
6) The preliminary report: Shibuya H., Bohgaki T., Ohashi K., *Chem. Pharm. Bull*., **47**, 911—912 (1999).
7) Lyckander I. M., Malterud K. E., *Acta Pharm. Nord.*, **4**, 159—166 (1992).
8) *a*) Abe A., Karaki H., *Jpn. J. Pharmacol*., **60**, 389—392 (1992); *b*) Matsubara T., Bohgaki T., Watarai M., Suzuki H., Ohashi K., Shibuya H., *Biol. Pharm. Bull.*, **22**, 1083—1088 (1999).
9) Demarco P. V., Farkas E., Doddrell D., Mylari B. L., Wenkert E., *J. Am. Chem. Soc*., **90**, 5480—5486 (1968).
10) *a*) Harada N., Chen Sow-Mei L., Nakanishi K., *J. Am. Chem. Soc.*, **97**, 5345—5352 (1975); *b*) Harada N., Iwabuchi J., Yokota Y., Uda H., Nakanishi K., *ibid*, **103**, 5590—5591 (1981).