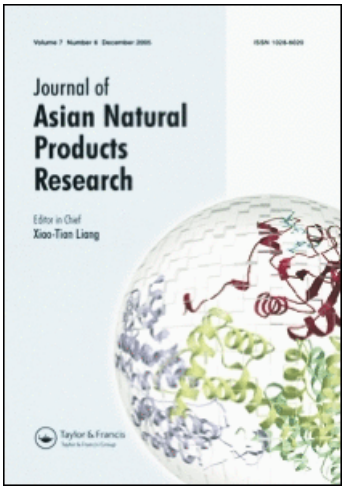

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Pan, Shu and Hou, Ai-Jun(2009) 'New long-chain hydroxyalkyl ferulates from the root bark of *Lycium chinense* Mill.', Journal of Asian Natural Products Research, 11: 7, 681 — 685
To link to this Article: DOI: 10.1080/10286020903002139
URL: http://dx.doi.org/10.1080/10286020903002139

Journal of Asian Natural Products Research
Vol. 11, No. 7, July 2009, 681–685

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New long-chain hydroxyalkyl ferulates from the root bark of *Lycium chinense* Mill.

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(*Received 5 March 2009; final version received 27 April 2009*)

Two new long-chain hydroxyalkyl ferulates, (*E*)-22-hydroxydocosyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (lyciumol A, **1**) and (*E*)-20-hydroxyeicosyl 3-(4-hydroxy-3-methoxyphenyl)acrylate (lyciumol B, **2**), together with eight known compounds, were isolated from the root bark of *Lycium chinense* Mill. Their structures were elucidated by spectroscopic methods, including 1D, 2D NMR, and HR-ESI-MS analysis.

Keywords: *Lycium chinense*; long-chain hydroxyalkyl ferulates; lyciumol A; lyciumol B

1. Introduction

Lycium chinense Mill. is a deciduous shrub widely distributed over China. Its dried root bark is used as traditional Chinese medicine 'Cortex Lycii' for the treatment of night sweat, cough, non-traumatic hemorrhage, bloody stranguria, hypertension, and ulcer. The modern pharmacological study showed that the crude extract from the root bark of *L. chinense* had hypotensive, hypoglycemic, antipyretic, and anti-stress ulcer activities in experimental animals [1]. A limited number of glycosides [2], cyclic peptides [3], terpenes [4], spermine alkaloid [5], cerebrosides [6], and phenolic amides [7,8] were isolated from this plant, some of these compounds exerting hypotensive, anti-hepatotoxic, anti-oxidative, and anti-fungal effects. Further phytochemical investigations on the root bark of *L. chinense* by our group afforded two new long-chain hydroxyalkyl ferulates, lyciumols A–B (**1**–**2**) (Figure 1), and eight known compounds, aurantiamide acetate (**3**), scopoletin (**4**), scoparone (**5**), vanillic acid (**6**), vanillin (**7**), isovanilin (**8**), *p*-hydroxybenzoic acid (**9**), and daucosterol (**10**). To our knowledge, compounds **1** and **2** are the first two ferulates bearing a long-chain hydroxyalkyl group from the plant genus *Lycium*, although ferulates being found quite commonly. Moreover, compounds **5** and **7–9** were isolated from the genus *Lycium* for the first time. In this paper, we mainly describe the structural elucidation of compounds **1** and **2**.

2. Results and discussion

Lyciumol A (**1**), a white amorphous powder, was assigned a molecular formula of $C_{32}H_{54}O_5$ by HR-ESI-MS at *m/z* 541.3884 $[M+Na]^+$. The IR spectrum of **1** exhibited absorptions for hydroxyl groups (3431 cm^{-1}), a carbonyl group (1711 cm^{-1}), and an aromatic ring (1602 and 1469 cm^{-1}). The UV absorption maxima at 321 and 241 nm resembled those of ferulic acid derivatives [9]. The ^1H NMR spectrum (Table 1) showed signals of an aromatic *ABX* spin system

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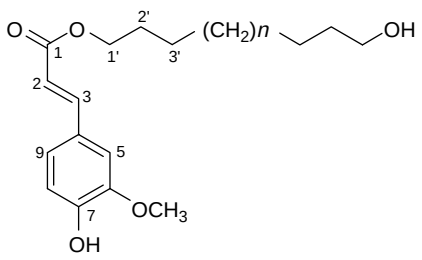
ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903002139
http://www.informaworld.com



1 $n = 16$
2 $n = 14$

Figure 1. Structures of compounds **1** and **2**.

at δ_H 7.07 (1H, dd, $J = 1.5$, 8.1 Hz), 7.04 (1H, br d, $J = 1.5$ Hz), and 6.92 (1H, d, $J = 8.1$ Hz), a *trans*-double bond conjugated with a carbonyl group at δ_H 7.61 and 6.29 (each 1H, d, $J = 16.0$ Hz), and a methoxy group at δ_H 3.92 (3H, s). These data suggested the presence of a *trans*-ferulyl group by comparison of the data with those reported previously [10]. Furthermore, the ^{1}H NMR spectrum also provided signals of two downfield methylene groups at δ_H 4.18 and 3.64 (each 2H, t, $J = 6.6$ Hz), two upfield methylene groups at δ_H 1.68 and 1.55 (each 2H, m), and 36 overlapped protons at δ_H 1.24–1.41, corresponding to an oxygenated long-chain alkyl group. The ^{13}C NMR spectrum (Table 1) exhibited a carbonyl signal at δ_C 167.4, eight sp^2-hybridized carbon signals at δ_C 109.3–147.4, and a methoxy group signal at δ_C 55.9, which were associated with the ferulyl moiety [10]. In addition, two downfield methylene signals at δ_C 64.6 and 63.0 and carbon signals at δ_C 25.7–32.8 were attributed to the oxygenated long-chain alkyl group, which was deduced to be a hydroxydocosyl group by the molecular formula of **1**. Analysis of HMQC and HMBC spectra assigned all ^{1}H and ^{13}C NMR signals (Table 1). The methoxy group at δ_H 3.92 was located at C-6, as established by its HMBC correlation with C-6 (δ_C 146.8). The methylene groups at δ_H 4.18 and 3.64 were assigned to be CH_2-1′ and CH_2-22′, respectively, according to their downfield chemical shifts and HMBC correlations

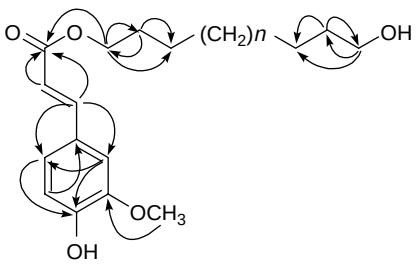
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** and **2** (in $CDCl_3$, δ in ppm, J in Hz).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	–	167.4	–	167.4
2	6.29 (d, $J = 16.0$)	115.6	6.29 (d, $J = 16.0$)	115.7
3	7.61 (d, $J = 16.0$)	144.7	7.61 (d, $J = 16.0$)	144.6
4	–	127.0	–	127.1
5	7.04 (br d, $J = 1.5$)	109.3	7.03 (br d, $J = 1.2$)	109.3
6	–	146.8	–	146.9
7	–	147.4	–	147.9
8	6.92 (d, $J = 8.1$)	114.7	6.92 (d, $J = 8.2$)	114.7
9	7.07 (dd, $J = 1.5$, 8.1)	123.0	7.08 (dd, $J = 1.2$, 8.2)	123.1
6-OMe	3.92 (s)	55.9	3.93 (s)	56.0
1′	4.18 (t, $J = 6.6$)	64.6	4.19 (t, $J = 6.6$)	64.6
2′	1.68 (m)	28.7	1.68 (m)	28.8
3′	1.24–1.41 (overlap)	26.0	1.24–1.40 (overlap)	26.0
4′–17′	1.24–1.41 (overlap)	29.3–29.7	1.24–1.40 (overlap)	29.3–29.7
18′	1.24–1.41 (overlap)	29.3–29.7	1.24–1.40 (overlap)	25.8
19′	1.24–1.41 (overlap)	29.3–29.7	1.55 (m)	32.8
20′	1.24–1.41 (overlap)	25.7	3.64 (t, $J = 6.6$)	63.1
21′	1.55 (m)	32.8	–	–
22′	3.64 (t, $J = 6.6$)	63.0	–	–

from CH_2-1′ (δ_H 4.18) to C-1 (δ_C 167.4). The methylene groups at δ_H 1.68 and 1.55 were determined to be CH_2-2′ and CH_2-21′, respectively, as supported by HMBC correlations from CH_2-2′ (δ_H 1.68) to C-1′ (δ_C 64.6) and C-3′ (δ_C 26.0) and from CH_2-21′ (δ_H 1.55) to C-22′ (δ_C 63.0) and C-20′ (δ_C 25.7) (Figure 2). Thus, the structure of **1** was elucidated as (*E*)-22-hydroxydocosyl 3-(4-hydroxy-3-methoxyphenyl)acrylate, and named lyciumol A (Figure 1).

Lyciumol B (**2**), a white amorphous powder, was assigned a molecular formula of $C_{30}H_{50}O_5$ by HR-ESI-MS at *m/z* 513.3544 $[M+Na]^+$. The UV and IR data of **2** were very similar to those of **1**. Comparison of the ^1H and ^{13}C NMR spectral data of these two compounds (Table 1) showed that they shared the same ferulyl moiety, but had the different long-chain alkyl groups. Compound **2** contained a hydroxyeicosyl group rather than the hydroxydocosyl group in **1**, as supported by the molecular formula of **2**. Thus, the structure of **2** was elucidated as (*E*)-20-hydroxyeicosyl 3-(4-hydroxy-3-methoxyphenyl)acrylate, and named lyciumol B (Figure 1).

Eight known compounds were isolated and identified as aurantiamide acetate (**3**) [11], scopoletin (**4**) [12], scoparone (**5**) [13], vanillic acid (**6**) [14], vanillin (**7**) [15], isovanilin (**8**) [15], *p*-hydroxybenzoic acid (**9**) [16], and daucosterol (**10**) [16] by comparison of the spectroscopic data with those reported previously.



1 *n* = 16
2 *n* = 14

Figure 2. Selected HMBC (H → C) correlations of compounds **1** and **2**.

3. Experimental

3.1 General experimental procedures

The UV spectra were determined on a Shimadzu UV-2401PC spectrophotometer and IR spectra were measured on a Nicolet Avatar-360 spectrometer. The 1D and 2D NMR spectra were recorded on Bruker DRX-500 and Varian Mercury Plus 400 instruments, using residual solvent peaks of $CDCl_3$ (δ_H 7.27, δ_C 76.9) as the standard. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. EI-MS measurements were carried out on a VG Auto Spec-3000 spectrometer and HR-ESI-MS data were obtained on an AB QSTAR Pulsar mass spectrometer. Column chromatography was performed on silica gel H (10–40 μm and 200–300 mesh; Institute of Chemical Technology, Yantai, China), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), MCI gel CHP-20P (75–150 μm; Mitsubishi Chemical Co.), and Chromatorex RP-18 gel (20–45 μm; Fuji Silysia Chemical, Ltd, Kasugai, Japan). TLC analysis was run on precoated silica gel GF_{254} plates (10–40 μm; Institute of Chemical Technology). Preparative HPLC was performed on Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) and Sepax Amethyst C_{18} column (10.0 × 150 mm, 5 μm; Sepax Technologies, Inc., Newark, NJ, USA).

3.2 Plant material

The dried root bark of *L. chinense* Mill. was collected from Hebei Province, China, in 2007, and was identified by Dr Yun Kang, Fudan University. A voucher specimen (TCM 2007-07-09 Hou) is deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

3.3 Extraction and isolation

The dried and powdered root bark of *L. chinense* Mill. (9.5 kg) was percolated with 80% ethanol at room temperature. The filtrate was evaporated *in vacuo* to give a residue, which was suspended in H_2O and partitioned successively with $CHCl_3$ and EtOAc. The $CHCl_3$ extract (90 g) was subjected to column chromatography (CC) on silica gel eluted with a gradient of petroleum ether–Me_2CO (50:1 → 1:2) to afford fractions 1–14. Fraction 8 (6 g) was separated by CC on silica gel (petroleum ether–Me_2CO, 6:1) to give fractions 8.1–8.8. Fraction 8.6 (972 mg) was purified by CC on silica gel ($CHCl_3$–Me_2CO, 100:1) to afford fractions 8.6.1–8.6.9. Fraction 8.6.1 (12 mg) was separated by CC over silica gel (petroleum ether–Me_2CO, 8:1) to give **6** (3 mg). Fraction 8.6.4 (15 mg) was purified by CC on RP-18 (MeOH–H_2O, 40:60) to yield **7** (3 mg) and **8** (4 mg). Fraction 8.6.7 (25 mg) was purified by HPLC (flow rate 1 ml/min, UV detector 210 nm) using 98% MeOH to yield **1** (5 mg, t_R 63 min). Fraction 8.6.8 (32 mg) was purified by HPLC (flow rate 1 ml/min, UV detector 210 nm) using 96% CH_3CN to give **2** (8 mg, t_R 61 min). Fraction 8.7 (82 mg) was separated by CC over silica gel (petroleum ether–Me_2CO, 5:1) to afford **3** (5 mg). Fraction 10 (602 mg) was chromatographed over silica gel (petroleum ether–Me_2CO, 4:1), followed by CC on RP-18 (MeOH–H_2O, 60:40) to give **4** (9 mg). Fraction 12 (112 mg) was separated by CC on MCI gel CHP-20P (MeOH–H_2O, 60:40 → 90:10) to afford compound **9** (3 mg). Fraction 14 (9 g) was isolated by CC on Diaion HP-20 eluted with a gradient of MeOH–H_2O (30:70 → 100:0) to give **5** (4 mg) and **10** (1244 mg).

3.3.1 Lyciumol A (*1*)

A white amorphous powder; UV ($CHCl_3$) λ_{max} (log ε): 321 (4.05), 241 (3.88) nm; IR (KBr) ν_{max}: 3431, 2918, 2850, 1711, 1631, 1602, 1517, 1469, 1431, 1377, 847, 816 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* (%): 518 (55, $[M]^+$), 194 (67), 177 (100), 150 (44), 137 (39), 69 (13), 55 (8); HR-ESI-MS *m/z*: 541.3884 $[M+Na]^+$ (calcd for $C_{32}H_{54}O_5Na$, 541.3868).

3.3.2 Lyciumol B (*2*)

A white amorphous powder; UV ($CHCl_3$) λ_{max} (log ε): 327 (4.07), 236 (3.92) nm; IR (KBr) ν_{max}: 3441, 2918, 2850, 1710, 1631, 1602, 1518, 1469, 1430, 1377, 846, 816 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* (%): 490 (55, $[M]^+$), 194 (82), 177 (100), 150 (44), 137 (38), 117 (8), 69 (14), 55 (10); HR-ESI-MS *m/z*: 513.3544 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3555).

Acknowledgements

The authors thank Prof. Qin-Shi Zhao, Kunming Institute of Botany, Chinese Academy of Sciences, for his kind help. We are also thankful to the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences for recording the NMR spectra.

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