New Diels-Alder Type Adducts from *Morus macroura* and Their Anti-oxidant Activities

Sheng-Jun DAI, Yan WU, Ying-Hong WANG, Wen-Yi HE, Ruo-Yun CHEN, and De-Quan YU*

Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College; Beijing 100050, P. R. China. Received May 17, 2004; accepted July 5, 2004

Fractionation of the ethanolic extract of the stem bark of *Morus macroura* resulted in the isolation of four new Diels-Alder type adducts, named guangsangons K—N (1, 2, 5, 6), together with two known compounds, mulberrofuran G (3) and K (4). Their structures were determined on the basis of spectroscopic analyses and chemical methods. Furthermore, by means of $^1\text{H-NMR}$ variable temperature experiments and the Cotton curves in the circular dichroism (CD) spectra, the stereochemistry of four new compounds was elucidated. The isolated new compounds showed good activity on anti-oxidant *in vitro*, with the inhibitory rates of MDA being from 91.8 to 100.0% at concentrations of 10^{-5} mol/L.

Key words Moraceae; *Morus macroura*; guangsangon K; guangsangon L; guangsangon M; guangsangon N

Morus macroura MiQ. belongs to the economically and medically important genus *Morus*, because its leaves have been an indispensable food source for silk-worms, and its root barks have been used to treat diabetes, arthritis, and rheumatism in Chinese herbal medicine.¹⁾ Previously, many novel compounds, which were regarded biogenetically as Diels-Alder adducts of dehydroprenylphenols and chalcone derivatives, were isolated from *M. alba*, *M. lhou*, *M. bombycis*, and so on.^{2–8)} In the course of our seeking novel bioactive compound, we started phytochemical research on the stem bark of *M. macroura*. The EtOH extract of *M. macroura* was partitioned with CHCl_3 and EtOAc, and the EtOAc fraction was sequentially subjected to column chromatography over silica gel, silica gel RP-18 and Sephadex LH-20 to give six compounds. The present paper deals with the isolation and structure elucidation of these compounds, as well as the evaluation of anti-oxidant effect of new compounds.

Results and Discussion

Guangsangon K (1) was obtained as a brown amorphous powder, and gave a dark green color with the methanolic ferric chloride test, and reacted positively to the magnesium hydrochloric acid test. The molecular formula was determined to be $\text{C}_{35}\text{H}_{30}\text{O}_{11}$ by HR-FAB-MS, which showed a quasi-molecular ion peak at m/z 627.1873 $[\text{M}+\text{H}]^+$. The UV spectrum exhibited maxima at 232 (sh), 278 and 319 (sh) nm, and showed a bathochromic shift in the presence of aluminum chloride, indicating the presence of a chelated carbonyl group.⁹⁾ The IR spectrum displayed absorption bands at 3402, 1674, 1612, 1502 and 1462 cm^{-1} assignable to hydroxyl, conjugated carbonyl and benzene ring moieties. The $^1\text{H-NMR}$ spectrum of 1 displayed the following proton sig-

nals, a singlet at δ 13.32 (1H, s, HO-10") for a chelated hydroxyl group; one set of ABX aromatic protons assignable to a 2,4-dihydroxyphenyl moiety at δ 6.23 (d, $J=2.5$ Hz, H-17"), 6.11 (dd, $J=2.5, 9.0$ Hz, H-19") and 6.88 (d, $J=9.0$ Hz, H-20"); another set of ABX aromatic protons assignable to the 2,4-dihydroxybenzoyl unit at δ 5.96 (d, $J=2.0$ Hz, H-11"), 6.22 (dd, $J=2.0, 9.0$ Hz, H-13") and 7.46 (d, $J=9.0$ Hz, H-14"); other set of ABX aromatic protons at δ 6.72 (d, $J=2.5$ Hz, H-8), 6.59 (dd, $J=2.5, 9.0$ Hz, H-6) and 7.74 (d, $J=9.0$ Hz, H-5), a pair of *ortho*-coupled aromatic protons at δ 6.25 (d, $J=8.5$ Hz, H-5') and 7.09 (d, $J=8.5$ Hz, H-6'), as well as a pair of vicinal-coupled aliphatic protons at δ 4.72 (d, $J=12.0$ Hz, H-3) and 5.48 (d, $J=12.0$ Hz, H-2) attributable to the dihydroflavonol moiety; and protons for a trisubstituted methylcyclohexene ring at δ 5.54 (s, H-2"), 4.53 (br s, H-3"), 4.55 (br s, H-4"), 3.72 (br s, H-5"), 2.67 (m, H-6"), 2.22 (m, H-6") and 1.82 (3H, s, H-7"). The long-range correlation between H-20" and C-5" suggested that the 2,4-dihydroxyphenyl moiety was connected to the methylcyclohexene ring at C-5". Therefore, the 2,4-dihydroxybenzoyl and dihydroflavonol moieties were connected to the methylcyclohexene ring at C-4" and C-3".

In the $^1\text{H-NMR}$ spectrum (CD_3COCD_3), the proton signals of the methylcyclohexene ring were broadened, this phenomenon suggested that 1 existed as an equilibrium mixture of conformational isomers in solution,³⁾ and variable temperature studies were then carried out. Measured in $\text{DMSO}-d_6$ at 100°C , the $^1\text{H-NMR}$ spectrum of the methylcyclohexene ring was resolvable as shown in Fig. 1. Both coupling constants of H-4"/H-5" and H-4"/H-3" were observed to be 10.0 Hz, this suggested H-3", H-4" and H-5" to be axial and the relative configurations of three protons were all-*trans*.

Fig. 1. The Chemical Shifts and Key Coupling Constants (in Parentheses) of the Methylcyclohexene Ring for 1, 2, 5 and 6 (in $\text{DMSO}-d_6$, at 100°C)

* To whom correspondence should be addressed. e-mail: dqyu@imm.ac.cn

Chart 1. The Chemical Structures of **1**, **2**, **3**, **4**, **5** and **6** from *Morus macroura*

The stereochemistry of **1** with the absolute configurations of *R* (C-2), *R* (C-3), *R* (C-3''), *R* (C-4'') and *S* (C-5'') was determined by CD spectrum, which showed a positive Cotton effect at 347 nm, along with two negative Cotton effects at 282 and 287 nm.^{10,11)}

Guangsangon L (**2**) was isolated and purified as a yellow amorphous powder. The HR-FAB-MS showed a quasi-molecular ion peak at m/z 477.1558 $[M+H]^+$, consistent with a molecular formula of $C_{27}H_{24}O_8$. A comparison of NMR (1H , ^{13}C) spectra of **2** with those of **1** indicated the structure of **2** differed from **1** only in the moiety at C-3'' of the methylcyclohexene ring. Instead of a dihydroflavonol moiety of **1**, a 2,4-dihydroxybenzaldehyde moiety was present in **2**. In the 1H - 1H COSY spectrum (measured in $DMSO-d_6$, 100 °C), H-4'' was observed to be coupled with H-3'' (J , 10.0 Hz) and also with H-5'' (J , 10.0 Hz), this suggested that three protons were all axial and their relative configurations were all-*trans*. In the previous paper,¹⁰⁾ it was reported that the absolute configurations of three chiral centers on the methylcyclohexene ring in the all-*trans* adducts were established as *R* (C-3''), *R* (C-4'') and *S* (C-5''), while in the *cis-trans* adducts as *S* (C-3''), *R* (C-4'') and *S* (C-5''). Furthermore, the all-*trans* adducts exhibited negative optical rotation values while the *cis-trans* adducts showed positive ones. Since **2** displayed negative optical rotation value, the absolute configurations of three unsymmetrical carbons were assigned as *R* (C-3''), *R* (C-4'') and *S* (C-5''), respectively.

Guangsangon M (**5**) was obtained as a brown amorphous powder, and gave a dark green color with the methanolic ferric chloride test, and was positive to the magnesium hydrochloric acid test. The molecular formula was assigned as $C_{35}H_{30}O_{10}$ by HR-FAB-MS, which showed a quasi-molecular ion peak at m/z 611.1962 $[M+H]^+$. By means of NMR (1H , ^{13}C) data, the structure of **5** was found to be identical with **1** except for the difference of moiety at C-3'' of the methylcyclohexene ring. In contrast to the dihydroflavonol moiety of **1**, a flavanone unit was connected to the methylcyclohexene ring of **5**. 1H -NMR variable temperature experiment (Fig. 1) showed the relative configurations of H-3'', H-4'' and H-5'' were all-*trans*. In the CD spectrum, **5** gave a positive Cotton

Table 1. ^{13}C -NMR Data of **1**, **2**, **3** and **6** in Acetone- d_6 (125 MHz, 25 °C, δ in ppm)

No.	1	2	5	6
1		124.9		
2	79.3	163.4	75.3	75.5
3	72.8	104.0	43.3	43.4
4	193.7	164.9	190.3	190.2
5	128.4	116.0	129.6	128.5
6	112.0	136.2	110.7	110.5
7	165.6	195.6	165.1	165.0
8	104.1		103.2	103.2
9	165.1		164.4	164.7
10	117.1		114.4	114.7
1'	117.4		117.6	117.6
2'	156.1		155.8	155.7
3'	109.4		102.1	102.6
4'	157.4		153.4	153.2
5'	108.5		121.9	122.0
6'	128.2		128.2	128.0
1''	134.1	135.1	133.6	133.7
2''	125.2	125.3	125.6	125.5
3''	47.0	42.2	41.4	43.4
4''	49.7	50.9	50.1	50.8
5''	47.0	42.2	41.4	43.4
6''	37.7	37.7	37.6	37.2
7''	23.5	23.5	22.7	22.7
8''	208.7	209.0	208.7	208.6
9''	115.8	115.8	115.0	115.1
10''	166.6	166.2	165.1	165.1
11''	102.7	102.9	102.8	102.1
12''	165.8	163.9	164.7	164.4
13''	108.2	107.8	106.8	106.9
14''	129.8	134.0	128.7	128.7
15''	121.8	121.6	121.3	121.2
16''	156.6	157.5	156.6	156.5
17''	104.0	103.0	103.3	103.2
18''	156.5	156.7	155.5	155.3
19''	107.6	107.6	107.4	107.2
20''	134.2	133.1	133.8	133.8

effect at 305 nm, along with two negative Cotton effects at 285 and 326 nm, and corresponding to the absolute configurations of *R* (C-2), *R* (C-3''), *R* (C-4'') and *S* (C-5'').^{10,11,13)}

Guangsangon N (**6**) was obtained as a brown amorphous

powder. The molecular formula was determined to be $C_{35}H_{30}O_{10}$ by HR-FAB-MS, which displayed a quasi-molecular ion peak at m/z 611.1958 $[M+H]^+$. Similar to compound **5**, the NMR (1H , ^{13}C) spectra of **6** also exhibited signals assignable to the 2,4-dihydroxyphenyl, 2,4-dihydroxybenzoyl and flavanone moieties. According to the results of 1H -NMR variable temperature experiment (Fig. 1) and CD spectrum (with three negative Cotton effects at 269, 288 and 303 nm, and a positive Cotton effect at 333 nm), the relative configurations of H-3'', H-4'' and H-5'' were assigned as all-*trans*, and the absolute configurations of C-3'', C-4'', C-5'' and C-2 were assigned as *R*, *R*, *S* and *S*, respectively.^{10–12)}

In the case of the bioactivities of compounds **1**, **2**, **5**, **6** and Vit E, the inhibitory rates of MDA were 91.8%, 97.6%, 98.3%, 100.0% and 18.2% at concentrations of 10^{-5} mol/l, respectively. Thus, it could be concluded that these compounds had good anti-oxidant activities.

Phytochemical study of this plant also led to the isolation of two known constituents, mulberrofuran G¹⁴⁾ (**3**) and K¹⁵⁾ (**4**), and they were identified by comparison of their physical and spectral data ($[\alpha]_D$, UV, IR, 1H -NMR, ^{13}C -NMR, MS) with reported values or those of commercial samples.

Experimental

General Procedures UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. NMR spectra were recorded on a Varian Unity INOVA 500 at 500 MHz (1H) and 125 MHz (^{13}C), with TMS as the internal standard. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer with KBr disks. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. FAB-MS and HR-FAB-MS were recorded on an Autospec-Ultima ETOF MS spectrometer. CD spectra were on a JASCO J-725 spectrometer. Silica gel (200–300 mesh) for column chromatography and silica gel GF254 for preparative TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Precoated plates of silica gel GF254 and silica gel RP-18 F254s were used for TLC, and detected under UV. HPLC separation was performed on CONSTA METRIC 3200 and a UV detector at 254 nm.

Plant Material *Morus macroura* Miq. was collected in Xishuangbanna, Yunnan Province, Peoples Republic of China, in November 2002, and identified by Professor Kai-Jiao Jiang, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (EX97038) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation The air-dried stem bark (9.8 kg) of *Morus macroura* Miq. was finely cut and extracted with refluxing EtOH successively (10 l, 3 h, 3 times). Evaporation of the solvent under reduced pressure provided the ethanolic extract (684.2 g). The extract was dissolved and suspended in H_2O (3.0 l), and partitioned with $CHCl_3$ (3 $\times$ 4 l), and EtOAc (3 $\times$ 4 l). The EtOAc fraction (95.4 g) was subjected to column chromatography on silica gel (10 $\times$ 120 cm), eluted with $CHCl_3$ and MeOH in increasing polarity, and were combined according to TLC monitoring to eight fractions. Fraction I (1.8 g) was chromatographed over reversed-phase silica gel [eluted by MeOH– H_2O , 30 : 70–50 : 50, v/v] and purified by Sephadex LH-20 [eluted by MeOH– H_2O , 50 : 50, v/v], to give guangsangon K (**1**, 6 mg) and guangsangon L (**2**, 5 mg). Fraction III (3.3 g) was subjected to reversed-phase silica gel column chromatography [eluted by MeOH– H_2O , 40 : 60–50 : 50, v/v] and purified by preparative TLC ($CHCl_3$ –MeOH, 7 : 3, v/v) to give mulberrofuran G (**3**, 8 mg), mulberrofuran K (**4**, 15 mg) and a mixture (8 mg). The mixture was further separated by C_{18} HPLC (Alltech C-18, 250 $\times$ 10 mm, MeOH– H_2O , 35 : 65, v/v) to give guangsangon M (**5**, 4 mg, t_R = 26 min) and guangsangon N (**6**, 2 mg, t_R = 35 min).

Guangsangon K (**1**): Brown amorphous powder, $[\alpha]_D^{25}$ –178.5° (c = 0.14, MeOH). UV λ_{max} (MeOH) nm: 232 (sh), 278, 319 (sh). IR (KBr) ν_{max} cm^{-1} : 3392, 1678, 1610, 1508, 1462. FAB-MS m/z : 627.4 $[M+H]^+$. HR-FAB-MS m/z : 627.1873 $[M+H]^+$, (Calcd for $C_{35}H_{31}O_{11}$: 627.1866). CD (c = 7.19×10^{-4} M, MeOH) $\Delta\epsilon$ (nm): 282 (–25.6), 287 (–24.3), 347 (+0.8). 1H -NMR (500 MHz, CD_3COCD_3) δ : 13.32 (1H, s, HO-10''), 7.74 (1H, d, J = 9.0 Hz, H-5), 7.46 (1H, d, J = 9.0 Hz, H-14''), 7.09 (1H, d, J = 8.5 Hz, H-

6'), 6.88 (1H, d, J = 9.0 Hz, H-20''), 6.72 (1H, d, J = 2.5 Hz, H-8), 6.59 (1H, dd, J = 2.5, 9.0 Hz, H-6), 6.25 (1H, d, J = 8.5 Hz, H-5'), 6.23 (1H, d, J = 2.5 Hz, H-17''), 6.22 (1H, dd, J = 2.0, 9.0 Hz, H-13''), 6.11 (1H, dd, J = 2.5, 9.0 Hz, H-19''), 5.96 (1H, d, J = 2.0 Hz, H-11''), 5.54 (1H, s, H-2''), 5.48 (1H, d, J = 12.0 Hz, H-2), 4.72 (1H, d, J = 12.0 Hz, H-3), 4.55 (1H, brs, H-4''), 4.53 (1H, brs, H-3''), 3.72 (1H, brs, H-5''), 2.67 (1H, m, H-6''), 2.22 (1H, m, H-6''), 1.82 (3H, s, H-7''). ^{13}C -NMR data: see Table 1.

Guangsangon L (**2**): Yellow amorphous powder, $[\alpha]_D^{25}$ –389.3° (c = 0.14, MeOH). UV λ_{max} (MeOH) nm: 236 (sh), 280. IR (KBr) ν_{max} cm^{-1} : 3408, 1674, 1605, 1512, 1464. FAB-MS m/z : 477.2 $[M+H]^+$. HR-FAB-MS m/z : 477.1558 $[M+H]^+$, (Calcd for $C_{27}H_{25}O_8$: 477.1549). 1H -NMR (500 MHz, CD_3COCD_3) δ : 13.04 (1H, s, HO-10''), 11.19 (1H, s, OH-4), 7.58 (1H, d, J = 9.0 Hz, H-14'') 7.57 (1H, s, H-6), 6.89 (1H, d, J = 9.0 Hz, H-20''), 6.20 (1H, d, J = 2.0 Hz, H-17''), 6.12 (1H, s, H-3), 6.10 (1H, dd, J = 2.0, 9.0 Hz, H-19''), 6.09 (1H, dd, J = 2.0, 9.0 Hz, H-13''), 5.97 (1H, d, J = 2.0 Hz, H-11''), 5.35 (1H, s, H-2''), 4.42 (1H, brs, H-4''), 4.19 (1H, brs, H-3''), 3.63 (1H, brs, H-5''), 2.23 (1H, m, H-6''), 2.61 (1H, m, H-6''), 1.77 (1H, s, H-7''). ^{13}C -NMR data: see Table 1.

Guangsangon M (**5**): Brown amorphous powder, $[\alpha]_D^{25}$ –276.5° (c = 0.12, MeOH). UV λ_{max} (MeOH) nm: 230 (sh), 278, 319 (sh). IR (KBr) ν_{max} cm^{-1} : 3342, 1650, 1616, 1506, 1496, 1456, 1369. FAB-MS m/z : 611.2 $[M+H]^+$. HR-FAB-MS m/z : 611.1962 $[M+H]^+$, (Calcd for $C_{35}H_{31}O_{10}$: 611.1917). CD (c = 8.20×10^{-4} M, MeOH) $\Delta\epsilon$ (nm): 285 (–8.3), 305 (+0.7), 326 (–7.6). 1H -NMR (500 MHz, CD_3COCD_3) δ : 13.06 (1H, s, HO-10''), 7.72 (1H, d, J = 9.0 Hz, H-5), 7.64 (1H, d, J = 9.0 Hz, H-14''), 7.39 (1H, s, H-6'), 6.85 (1H, d, J = 9.0 Hz, H-20''), 6.68 (1H, d, J = 2.5 Hz, H-8), 6.52 (1H, dd, J = 2.5, 9.0 Hz, H-6), 6.35 (1H, dd, J = 2.0, 9.0 Hz, H-13''), 6.24 (1H, s, H-3'), 6.22 (1H, d, J = 2.5 Hz, H-17''), 6.08 (1H, dd, J = 2.5, 9.0 Hz, H-19''), 5.93 (1H, d, J = 2.0 Hz, H-11''), 5.61 (1H, brd, J = 13.5 Hz, H-2), 5.34 (1H, s, H-2''), 4.53 (1H, brs, H-4''), 4.36 (1H, brs, H-3''), 3.63 (1H, brs, H-5''), 2.96 (1H, dd, J = 13.5, 16.5 Hz, H-3a), 2.72 (1H, m, H-6''), 2.55 (1H, brd, J = 16.5 Hz, H-3b), 2.20 (1H, m, H-6''), 1.73 (3H, s, H-7''). ^{13}C -NMR data: see Table 1.

Guangsangon N (**6**): Brown amorphous powder, $[\alpha]_D^{21}$ –335.3° (c = 0.13, MeOH). UV λ_{max} (MeOH) nm: 206, 231 (sh), 276, 316 (sh). IR (KBr) ν_{max} cm^{-1} : 3406, 1675, 1616, 1505, 1456. FAB-MS m/z : 611.4 $[M+H]^+$. HR-FAB-MS m/z : 611.1958 $[M+H]^+$, (Calcd for $C_{35}H_{31}O_{10}$: 611.1917). CD (c = 8.20×10^{-4} M, MeOH) $\Delta\epsilon$ (nm): 269 (–6.7), 288 (–7.8), 303 (–7.0), 333 (+1.2). 1H -NMR (500 MHz, CD_3COCD_3) δ : 13.07 (1H, s, HO-10''), 7.74 (1H, d, J = 9.0 Hz, H-5), 7.48 (1H, d, J = 9.0 Hz, H-14''), 7.38 (1H, s, H-6'), 6.87 (1H, d, J = 9.0 Hz, H-20''), 6.58 (1H, dd, J = 2.5, 9.0 Hz, H-6), 6.54 (1H, d, J = 2.5 Hz, H-8), 6.25 (1H, d, J = 2.5 Hz, H-17''), 6.20 (1H, dd, J = 2.5, 9.0 Hz, H-19''), 6.19 (1H, s, H-3'), 6.06 (1H, dd, J = 2.0, 9.0 Hz, H-13''), 5.97 (1H, d, J = 2.0 Hz, H-11''), 5.67 (1H, brd, J = 13.0 Hz, H-2), 5.35 (1H, s, H-2''), 4.31 (1H, brs, H-4''), 4.23 (1H, brs, H-3''), 3.61 (1H, brs, H-5''), 3.04 (1H, dd, J = 13.9, 18.0 Hz, H-3a), 2.73 (1H, m, H-6''), 2.61 (1H, brd, J = 18.0 Hz, H-3b), 2.19 (1H, m, H-6''), 1.76 (3H, s, H-7''). ^{13}C -NMR data: see Table 1.

Anti-oxidation Bioassays The anti-oxidant activities of **1**, **2**, **5**, **6** and Vit E were determined by the content of MDA (malondialdehyde), a compound that was produced during microsomal lipid per-oxidation induced by ferrous-cysteine. MDA was detected by using the thiobarbituric acid (TBA) method. Briefly, 1 mg/ml microsomal protein, different concentration compound or vehicle and 0.2 mM cysteine in 0.1 M PBS were incubated for 15 min at 37 °C, added 0.5 mM ferrous, mixed and incubated for 15 min at 37 °C again. The equal volume of 20% TCA was added to terminate the reaction and centrifuged for 10 min at 3000 rpm. The supernatants reacted with 0.67% TBA for 10 min at 100 °C. After cooling, the MDA was quantified by determining the absorbance at 532 nm, and then calculated the inhibition rate.

References

- 1) Sun S.-G., Chen R.-Y., Yu D.-Q., *J. Asian Nat. Prod. Res.*, **3**, 253–259 (2001).
- 2) Nomura T., Fukai T., Junko M., Imashimizu A., Terada S., Hama M., *Planta Medica*, **46**, 167–174 (1982).
- 3) Nomura T., Fukai T., Hano T., Nemoto K., Terada S., Kuramochi T., *Planta Medica*, **47**, 151–156 (1983).
- 4) Hano Y., Hirakura K., Nomura T., Terada S., Fukushima K., *Planta Medica*, **48**, 127–130 (1984).
- 5) Hirakura K., Fukai T., Hano Y., Nomura T., *Phytochemistry*, **24**, 159–161 (1985).
- 6) Hano Y., Aida M., Nomura T., *J. Nat. Prod.*, **53**, 391–395 (1990).
- 7) Shen R.-C., Lin M., *Phytochemistry*, **57**, 1231–1235 (2001).

- 8) Hiracura K., Hano Y., Fukai T., Nomura T., Uzawa J., Fukushima K., *Chem. Pharm. Bull.*, **33**, 1088—1096 (1985).
- 9) Sherif C. A., Gupta R. K., Krishnamurti M., *Tetrahedron Lett.*, **21**, 641—642 (1980).
- 10) Hano Y., Suzuki S., Nomura T., Iitaka Y., *Heterocycles*, **27**, 2315—2324 (1988).
- 11) Gaffield W., *Tetrahedron*, **26**, 4093—4108 (1970).
- 12) Hano Y., Suzuki S., Kohno H., Nomura T., *Heterocycles*, **27**, 75—81 (1986).
- 13) Snatzke G., Znatke F., *Tetrahedron*, **29**, 909—912 (1973).
- 14) Fukai T., Hano Y., Hirakura K., Nomura T., Fukushima K., *Chem. Pharm. Bull.*, **33**, 3195—3204 (1985).
- 15) Hano Y., Kohno H., Nomura T., *Chem. Pharm. Bull.*, **33**, 5294—5300 (1985).