New Pyrrole Alkaloids with Bulky *N*-Alkyl Side Chains Containing Stereogenic Centers from *Lycium chinense*

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Four new pyrrole alkaloids, methyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]propanoate (**1**), methyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-3-(4-hydroxyphenyl)propanoate (**2**), dimethyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanedioate (**3**), and dimethyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]pentanedioate (**4**), were isolated from the AcOEt extract of the fruits of *Lycium chinense* MILLER (Solanaceae). The stereogenic center C(2) in the bulky *N*-alkyl side chain in each of **1**–**4** seems to hold the H-atoms of nearby CH_2 groups, $CH_2(7')$ and $CH_2(3)$ (if $R \neq H$), leading to two different chemical shifts in the ^{1}H-NMR spectrum due to their diastereotopic characteristics. In the ^{1}H-NMR data of each of **2**–**4**, the enhancement of H–C(2) signal was inhibited by the R group, probably due to steric hindrance, and its chemical shift was influenced by the anisotropy effect. The structures of **1**–**4** were elucidated by analysis of various spectroscopic data, including 1D- and 2D-NMR.

Introduction. – The fruits of *Lycium chinense* MILLER (Lycii Fructus, Solanaceae) are being used as a traditional tonic medicine for treating liver and kidney deficiency, and for moistening lungs [1]. There exist reports on the constituents of this species, including betaine [2], cerebroside [3], pyrrole alkaloid [4], and zeaxanthin [5], together with their biological effects such as antihepatotoxic [3–5], antioxidant [6], and neuroprotective activities [2]. In the present study, the AcOEt fraction of a Lycii Fructus extract was investigated and afforded the four new pyrrole alkaloids, **1**–**4** (*Fig. 1*).

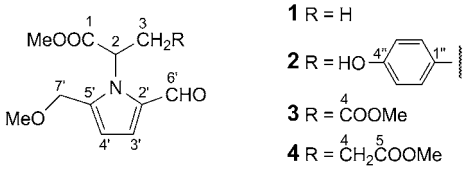

Fig. 1. *Structures of Compounds* **1**–**4** *isolated from the fruits of* L. chinense

[1]) These co-authors contributed equally to this work.

Results and Discussion. – Compound **1** was obtained as an optically active white powder, which showed a molecular-ion peak at *m/z* 225.1002 (M^+) in the HR-EI-MS, corresponding to the formula $C_{11}H_{15}NO_4$. The UV spectrum of **1** exhibited an absorption maximum at 260 nm ($\log \varepsilon$ 3.9), indicating the presence of a conjugated system [7]. The ^{1}H-NMR spectrum of **1** (*Table*) showed a set of two *doublet*s at δ(H) 6.32 (H–C(4′)) and 7.07 (H–C(3′)) with a vicinal coupling constant of 4.2 Hz. The combined evidence indicated the presence of a 2,5-disubstituted pyrrole derivative [4][8]. The ^{1}H- and ^{13}C-NMR spectra exhibited signals of one O-bearing CH_2 group (δ(H) 4.54 (*d*, *J* = 13.0, H_a–C(7′)) and 4.48 (*d*, *J* = 13.0, H_b–C(7′))/δ(C) 66.5 (C(7′))), an CHO group (9.34 (*s*, 1 H)/180.7 (C(6′))), and a MeO group (3.31 (*s*, 3 H)/58.0 (*Me*O–C(7′))), which, taken together, all clearly indicated the presence of a 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde derivative [8][9]. Signals of a methyl propanoate moiety appeared at δ(H) 5.37 (*q*, *J* = 6.8, 1 H)/δ(C) 55.9 (C(2)); 1.64 (*d*, *J* = 6.8, 3 H)/18.0 (C(3)); 3.66 (*s*, 3 H)/52.9 (*Me*OOC(1)); and δ(C) 172.4 (C(1)). The HMBC experiment of **1** showed three-bond connectivities H–C(2)/C(2′) and C(5′), which provided strong evidence for the assignment of C(2) at the N-atom in the pyrrole ring (*Fig. 2*). The stereogenic center C(2) seems to hold the H-atoms of CH_2(7′), leading to two different chemical shifts in the ^{1}H-NMR spectrum of **1** due to their diastereotopic characteristics. In case of previously reported pyrrole alkaloids containing bulky *N*-alkyl groups without stereogenic centers, two H-atoms of CH_2(7′) gave rise to identical peaks in their ^{1}H-NMR spectra [4]. As a result, the structure of **1** was elucidated as methyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]propanoate.

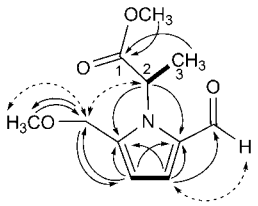

Fig. 2. *Important* ^{1}*H,*^{1}*H-COSY* (—), *NOESY* (H ↔ H), *and HMB* (H → C) *correlations of* **1**

Compound **2** was obtained as a white powder. Its molecular formula was established as $C_{17}H_{19}NO_5$ on the basis of its molecular-ion peak at *m/z* 317.1258 (M^+) in the HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of compound **2** were similar to those of **1**, except for the following peaks: the signals of a 1,4-disubstituted benzene ring system appeared at δ(H) 6.63 (*d*, *J* = 8.4, 2 H)/δ(C) 131.2 (C(2″,6″)); 6.56 (*d*, *J* = 8.4, 2 H)/116.3 (C(3″,5″)); δ(C) 157.5 (C(4″)), and 129.6 (C(1″)), together with those of a CH_2 group at δ(H) 3.56 (*dd*, *J* = 14.3, 3.4, H_a–C(3)), 3.13 (*dd*, *J* = 14.3, 11.2, H_b–C(3))/δ(C) 38.1 (C(3)), instead of the Me group at C(3) in **1**. The HMBCs CH_2(3)/C(1), C(2), and C(2″,6″), and H–C(2″,6″)/C(3) and C(4″) suggested that the 4-hydroxyphenyl moiety was connected to C(3), thus extending the former methyl propanoate functionality of **1** in compound **2**. In the ^{1}H-NMR spectrum, the two H-atoms of CH_2(7′) attached to the pyrrole ring in **2**, resonated at δ(H) 3.87 and 3.65, whereas the two H-atoms in **1** appeared at δ(H) 4.54

Table. *^{1}H- and ^{13}C-NMR Data* (at 400 and 100 MHz, resp.; CD_3OD) *of Compounds* **1**–**4**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		172.4		171.4		172.9		171.7
2	5.37 (*q*, *J* = 6.8)	55.9	5.24 (br. *s*)	62.8	5.72 (br. *s*)	56.6	5.34 (br. *s*)	59.2
3	1.64 (*d*, *J* = 6.8)	18.0	3.56 (*dd*, *J* = 14.3, 3.4), 3.13 (*dd*, *J* = 14.3, 11.2)	38.1	3.47 (*dd*, *J* = 17.1, 5.8), 2.93 (*dd*, *J* = 17.1, 7.6)	37.9	2.64–2.73 (*m*)	27.9
4						170.9	2.13–2.25 (*m*)	31.1
5								174.9
2′		133.8		133.9		133.8		134.1
3′	7.07 (*d*, *J* = 4.2)	127.1	7.11 (*d*, *J* = 4.2)	127.6	7.10 (*d*, *J* = 4.0)	127.5	7.10 (*d*, *J* = 4.2)	127.5
4′	6.32 (*d*, *J* = 4.2)	113.0	6.13 (*d*, *J* = 4.2)	112.3	6.32 (*d*, *J* = 4.0)	113.0	6.34 (*d*, *J* = 4.2)	113.2
5′		141.2		142.7		142.3		142.1
6′	9.34 (*s*)	180.7	9.41 (*s*)	180.8	9.34 (*s*)	180.8	9.35 (*s*)	180.8
7′	4.54, 4.48 (2*d*, *J* = 13.0)	66.5	3.87, 3.65 (2*d*, *J* = 13.4)	66.5	4.78, 4.45 (2*d*, *J* = 13.0)	66.8	4.49, 4.44 (2*d*, *J* = 13.0)	66.5
1″				129.6				
2″, 6″			6.63 (*d*, *J* = 8.4)	131.2				
3″, 5″			6.56 (*d*, *J* = 8.4)	116.3				
4″				157.5				
*Me*O–C(7′)	3.31 (*s*)	58.0	3.11 (*s*)	57.9	3.30 (*s*)	58.1	3.30 (*s*)	58.1
*Me*OOC(1)	3.66 (*s*)	52.9	3.70 (*s*)	52.9	3.62 (*s*)	52.5	3.67 (*s*)	53.0
*Me*OOC(4)					3.66 (*s*)	53.2		
*Me*OOC(5)							3.60 (*s*)	52.2

and 4.48. The H–C(2) of compound **2** appeared at δ(H) 5.24, whereas that of compound **1** appeared at δ(H) 5.37. These shielding effects of H-atoms at C(7′) and C(2) are probably due to the magnetic anisotropic effect of the phenyl ring in **2**. In addition, a steric hindrance of the phenyl ring seems to be responsible for the weak intensity of H–C(2) in **2**, which appeared as a broad *singlet*, whereas that of **1** resonated as a *quadruplet* with normal intensity. This phenomenon was also observed for a similar compound, methyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-3-phenylpropanoate [8], which was different from **2** only in the presence of a OH group at C(4″). The H-atoms of CH_2(7′) and CH_2(3) showed diastereotopic characteristics in the ^{1}H-NMR spectrum of **2** due to the presence of a stereogenic center C(2) as described for **1**. Thus, **2** was determined as methyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-3-(4-hydroxyphenyl)propanoate.

Compound **3** was obtained as a white powder and showed a molecular-ion peak at *m*/*z* 283.1059 (M^+) in the HR-EI-MS, consistent with the formula $C_{13}H_{17}NO_6$. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **1**, except for the presence of COOMe signals at δ(H) 3.66 (*s*, 3 H)/δ(C) 53.2 (*Me*OOC(4)), and δ(C) 170.9 (C(4)), along with those of a CH_2 group, forming part of an *ABX*-system at δ(H) 3.47 (*dd*, J = 17.1, 5.8, H_a–C(3)) and 2.93 (*dd*, J = 17.1, 7.6, H_b–C(3))/δ(C) 37.9 (C(3)), instead of the Me group at C(3) in **1**. These peaks, along with signals of a CH group at δ(H) 5.72 (br. *s*, 1 H)/δ(C) 56.6 (C(2)), of a MeO group at δ(H) 3.62 (*s*, 3 H)/δ(C) 52.5 (*Me*OOC(1)), and of another ester COOMe C-atom at δ(C) 172.9 (C(1)) indicated a dimethyl butanedioate moiety in compound **3** [10]. The presence of this group was supported by the molecular-ion peak at *m*/*z* 283 (M^+) and the base peak at *m*/*z* 138 ([M – 145 (MeOOCCHCH$_2$COOMe)]$^+$) for the pyrrole moiety (loss of a dimethyl butanedioate group) in the EI-MS spectrum. The HMBC features *Me*OOC(1)/C(1), *Me*OOC(4)/C(4), and CH_2(3)/C(2) and C(4) provided unambiguous evidence for a substituted dimethyl butanedioate moiety. On the other hand, the ^{1}H-NMR data of **3** showed a weak broad *singlet* at δ(H) 5.72 for H–C(2) which was much more deshielded than that of compound **1**, probably due to the anisotropy effect of the C=O functionality of the R group in **3**. The weak enhancement of the H–C(2) signal seems to be also affected by the R group, COOMe functionality in **3** [8]. In addition, each H-atom of CH_2(7′) and CH_2(3) in **3**, appeared at two different chemical shifts due to the stereogenic center C(2). Therefore, **3** was identified as dimethyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanedioate.

Compound **4** was obtained as a white powder which exhibited a molecular-ion peak at *m*/*z* 297.1215 (M^+) in the HR-EI-MS, corresponding to the formula $C_{14}H_{19}NO_6$. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3**, except for the presence of an CH_2CH_2 group resonating at δ(H) 2.64 – 2.73 (*m*, 2 H)/δ(C) 27.9 (C(3)), and 2.13 – 2.25 (*m*, 2 H)/31.1 (C(4)), instead of the CH_2 group at C(3) in **3**. The molecular-ion peak at *m*/*z* 297 (M^+) and the major fragment-ion peak at *m*/*z* 138 ([M – MeOOCCH(CH$_2$)$_2$COOMe]$^+$) in the EI-MS spectrum of **4** supported the presence of a dimethyl pentanedioate moiety. The H–C(2) signal of **4** showed a very weak enhancement, probably due to the steric hindrance by the R group, methyl acetate [8]. The stereogenic center C(2) in the bulky *N*-alkyl side chain in **4** also seems to hold the H-atoms of nearby CH_2 groups, CH_2(7′), resulting in two different chemical shifts of

each CH_2 in the ^{1}H-NMR spectrum of **4**. Therefore, the structure of **4** was elucidated as dimethyl 2-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]pentanedioate.

All the ^{1}H- and ^{13}C-NMR resonances of **1**–**4** were assigned unambiguously according to the 1D- and 2D-NMR data, including COSY, NOESY, HSQC, and HMBC experiments. The absolute configuration at C(2) of **1**–**4** could not be determined.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 230–400 mesh, *Merck*, Germany). TLC: silica gel *60 F_{254}* plates (*Merck*, Germany). HPLC: Prep. HPLC *Acme 9000* (*Young Lin*, Republic of Korea) equipped with *YMC-pack Pro C_{18}* column (S-5 μm, 250 mm × 20 mm; *YMC Co., Ltd.*, Japan); t_R in min. UV Spectra: *Hitachi U3000* spectrophotometer (*Hitachi*, Japan); λ_{max} (log ε) in nm. Circular dichroism (CD) spectra: *Jasco J-810* CD-ORD spectropolarimeter (Tokyo, Japan); λ_{max} ($\Delta\varepsilon$) in nm. NMR Spectra: *Varian UNITY INOVA 400* MHz FT-NMR instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-EI-MS: *JEOL JMS-700 Mstation* mass spectrometer; in *m*/*z*.

Plant Material. The fruits of *L. chinense* were collected in Cheongyang-gun, Chungcheongnam-do, Korea, in May 2009, and were identified by one of the authors, *J.-H. L.* A voucher specimen (No. EAC274) has been deposited with the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The fruits of *L. chinense* (15 kg) were extracted with MeOH (2 × 50 l) under reflux for 4 h. The solvent was concentrated *in vacuo* to yield a MeOH extract (4800 g), which was suspended in dist. H_2O (4 l) and successively fractionated with hexane (3 × 4 l), AcOEt (3 × 4 l), and BuOH (3 × 4 l). The AcOEt extract (70 g) was subjected to CC (SiO_2 (2.5 kg); $CHCl_3$/MeOH 100 : 0 → 5 : 5): *Frs. 1–14*. *Fr. 5* (2.0 g), eluted with 100% $CHCl_3$, was subjected to CC (SiO_2 (2.5 kg); $CHCl_3$/MeOH 100 : 0 → 9 : 1): *Frs. 5.1–5.10*. *Fr. 5.2* (25 mg) was subjected to a prep. HPLC (*RP-C_{18}*; MeOH/H_2O 3 : 7 → 4 : 1; 1 ml/min): **1** (t_R 64; 2 mg), **2** (t_R 37; 1 mg), and **4** (t_R 34; 0.5 mg). On the other hand, *Fr. 5.6* (35 mg) was further separated by prep. HPLC (*RP-C_{18}*; MeOH/H_2O 3 : 7 → 4 : 1; 1 ml/min): **3** (t_R 70; 0.5 mg).

*Methyl 2-[2-Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]propanoate* (**1**). White powder. UV (MeOH): 260 (3.9). CD (MeOH): 332 (–13.4). ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 225 (100, M^+), 196 (55), 150 (30), 138 (62), 134 (85). HR-EI-MS: 225.1002 (M^+, $C_{11}H_{15}NO_4^+$; calc. 225.1001).

*Methyl 2-[2-Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-3-(4-hydroxyphenyl)propanoate* (**2**). White powder. UV (MeOH): 260 (3.7). CD (MeOH): 255 (+17.1), 324 (–9.4). ^{1}H- and ^{13}C-NMR: *Table*. HMBCs: *Me*OOC(1)/C(1); CH_2(3)/C(1), C(2), C(1″), C(2″), C(6″); H–C(3′)/C(2′), C(4′), C(5′), C(6′); H–C(4′)/C(2′), C(3′), C(5′); H_a–C(7′)/C(4′), *Me*O–C(7′); H_b–C(7′)/C(5′), *Me*O–C(7′); *Me*O–C(7′)/C(7′); H–C(2″,6″)/C(4″); H–C(3″,5″)/C(1″), C(4″), EI-MS: 317 (65, M^+), 179 (15), 178 (95), 140 (100), 138 (10), 120 (30), 108 (45). HR-EI-MS: 317.1258 (M^+, $C_{17}H_{19}NO_5^+$; calc. 317.1263).

*Dimethyl 2-[2-Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanedioate* (**3**). White powder. UV (MeOH): 260 (4.2). CD (MeOH): 329 (–15.9). ^{1}H- and ^{13}C-NMR: *Table*. HMBCs: *Me*OOC(1)/C(1); CH_2(3)/C(1), C(2), C(4); *Me*OOC(4)/C(4); H–C(3′)/C(2′), C(4′), C(5′), C(6′); H–C(4′)/C(2′), C(5′), C(7′); H_a–C(7′)/C(5′), *Me*O–C(7′); H_b–C(7′)/C(4′), C(5′), *Me*O–C(7′); *Me*O–C(7′)/C(7′). EI-MS: 283 (80, M^+), 254 (100), 252 (38), 192 (52), 138 (80). HR-EI-MS: 283.1059 (M^+, $C_{13}H_{17}NO_6^+$; calc. 283.1056).

*Dimethyl 2-[2-Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]pentanedioate* (**4**). White powder. UV (MeOH): 260 (4.0). CD (MeOH): 329 (–9.6). ^{1}H- and ^{13}C-NMR: *Table*. HMBCs: *Me*OOC(1)/C(1); CH_2(3)/C(2), C(4), C(5); CH_2(4)/C(2), C(3), C(5); *Me*OOC(5)/C(5); H–C(3′)/C(2′), C(4′), C(5′), C(6′); H–C(4′)/C(2′), C(3′), C(5′), C(7′); H_a–C(7′)/C(5′), *Me*O–C(7′); H_b–C(7′)/C(4′), C(5′),

*Me*O–C(7′); *Me*O–C(7′)/C(7′). EI-MS: 297 (95, M^+), 268 (100), 206 (65), 159 (8), 146 (37), 138 (85). HR-EI-MS: 297.1215 (M^+, $C_{14}H_{19}NO_6^+$; calc. 297.1212).

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