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Hepatoprotective Pyrrole Derivatives of *Lycium chinense* Fruits

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Abstract—As a part of our search for hepatoprotective compounds from *Lycium chinense* fruits, three new pyrrole derivatives (**1**–**3**) were isolated. These compounds and a related synthetic methylated compound (**4**) were evaluated for their biological activity and structure–activity relationship, and compounds **1** and **2** showed hepatoprotective effects comparable to silybin at the concentration of 0.1 μM (64.4 and 65.8%, respectively).

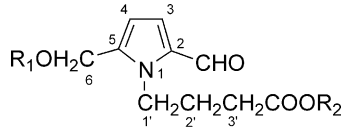
Fruits of *Lycium chinense* Miller (Solanaceae), distributed in northeast Asia, have been used as a tonic in traditional Oriental medicine and were reported to exhibit hypotensive, hypoglycemic and antipyretic activities and to prevent stress-induced ulceration in experimental animals.[1,2]

A number of neutral volatile compounds and steroids have been reported as constituents of the fruits of this plant.[3,4] In particular, cerebrosides which showed anti-hepatotoxic activity were isolated from the fruits in our laboratory.[5] Kukoamine A, (*S*)-9-hydroxy-*E*-10,*Z*-12-octadecadienoic and (*S*)-9-hydroxy-*E*-10,*Z*-12,*Z*-15-octadecatrienoic acids were also isolated from the root barks of *L. chinense*.[6] Since we previously found that the EtOAc fraction of these fruits showed a hepatoprotective activity,[5] we continued to search for new and bioactive compounds from this fraction. As a result, we herein report three new compounds (**1**–**3**) from the fruits of *L. chinense* and have evaluated these compounds along with a synthetic analogue (**4**) for their hepatoprotective effect.

The dried fruits of *L. chinense* (10 kg) were extracted with MeOH (20 L) by sonication. After filtration and concentration, the resultant extract was suspended in H_2O and then partitioned with *n*-hexane and EtOAc. This EtOAc extract was separated with reversed-phase (C_{18}) silica gel, silica gel and Sephadex LH-20® column chromatography and finally HPLC to give three pyrrole derivatives (**1**–**3**) (Fig. 1).[7]

The elemental formula of compound **1**, $C_{10}H_{13}O_4N$ was deduced by EI-HRMS measurement of the $[M]^+$ ion at m/z 211.0840. Absorption bands at 1730 and 1659 cm^{-1} in the IR spectrum of **1** were assigned to a carboxylic acid and an aldehyde group, respectively. The UV spectrum of **1** exhibited an absorption maximum at 292 nm, which is characteristic of pyrrole-2-aldehyde.[8] Chemical shifts and coupling constants of signals at δ_H 6.98 (1H, d, $J = 3.9$ Hz, H-3) and 6.26 (1H, d, $J = 3.9$ Hz, H-4) implied the presence of a heterocyclic ring containing a nitrogen atom and their coupling constants indicated 2,5 di-substitution of the pyrrole ring.[9] If **1** were either a 2,3 or a 2,4 substituted pyrrole compound, their coupling constants would be 1.3–2.9 or 2.3–3.2 Hz, respectively.

The peaks at δ_H 9.44 in the ^1H NMR spectrum and δ_C 179.6 in the ^{13}C NMR spectrum were assigned to an



	R_1	R_2
1	H	H
2	CH_3	H
3	CH_3	CH_3
4	H	CH_3

Figure 1. Structures of compounds **1**–**4**.

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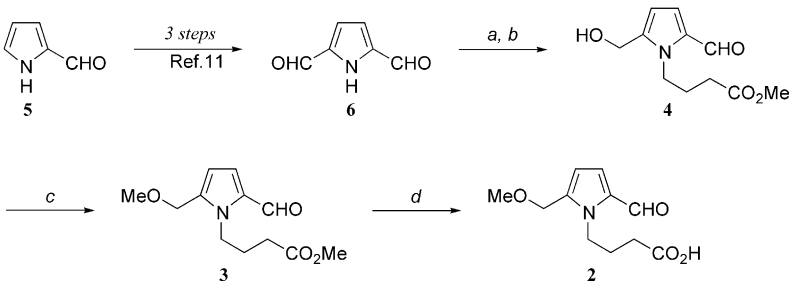

Scheme 1. Reagents and conditions: (a) methyl 4-iodobutyrate, NaOH, CH_3CN, reflux, 50%; (b) $NaBH_4$, MeOH, 0 °C, 90%; (c) Ag_2O, MeI, CH_3CN, reflux, 85%; (d) LiOH–H_2O, THF/H_2O (1:1), 0 °C, 93%.

aldehyde and a singlet signal at δ_H 4.63 was resulted from a hydroxymethyl group. A butanoic acid moiety appeared at δ_H 4.39 (t, $J=7.3$) and 2.31 (t, $J=7.3$), 2.00 (q, $J=7.3$), confirmed by HMBC cross peaks of H-3′ (δ_H 2.31) and H-2′ (δ_H 2.00) to COOH (δ_C 177.0). The connection between N and the butanoic acid moiety was suggested by observing correlation peaks through three bonds from δ_H 4.39 (H-1′) to δ_C 141.7 (C-5) and δ_C 132.4 (C-2) in the HMBC spectra. These data clearly indicated that a butanoic acid moiety was attached to N of the pyrrole ring. A hydroxymethyl group was connected to C-5, which was further proved by an HMBC cross peak of δ_H 4.63 to δ_C 141.7. On the basis of these data, the structure of **1** was determined as a 4-[formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]butanoic acid and confirmed by comparison with reference data.[10]

The molecular ion peak at *m*/*z* 225.0994 in the EI-HRMS indicated that the molecular formula of **2** was $C_{11}H_{15}O_4N$. The spectral data of **2** were similar to **1** except for a methoxy peak at δ_H 3.36 and δ_C 57.9 in the ^1H and ^{13}C NMR spectra, respectively, suggesting that **2** had an additional methoxy group. The HMBC spectrum exhibited the connectivites of methoxy protons (δ_H 3.36) to the methylene carbon (C-6), which was shifted to 65.5 ppm (9.3 ppm downfield) by substitution of a hydroxy with a methoxy group. Therefore, the structure of this compound was determined as a 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanoic acid.

Compound **3** was shown to possess the molecular formula of $C_{12}H_{17}O_4N$ by EI-HRMS measurement of the $[M]^+$ ion at *m*/*z* 239.1162. Compound **3** was assigned to possess an additional methoxy group (δ_H 3.65, δ_C 52.9) by comparison with **2** in the ^1H and ^{13}C NMR spectra, respectively. The HMBC spectral analysis displayed correlation peaks between a methoxy group (δ_H 3.65) and butanoic acid (δ_C 175.9), which revealed that a methoxy group was connected to a butanoic acid moiety. The structure of compound **3** was elucidated as a 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl] butanoate. Compounds **1**–**3** were isolated for the first time from a natural source although **1** was synthesized previously.[10]

Since only small amounts of compounds **1**–**3** could be isolated from their natural source, it was difficult to evaluate their biological activity and establish structure–activity relationship. Therefore, we decided to synthesize the related compounds as shown in Scheme 1 from the known pyrrole-2,5-dicarboxaldehyde **6**, which was prepared according to the reported procedure.[11] *N*-Alkylation of the pyrrole **6** with methyl 4-iodobutyrate, followed by mono-reduction of the resulting dialdehyde, gave the compound **4**. Subsequently, methylation of an alcohol functionality of **4** afforded the compound **3** and hydrolysis of the resulting ester **3** yielded the compound **2**. Furthermore, compound **1** could be obtained by hydrolysis of ester **4**.

Identification of synthetic compounds **1-3** was carried out by comparison of their ^1H and ^{13}C NMR spectral data with those of the naturally occurring compounds. The structure of compound **4** was analyzed by detailed NMR including HMQC and HMBC. Its ^1H and ^{13}C NMR spectra revealed that **4** was a homologue of **2** and **3**. It was found that only one methoxy peak appeared at δ_H 3.64 in **4**, whereas there were two methoxy peaks at δ_H 3.34 and 3.65 in **3**. These data suggested that a methoxy group attached to C-6 was missing in **4**. The location of a carbomethoxy group in **4** was confirmed by a cross peak between δ_H 3.64 and δ_C 174.2 in the HMBC spectrum. On the basis of these data, the structure of compound **4** was determined as a 4-[formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]butanoate.[13]

The hepatoprotective activities of compounds **1**–**4** were

Table 1. Effects of compounds **1**–**4** on the CCl_4-induced toxicity in the primary cultures of rat hepatocytes

Compd	Relative protection[b] (%)			
	0.01 μM	0.1 μM	1 μM	10 μM
Control	100±0.6	100±0.6	100±0.6	100±0.6
CCl_4-treated	0.0±3.0	0.0±3.0	0.0±3.0	0.0±3.0
1	12.4±4.0	64.4±3.9**	39.7±4.2*	3.2±1.0
2	36.0±9.2	65.8±5.6**	29.2±3.8	14.2±2.2
3	8.9±0.8	38.5±4.9*	13.2±5.7	15.9±1.7
4	5.1±1.2	30.8±7.6	40.8±2.2*	20.8±1.0
Silybin[a]			14.6±6.2	28.8±7.4

Primary cultures of rat hepatocytes were exposed to 5 mM CCl_4 with or without each compound. The activity of GPT in the culture medium was measured as described in ref 12. The control was the value for cultured hepatocytes not challenged with CCl_4. The control and CCl_4-treated values for GPT were 13.7±2.7 and 56.1±5.9 IU/L, respectively. Each value represents the mean±SD ($n=3$).

*$p<0.05$; **$p<0.01$.

[a]Silybin showed the optimal hepatoprotective activity at a concentration of 50 μM (45.5±6.2%, $p<0.05$).

[b]The relative percent protection (%) was calculated as 100×(value of CCl_4-treated−value of sample)/(value of CCl_4-treated−value of control).

assessed by measuring their effects on the release of glutamic pyruvic transaminase (GPT) from the primary cultures of rat hepatocytes injured by CCl_4.[12] Compounds **1** and **2** markedly blocked the release of GPT from CCl_4-injured hepatocytes at the concentration of 0.1 μM (Table 1). The hepatoprotective activities of **1** and **2** at a concentration of 0.1 μM were comparable to that of silybin, which was used as a positive control (45.5% at 50 μM). We have also conducted a study on the relationship between the structures of these four compounds and their activities. The results showed that the presence of the carboxylic acid in **1** and **2** seemed to be important because these compounds exert higher activities than **3** and **4** which are esterfied with a methyl group in the carboxylic acid functionality. Methylation in hydroxyl group of **2** and **3** seemed to have no influence on the hepatoprotective activity.

Acknowledgements

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7. The EtOAc extract was fractionationed using reversed-phase (C_{18}) silica gel eluted with MeOH to give seven fractions. Fraction 4 was further purified by silica gel ($CHCl_3$–MeOH = 10:1→0:1) and Sephadex LH-20® (MeOH) column chromatography and finally HPLC (AcCN–H_2O = 25:75) using a J'sphere ODS column to afford **1** (2.0 mg), **2** (2.2 mg), and **3** (1.8 mg).
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13. Physical and spectroscopic data: **4-[formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]butanoic acid** (**1**): UV (EtOH) λ_{max} nm (log ε) 292 (3.55); IR (KBr) ν_{max} 3442, 2920, 1730, 1659 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.41 (1H, s, CHO), 6.98 (1H, d, J = 4.1 Hz, H-3), 6.26 (1H, d, J = 4.1 Hz, H-4), 4.63 (2H, s, H-6), 4.39 (2H, t, J = 7.3 Hz, H-1′), 2.31 (2H, t, J = 7.3 Hz, H-3′), 2.00 (2H, q, J = 7.3 Hz, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.6 (CHO), 177.0 (COOH), 141.7 (C-5), 132.4 (C-2), 124.7 (C-3), 110.8 (C-4), 56.2 (C-6), 44.6 (C-1′), 30.2 (C-3′), 25.9 (C-2′); EI-HRMS m/z calcd for $C_{10}H_{13}O_4N$ 211.0845, found 211.0840.

4-[Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl] butanoic acid (**2**): UV (EtOH) λ_{max} nm (log ε) 291 (3.76); IR (KBr) ν_{max} 2928, 1730, 1660 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.44 (1H, s, CHO), 6.98 (1H, d, J = 4.0 Hz, H-3), 6.28 (1H, d, J = 4.0 Hz, H-4), 4.49 (2H, s, H-6), 4.36 (2H, t, J = 7.3 Hz, H-1′), 3.36 (3H, s, OCH_3), 2.29 (2H, t, J = 7.3 Hz, H-3′), 2.00 (2H, q, J = 7.3 Hz, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5 (CHO), 176.0 (COOH), 143.9 (C-5), 132.5 (C-2), 124.3 (C-3), 111.7 (C-4), 65.5 (C-6), 57.9 (OCH_3), 44.7 (C-1′), 30.3 (C-3′), 26.0 (C-2′); EI-HRMS m/z calcd for $C_{11}H_{15}O_4N$ 225.0997, found 225.0994.

4-[Formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl] butanoate (**3**): UV (EtOH) λ_{max} nm (log ε) 286 (3.39); IR (KBr) ν_{max} 2918, 1731, 1660 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.44 (1H, s, CHO), 6.98 (1H, d, J = 3.9 Hz, H-3), 6.28 (1H, d, J = 3.9 Hz, H-4), 4.48 (2H, s, H-6), 4.35 (2H, t, J = 7.3 Hz, H-1′), 3.65 (3H, s, $COOCH_3$), 3.34 (3H, s, OCH_3), 2.35 (2H, t, J = 7.3 Hz, H-3′), 2.00 (2H, q, J = 7.3 Hz, H-2′); ^{13}C NMR (100 MHz, CD_3OD) δ 181.9 (CHO), 175.9 (COOH), 141.8 (C-5), 134.6 (C-2), 124.2 (C-3), 110.2 (C-4), 67.1 (C-6), 59.0 (OCH_3), 52.9 ($COOCH_3$), 46.6 (C-1′), 32.4 (C-3′), 28.3 (C-2′); EI-HRMS m/z calcd for $C_{12}H_{17}O_4N$ 239.1163, found 239.1162.

4-[Formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl] butanoate (**4**): UV (EtOH) λ_{max} nm (log ε) 297 (3.72); IR (KBr) ν_{max} 3120, 2952, 1734, 1658 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.49 (1H, s, CHO), 6.86 (1H, d, J = 3.9 Hz, H-3), 6.21 (1H, d, J = 3.9 Hz, H-4), 4.67 (2H, s, H-6), 4.35 (2H, t, J = 7.3 Hz, H-1′), 3.64 (3H, s, $COOCH_3$), 2.34 (2H, t, J = 7.3 Hz, H-3′), 1.99 (2H, q, J = 7.3 Hz, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5 (CHO), 174.2 (COO), 141.8 (C-5), 132.4 (C-2), 123.9 (C-3), 110.7 (C-4), 56.2 (C-6), 51.9 ($COOCH_3$), 44.7 (C-1′), 30.5 (C-3′), 25.9 (C-2′); EI-HRMS m/z calcd for $C_{11}H_{15}O_4N$ 225.0997, found 225.0994.