

## Two New Hydroxy Chalcone Derivatives from *Thymus cilicicus*

Bahar Ahmed<sup>a</sup> and Tawfeq A. Al-Howiriny<sup>b</sup>

<sup>a</sup> Department of Pharmaceutical Chemistry, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi-110062, India

<sup>b</sup> Medicinal, Aromatic and Poisonous Plants Research Center, Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Reprint requests to Dr. Bahar Ahmed. Fax + (91)-11-26059663. E-mail: drbahmed@rediffmail.com

Z. Naturforsch. **2007**, 62b, 121 – 124; received May 12, 2006

The aerial part of *Thymus cilicicus* Linn. (Labiatae) has afforded two new hydroxy chalcone derivatives, characterized as 4, 2', 4', 6', 7, 8-hexahydroxy-7 (8)-dihydro-chalcone (**1**), and 3, 4, 2', 4', 6', 7, 8-heptahydroxy-7 (8)-dihydro-chalcone (**2**). The structures of the isolated compounds have been elucidated based on various spectral studies.

**Key words:** *Thymus cilicicus*, Labiatae, 7, 8-Dihydro Chalcone Derivatives

### Introduction

The plant *Thymus cilicicus* Linn. (Labiatae), commonly known as cilician thyme, is an evergreen aromatic shrub growing to 0.15–0.4 m height, and is distributed in South East Asia, East Aegean, Greece, Saudi Arabia and Syria [1]. The shrub is used in traditional medicine as an antiseptic, deodorant and disinfectant [2, 3]. A literature survey indicated that only some essential oils produced from the plant have been reported [4, 5]. We have isolated two new chalcone derivatives from the aerial parts of the plant, which have been characterized as 4, 2', 4', 6', 7, 8-hexahydroxy-7, (8)-dihydro-chalcone (**1**), and 3, 4, 2', 4', 6', 7, 8-heptahydroxy-7, (8)-dihydro-chalcone (**2**). Their structures were elucidated with the help of different spectral studies. 7(8)-Dihydro-chalcone derivatives are very rarely found in nature, and only a few reports are available [6].

### Results and Discussion

The compound **1** named cilicone-a obtained as a yellowish powder has a molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>7</sub> (306.0739), as established on the basis of HR-MS, elemental analysis, <sup>13</sup>C NMR and DEPT spectra. It gave a positive Shinoda test indicating it to be a chalcone derivative. The IR spectrum indicated the presence of a hydroxyl group (3450–3500 cm<sup>-1</sup>) and a double bond (1550 cm<sup>-1</sup>). The <sup>13</sup>C NMR and DEPT spectra [7] showed 15 carbon atoms for the molecule consisting of six aromatic methines, six quaternary, two carbino-

lic and one carbonyl carbon atom (in total C<sub>15</sub>H<sub>8</sub>). The sequential assignments of protons and carbon atoms were made with the help of <sup>1</sup>H-<sup>1</sup>H COSY, and HMQC experiments [8] starting with the easily distinguishable carbinolic protons at  $\delta_H = 4.56$  (d,  $J = 12.0$  Hz,  $\delta_C = 73.6$ ) and  $4.98$  (d,  $J = 12.0$  Hz,  $\delta_C = 85.0$ ), which correlated with each other indicating their vicinal nature at positions 7 and 8, respectively. The HMBC spectrum showed long-range correlation of H-8 with C-7 and C-9 ( $\delta_C = 198.5$ , C=O), whereas H-7 displayed correlations with C-8, C-9, C-1 ( $\delta_C = 129.3$ ), C-2 (130.4) and C-6 (130.4) indicating the presence of a carboxyl group at position 9 and attachment of ring A with carbon C-7. Had the double bond been present in place of the hydroxyl groups at positions 7 and 8, the doublets ( $J = 16$  Hz each) of H-7 and H-8 would have appeared in the range of  $\delta_H = 7.0$  and  $\delta_H = 8.5$  ppm, and consequently the compounds would have been the chalcones.

The <sup>1</sup>H-<sup>1</sup>H COSY spectrum exhibited couplings of H-2 ( $\delta_H = 7.37$ , dd,  $J = 8.5, 2.0$  Hz;  $\delta_C = 130.4$ ) with H-3 ( $\delta_H = 6.85$ , dd,  $J = 8.5, 2.0$  Hz;  $\delta_C = 116.5$ ) and of H-5 ( $\delta_H = 6.85$ , dd,  $J = 8.5, 2.0$  Hz;  $\delta_C = 116.5$ ) with H-6 ( $\delta_H = 7.37$ , dd,  $J = 8.5, 2.0$  Hz) indicating their vicinal positions, respectively, in ring A. The long-range couplings in the HMBC spectrum also substantiated the assignments, wherein H-2 displayed correlations with C-3 and C-4 ( $\delta_C = 159.2$ ), H-3 with C-2 and C-4, H-5 with C-1, C-4 and C-6, while H-6 correlated with C-1, C-4 and C-5. The <sup>1</sup>H NMR spectrum displayed two doublets at  $\delta_H =$

Position	<sup>1</sup> H NMR*	<sup>13</sup> C NMR HMQC**	DEPT***	<sup>1</sup> H- <sup>1</sup> H COSY*	HMBC**** <sup>2</sup> J <sub>CH</sub>	<sup>3</sup> J <sub>CH</sub>
1	–	129.3 s	C	–	–	–
2	7.37 d (8.5)	130.4 d	CH	H-3	C-3	C-4
3	6.85 d (8.5)	116.5 d	CH	H-2	C-2, C-4	–
4	–	159.2 s	C	–	C-5	–
5	6.85 d (8.5)	116.5 d	CH	H-6	C-6, C-4	C-1
6	7.37 d (8.5)	130.4 d	CH	H-5	C-5, C-1	C-4
7	4.56 d (12.0)	73.6 d	CH	H-8	C-1, C-8	C-6, C-2, C-9
8	4.98 d (12.0)	85.0 d	CH	H-7	C-7, C-9	–
9	–	198.5 s	C	–	–	–
1'	–	101.8 s	C	–	–	–
2'	–	165.5 s	C	–	–	–
3'	5.90 d (2.0)	97.3 d	CH	–	C-2', C-4'	C-1', C-5'
4'	–	164.8 s	C	–	–	–
5'	5.94 d (2.0)	97.0 d	CH	–	C-4', C-6'	C-1'
6'	–	168.7 s	C	–	–	–

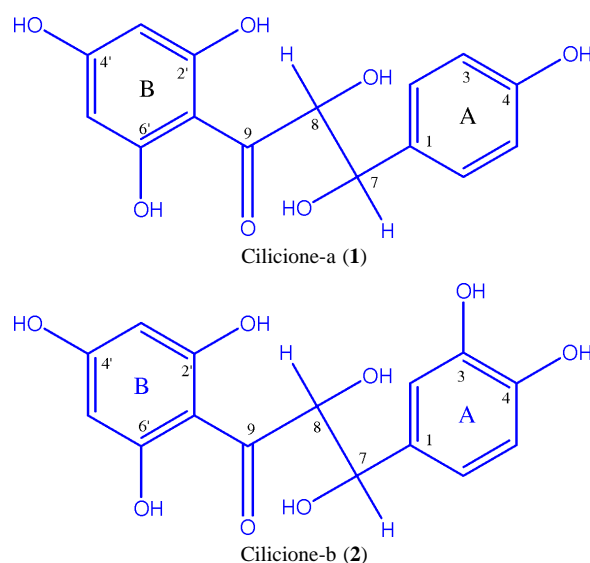
Table 1. 1D- and 2D NMR data of cilicone-a (1).

\* Assignments were based on COSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s: singlet, d: doublet; \*\* C-multiplicities were established by a HMQC experiment; s = C, d = CH, t = CH<sub>2</sub>, q = CH<sub>3</sub>; \*\*\* DEPT: methylene carbon signals appeared downward, whereas methyl and methine carbon signal appeared upward; \*\*\*\* the correlations in HMBC have been shown from protons to carbons.

5.90 ( $J = 2.0$  Hz;  $\delta_c = 97.3$ ) and 5.94 ( $J = 2.0$  Hz;  $\delta_c = 97.0$ ) attributable to positions 3' and 5' of the ring B. H-3' showed long-range correlations in the HBMBC spectrum with C-2', C-4', C-1' and C-5', whereas H-5' displayed correlations with C-6', C-4' and C-1' substantiating the structure of ring B. The downfield shift of C-2' ( $\delta_c = 165.5$ ), C-4' ( $\delta_c = 164.8$ ), C-6' (168.7) and C-4 (159.2) indicated their linkage with oxygen atoms of three hydroxyl groups in ring A and one hydroxyl group in ring B, respectively (Table 1).

The mass spectrum also supported the proposed structure of the compound, giving prominent peaks at  $m/z = 306$  ( $M^+$ ), 164, 181, 153 and 123. Thus, on the basis of the above spectral studies, the structure of the compound has been elucidated as 4, 2', 4', 6', 7, 8-hexahydroxy-7(8)-dihydro-chalcone and has been designated as cilicone-a (1) (Scheme 1).

The compound 2 named as cilicone-b obtained as a yellowish powder has a molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>8</sub> (322.0686) as established on the basis of HR-MS, elemental analysis, <sup>13</sup>C NMR and DEPT spectra. It gave a positive Shinoda test indicating it to be a chalcones derivative. The IR spectrum indicated the presence of a hydroxyl group (3450–3500 cm<sup>-1</sup>) and of a double bond (1552 cm<sup>-1</sup>). The <sup>13</sup>C NMR and DEPT spectra [6] showed 15 carbon atoms for the molecule, consisting of five aromatic methines, seven quaternary and two carbinolic carbon atoms and one carbonyl carbon atom (in total C<sub>15</sub>H<sub>8</sub>). The sequential assignments of protons and carbon atoms were made with the help of <sup>1</sup>H-<sup>1</sup>H COSY and HMQC experiments [7], and were further confirmed with the help of long-range couplings in the HMBC spectrum.



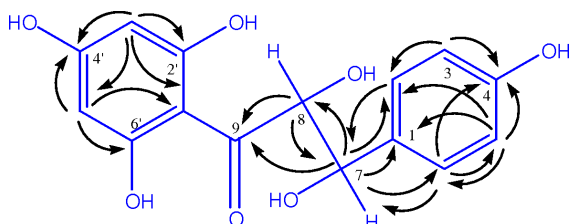
Scheme 1.

The structure of compound 2 was found to be similar to the structure of compound 1, except for an additional hydroxyl group at position 3. The proton and carbon signals at  $\delta_H = 6.85$  and  $\delta_C = 116.5$  due to H-3 and C-3, respectively, have disappeared. However, C-3 appeared downfield at  $\delta_C = 146.3$  indicating that an additional hydroxyl group was present at position 3. Further confirmation was accomplished with the help of long-range correlations in the HMBC spectrum (Table 2). The mass spectrum also supported the proposed structure of the compound, giving prominent peaks at  $m/z = 322$  ( $M^+$ ), 164, 197 and 139. Thus, on the basis of the above spectral studies, the structure of the compound has been elucidated as 3, 4, 2', 4', 6', 7,

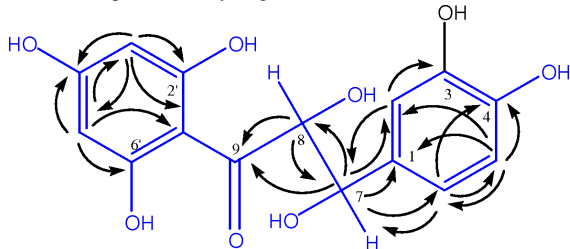
Position	$^1\text{H}$ NMR*	$^{13}\text{C}$ NMR HMQC**	DEPT***	$^1\text{H}$ - $^1\text{H}$ COSY*	HMBC**** $^2J_{\text{CH}}$	$^3J_{\text{CH}}$
1	–	129.8 s	C	–	–	–
2	6.86 d (2.0)	115.9 d	CH	–	C-3	C-4, C-7
3	–	146.3 s	C	–	–	–
4	–	147.2 s	C	–	–	–
5	6.70 d (8.0)	116.1 d	CH	H-6	C-4, C-6	C-1
6	6.75 d (8.0)	120.9 d	CH	H-5	C-5	C-7, C-4, C-2
7	4.41 d (11.5)	73.6 d	CH	H-8	C-8, C-1	C-9, C-2, C-6
8	4.82 d (11.5)	85.1 d	CH	H-7	C-7, C-9	C-1
9	–	198.4 s	C	–	–	–
1'	–	101.8 s	C	–	–	–
2'	–	165.3 s	C	–	–	–
3'	5.77 d (2.5)	96.3 d	CH	–	C-2', C-4'	C-1', C-5'
4'	–	164.5 s	C	–	–	–
5'	5.82 d (2.5)	97.3 d	CH	–	C-6', C-4'	C-1' C-3'
6'	–	168.7 s	C	–	–	–

Table 2. 1D- and 2D NMR data of cilicicone-b (2).

\* Assignments were based on COSY and HMQC experiments; coupling constants in Hertz are given in parentheses; s: singlet, d: doublet; \*\* C-multiplicities were established by a HMQC experiment; s = C, d = CH, t = CH<sub>2</sub>, q = CH<sub>3</sub>; \*\*\* DEPT: methylene carbon signals appeared downward, whereas methyl and methine carbon signal appeared upward; \*\*\*\* the correlations in HMBC have been shown from protons to carbons.



Heteronuclear multiple bond correlations (HMBC) for cilicicone-a (1). Arrows point from hydrogen to carbon atoms.



Heteronuclear multiple bond correlations (HMBC) for cilicicone-b (2). Arrows point from hydrogen to carbon atoms.

8-heptahydroxy-7 (8)-dihydro-chalcone and has been designated as cilicicone-b (2).

## Experimental Section

### General

The IR spectra were recorded as KBr pellets on a PYE UNICAM spectrophotometer. The mass spectra were recorded on a Finnegan MAT 300 mass spectrometer, and relative intensities have been given in parentheses. The  $^1\text{H}$  NMR (500 MHz) as well as the  $^{13}\text{C}$  NMR and DEPT 90 and 135 NMR (125 MHz) and 2D NMR spectra (COSY, HMBC and HMQC) were recorded on a Bruker DRX 500 spectrometer in MeOH- $d_4$  using TMS as an internal standard reference. Chemical shifts are in  $\delta$  (ppm) and cou-

pling constants ( $J$  values) are in Hz. The elemental analysis was performed on a Perkin Elmer CHNSO analyzer, model no. 2400. Column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh) as an adsorbent. TLC was performed on silica gel 60 F<sub>254</sub> Merck plates and sprayed with vanillin- $\text{H}_2\text{SO}_4$  reagents for visualization of the spots.

### Plant material

The aerial parts of *Thymus cilicicus* were collected on June 8, 2000 from Syria and were identified by a taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen no. 14140 has been deposited in the herbarium of the center for future reference.

### Extraction and isolation

The dried aerial parts (70 g) were crushed to a coarse powder and extracted exhaustively with petroleum ether (1.4 g), and consecutive with chloroform (1.2 g) and 95% alcohol (3.5 g) in a percolator. The alcoholic extract was concentrated and dried under reduced pressure to get a viscous mass (3.5 g). The petroleum ether and chloroform extracts were not investigated as there was no promising TLC pattern of the compounds. The alcoholic extract was chromatographed on a column of silica gel, and successively eluted with petroleum ether, chloroform and methanol with increasing order of polarity. The eluent  $\text{CHCl}_3$ -MeOH (97:3) afforded compound 1 (cilicicone-a, 25 mg). Further elution of the column with  $\text{CHCl}_3$ -MeOH (95:5) yielded compound 2 (cilicicone-b, 14 mg).

**Cilicicone-a (1):** Yellow solid (25 mg). –  $R_f$  = 0.47 ( $\text{CHCl}_3$ -MeOH = 9:1). – IR (KBr)  $\nu_{\text{max}}$  = 3450 (OH), 3050 (CH=CH), 1710 (C=O), 1550 (C=C), 1462, 1450, 1370,

1245, 1205, 1115 (C-O, phenolic), 1050 (C-O, alcoholic), 940, 690 (CH=CH)  $\text{cm}^{-1}$ . – 1D and 2D NMR data (see Table 1). – MS (EI, 70 eV):  $m/z$  (%) = 306(80) [ $\text{M}^+$ ], 164 (100), 181 (50), 153 (40), 123 (30). – HRMS:  $m/z$  = 306.0739. –  $\text{C}_{15}\text{H}_{14}\text{O}_7$  (306.0737): calcd. C 58.82, H 4.61; found C 58.80, H 4.62.

*Cilicicone-b* (2): Yellow solid (14 mg). –  $R_f$  = 0.36 ( $\text{CHCl}_3$ -MeOH=9:1). – IR (KBr)  $\nu_{\text{max}}$  = 3455 (OH), 3053 (CH=CH), 1713 (C=O), 1550 (C=C), 1461, 1452, 1371, 1240, 1202, 1117 (C-O, phenolic), 1052 (C-O, al-

coholic), 390, 691 (CH=CH)  $\text{cm}^{-1}$ . – 1D and 2D NMR data (see Table 2). – MS (EI, 70 eV):  $m/z$  (%) = 322 (85) [ $\text{M}^+$ ], 164 (100), 197 (50), 139 (30). – HRMS:  $m/z$  = 322.0686. –  $\text{C}_{15}\text{H}_{14}\text{O}_8$  (322.0689): calcd. C 55.90, H 4.38; found C 55.91, H 4.37.

#### Acknowledgement

The authors are thankful to Mr. M. Mukhiar (MAPPRC) for technical assistance.

- 
- [1] O. Tzakou, T. Constantinidis, *Biochemical Systematics and Ecology* **2005**, 33, 1131.
  - [2] A. Huxley, *The New RHS Dictionary of Gardening*, MacMillan Press, **1992**.
  - [3] D. Bown, *Encyclopedia of Herbs and their Uses*, Dordling Kindersley, London, **1995**.
  - [4] A. Akgul, M. Ozcan, F. Chialva, F. Monguzzi, *J. Essential Oil Res.* **1999**, 11, 209.
  - [5] G. Tumen, M. Koyuncu, N. Kirimer, K.H.C. Baser, *J. Essential Oil Res.* **1994**, 6, 97.
  - [6] G.M. Woldemichael, M.T. Gutierrez-Lugo, S.G. Franzblau, Y. Wang, E. Suarez, B.N. Timmermann, *J. Nat. Prod.* **2004**, 67, 598.
  - [7] D. J. Pegg, D. M. Dedrell, M. E. Bendal, *J. Chem Phys.* **1982**, 25, 2745.
  - [8] K. Nakanishi, *One-dimensional and Two-dimensional NMR Spectra by Modern Pulse Technique*, University Science Books, Kodansha, Tokyo, **1990**.