

## Nepethalates A and B: Two New Phthalate Derivatives from *Nepeta clarkei*

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The structure elucidation of two new phthalate derivatives named nepethalates A (**1**) and B (**2**) is reported. Both of these secondary metabolites were isolated from the MeOH extract of *Nepeta clarkei*. HR-EI-MS, IR and UV absorption spectrometry, and NMR experiments including COSY, HMQC, and HMBC were used for the determination of the structures and complete <sup>1</sup>H- and <sup>13</sup>C-NMR assignments.

**Introduction.** – The genus *Nepeta* is one of the largest genera within the family labiatae (Lamiaceae) having ca. 250 species distributed mainly in Southwest and Central Asia, Europe, North Africa, and North America [1]. About 67 species are found in Iran and 58 in Pakistan. Members of the genus *Nepeta* are sub-shrubs, perennial or annual herbs, monoecious or dioecious, and usually aromatic in nature [1]. *Nepeta* species are used as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue, sedative, insecticidal, acaricidal, antiviral, anti-inflammatory, and antioxidant agents [2]. Some of the Iranian *Nepeta* species having great influence in Iranian folk and traditional medicines are used for the treatment of various disorders such as nervous, respiratory, and gastrointestinal diseases [1]. In the course of phytochemical studies of medicinal plants from Pakistan [3][4], we investigated *Nepeta clarkei* and report now on the structure elucidation and NMR assignments of two new phthalate derivatives, namely nepethalates A (**1**) and B (**2**) (Fig. 1).

**Results and Discussion.** – The molecular formula of compound (**1**) was determined to be C<sub>46</sub>H<sub>82</sub>O<sub>6</sub> (six degrees of unsaturation) by HR-EI-MS analysis (*m/z* 730.6101), which was supported by <sup>1</sup>H- and <sup>13</sup>C-NMR and <sup>13</sup>C-DEPT data. The IR spectrum of **1** showed absorptions of an alkane (2925 cm<sup>-1</sup>, C–H stretch), a C=O (1720 cm<sup>-1</sup>), an aromatic (1600, 1575, 1480, and 740 cm<sup>-1</sup>), and an ester moiety (1264, 1115, 1072, and 1033 cm<sup>-1</sup>). The UV spectrum of **1** exhibited the presence of a conjugated system at 274 nm. Analysis of the <sup>1</sup>H- and <sup>13</sup>C-NMR, DEPT, and HMQC data of **1** revealed the presence of two Me groups, 15 CH<sub>2</sub> units (two O-bearing), two aromatic sp<sup>2</sup> C-atoms,

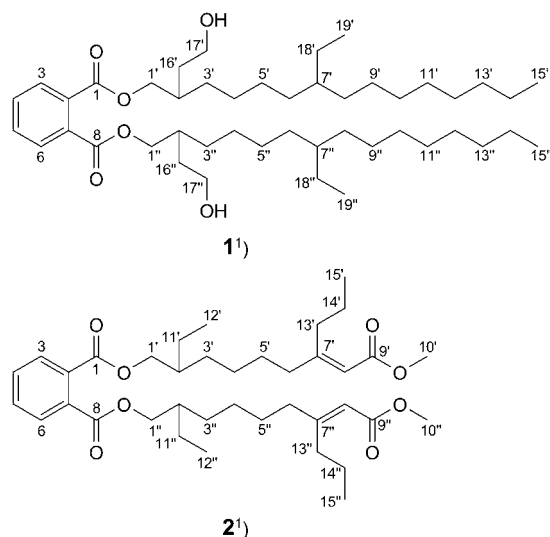


Fig. 1. *Nepethalates A (1) and B (2), isolated from Nepeta clarkei*

two CH groups, one quaternary  $sp^2$  C-atom, and one C=O group for half of the molecule. An  $AA'BB'$  system was present in the  $^1\text{H}$ -NMR spectrum at  $\delta(\text{H})$  7.68 (*dd*,  $J = 6.0, 2.0$  Hz, 2 H) and 7.51 (*dd*,  $J = 6.0, 2.0$  Hz, 2 H). These data required that the compound had one benzene ring and side chains with two C=O groups, consistent with the six degrees of unsaturation. The NMR data of **1** were characteristic of a disubstituted benzene structure. Analysis of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR chemical shifts and the coupling constants observed for the four aromatic H-atoms led to the identification of an *ortho*-substituted aromatic ring. These assignments were further confirmed by relevant HMBC features of **1** (Fig. 2). Two aromatic H-atoms ( $\delta(\text{H})$  7.68) present in **1** showed HMBC cross-peaks with C(1) and C(8), C(2) and C(7), and C(4) and C(5), respectively, indicating that two C=O groups were directly connected to aromatic quaternary  $sp^2$  C-atoms (C(2) and C(7)). Due to the  $^{13}\text{C}$ -NMR chemical shift of these two C=O groups and  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of two O-bearing  $\text{CH}_2$  units, the structure of compound **1** was suggested to be a phthalate derivative [5–8] which was supported by HMBC cross-peaks of  $\text{CH}_2(1')$  and  $\text{CH}_2(1'')$  with C(1) and C(8), respectively. The connections of the two side-chain moieties were completely assigned by HMBC and COSY features (Fig. 2). The chain length and positions of the different groups were further confirmed by the fragmentation pattern in the EI-MS, which is illustrated in Fig. 3. On the basis of these data, the constitution of nepathlate A (**1**) was established as bis[7-ethyl-2-(2-hydroxyethyl)pentadecyl] phthalate.

The molecular formula of nepethalate B (**2**) was determined to be  $\text{C}_{38}\text{H}_{58}\text{O}_8$  (ten degrees of unsaturation) by HR-EI-MS analysis ( $m/z$  642.4130), and this conclusion was supported by 1D-NMR data. The IR spectrum of **2** showed an alkane ( $2924\text{ cm}^{-1}$ , C–H stretch), a C=O ( $1720\text{ cm}^{-1}$ ), an aromatic ( $1604, 1570, 1480$ , and  $745\text{ cm}^{-1}$ ), and

<sup>1)</sup> Arbitrary atom numbering; for systematic names, see *Exper. Part*.

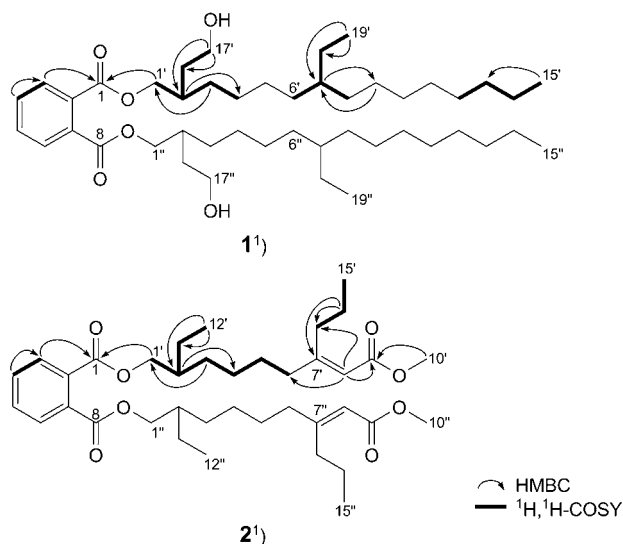


Fig. 2. Important  $^1\text{H}, ^1\text{H}$ -COSY and HMBC features of nepethalates A (**1**) and B (**2**)

an ester moiety (1265, 1115, 1070, and 1030  $\text{cm}^{-1}$ ). The UV spectrum of **2** revealed an absorption at 276 nm indicating the presence of a conjugated system. Analysis of the NMR spectra including 1D and 2D data showed the presence of two Me, one MeO, and eight  $\text{CH}_2$  units (one O-bearing  $\text{CH}_2$ ), four aromatic  $\text{sp}^2$  C-atoms, two CH groups (including one olefinic), three quaternary  $\text{sp}^2$  C-atoms, and two  $\text{C}=\text{O}$  groups for half of the molecule. The  $^1\text{H}$ -NMR spectrum of **2** showed an  $AA'BB'$  system at  $\delta(\text{H})$  7.68 (*dd*,  $J = 6.0, 2.0$  Hz) and 7.51 (*dd*,  $J = 6.0, 2.0$  Hz) and also signals for one olefinic H-atom ( $\delta(\text{H})$  5.84 (s)) and one MeO group ( $\delta(\text{H})$  3.80 (s)). The  $^{13}\text{C}$ -NMR spectrum of **2** exhibited signals for two ester groups including a methyl ester. These data required that the compound had one benzene ring, side chains with four  $\text{C}=\text{O}$  groups, and two  $\text{C}=\text{C}$  bonds, consistent with the 10 degrees of unsaturation. The NMR data of **2** were characteristic of a disubstituted benzene structure. Analysis of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR chemical shifts and the  $J$  values observed for the four aromatic H-atoms led to the identification of an *ortho*-substituted aromatic ring. These assignments were further confirmed by relevant HMBC features (Fig. 2). Two aromatic H-atoms ( $\delta(\text{H})$  7.68) present in **2** showed HMBC cross-peaks with C(1) and C(8), C(2) and C(7), and C(4) and C(5), respectively, indicating that two  $\text{C}=\text{O}$  groups were directly connected to aromatic quaternary  $\text{sp}^2$  C-atoms (C(2) and C(7)). Due to the  $^{13}\text{C}$ -NMR chemical shifts of these two  $\text{C}=\text{O}$  groups as well as  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of two O-bearing  $\text{CH}_2$  units, the structure of compound **2** was suggested to be a phthalate derivative, which was supported by HMBC cross-peaks of  $\text{CH}_2(1')$  and  $\text{CH}_2(1'')$  with C(1) and C(8), respectively. The connections of the two side-chain moieties and the positions of the methyl ester, ethyl, and propyl groups in the side chains were completely assigned by HMBC and COSY features (Fig. 2). The chain length and positions of the different groups were further confirmed by the fragmentation pattern in the EI-MS, which is illustrated in Fig. 3. Therefore, the comprehensive analyses of the MS and 1D- and 2D-

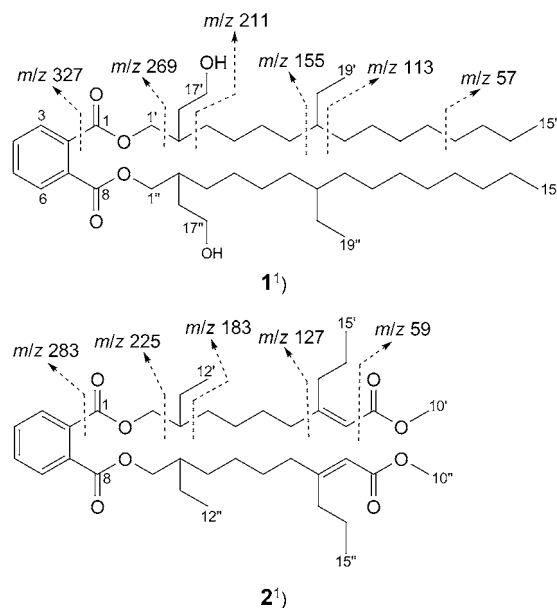


Fig. 3. Key fragmentation pattern in the EI-MS of nepethalates A (**1**) and B (**2**)

NMR data unambiguously elucidated the structure of nepethalate B (**2**) as bis[(7*E*)-2-ethyl-7-(2-methoxy-2-oxoethylidene)decyl] phthalate.

#### Experimental Part

**General.** Column chromatography (CC): silica gel (SiO<sub>2</sub>; 0.040–0.063 mm; Merck) and Sephadex LH-20 (Amersham Biosciences). Anal. and prep. TLC: precoated SiO<sub>2</sub> plates (G60 F-254 or G50 UV-254, resp.; Merck). UV Spectra: Shimadzu UV-2101PC spectrophotometer;  $\lambda_{\text{max}}$  (log  $\epsilon$ ) in nm. IR Spectra: Nicolet-510P spectrophotometer;  $\tilde{\nu}$  in cm<sup>-1</sup>. <sup>1</sup>H- and <sup>13</sup>C-NMR Spectra: Bruker-Avance-500 spectrometer; at 500 for (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C); residual CDCl<sub>3</sub> ( $\delta$ (H) 7.26;  $\delta$ (C) 77.0) as internal standard,  $\delta$  in ppm, *J* in Hz. EI-MS and HR-EI-MS: MAT-8200 and Micromass-LCT mass spectrometers; in *m/z*.

**Plant Material.** The entire plant of *N. clarkei* was collected at the Parachinar Kurram Agency, Khyber pakhtunkhwa, Pakistan, in 2005, and was identified by Mr. Muhammad Siraj (plant taxonomist) at the Department of Botany, Govt. Jehanzeb Post Graduate College, Saidu Sharif, Swat. A specimen of this plant (KN-375) was deposited with the Herbarium of the College.

**Extraction and Isolation.** The dried and powdered whole plant of *N. clarkei* (6.5 kg) was extracted with MeOH at r.t. for two weeks. The crude extract was suspended in H<sub>2</sub>O and extracted with hexane, CHCl<sub>3</sub>, and AcOEt. The CHCl<sub>3</sub> fraction (55 g) was then subjected to CC (hexane, hexane/CHCl<sub>3</sub> and CHCl<sub>3</sub>/MeOH, in the order of increasing polarity): Fractions 1–11. Fr. 5 (4.6 g) was then subjected to repeated CC (hexane/CHCl<sub>3</sub> 3:7): nepethalates A (**1**; 13 mg) and B (**2**; 12.5 mg).

**Nepethalate A** (= Bis[7-ethyl-2-(2-hydroxyethyl)pentadecyl] Benzene-1,2-dicarboxylate; **1**): Yellowish oil. UV (CHCl<sub>3</sub>): 274 (2.79). IR: 2925, 1720, 1600, 1575, 1480, 1264, 1115, 1072, 1033, 740. <sup>1</sup>H-NMR: 7.68 (dd, *J* = 6.0, 2.0, H-C(3), H-C(6)); 7.51 (dd, *J* = 6.0, 2.0, H-C(4), H-C(5)); 4.17–4.21 (*m*, CH<sub>2</sub>(1'), CH<sub>2</sub>(1'')); 3.60–3.63 (*m*, CH<sub>2</sub>(17'), CH<sub>2</sub>(17'')); 1.48–1.51 (*m*, H-C(2'), H-C(2''), CH<sub>2</sub>(16'), CH<sub>2</sub>(16'')); 1.38–1.41 (*m*, H-C(7'), H-C(7'')); 1.29–1.32 (*m*, CH<sub>2</sub>(4'), CH<sub>2</sub>(4'')); 1.21–1.22 (*m*, CH<sub>2</sub>(3'), CH<sub>2</sub>(5'), CH<sub>2</sub>(6'), CH<sub>2</sub>(9') to CH<sub>2</sub>(14'), CH<sub>2</sub>(18'), CH<sub>2</sub>(18''), CH<sub>2</sub>(3''), CH<sub>2</sub>(5''), CH<sub>2</sub>(6''), CH<sub>2</sub>(9'') to CH<sub>2</sub>(14'')); 0.86 (*t*, *J* = 6.0, Me(15'), Me(15'')); 0.90 (*t*, *J* = 6.0, Me(19'), Me(19'')). <sup>13</sup>C-NMR: 167.2 (C(1), C(8));

133.4 (C(2)); 132.4 (C(7)); 130.9 (C(4), C(5)); 128.8 (C(3), C(6)); 68.1 (C(1'), C(1'')); 63.1 (C(17'), C(17'')); 38.7 (C(2'), C(2'')); 32.8 (C(7'), C(7'')); 31.9 (C(8'), C(8'')); 29.5 (C(9') to C(14'), C(9'') to C(14'')); 29.6 (C(6'), C(6'')); 28.9 (C(5'), C(5'')); 25.7 (C(18'), C(18'')); 23.7 (C(3'), C(3'')); 22.7 (C(16'), C(16'')); 22.9 (C(4'), C(4'')); 14.1 (C(15'), C(15'')); 10.9 (C(19'), C(19'')). HR-EI-MS: 730.6101 ( $M^+$ ,  $C_{46}H_{82}O_6^+$ ; calc. 730.6111).

*Nepethalate B* (= *Bis*[(7*E*)-2-ethyl-7-(2-methoxy-2-oxoethylidene)decyl] *Benzene-1,2-dicarboxylate*; **2**): Yellowish oil. UV ( $CHCl_3$ ) 273 (2.85). IR: 2924, 1720, 1604, 1570, 1480, 1265, 1115, 1070, 1030, 745.  $^1H$ -NMR: 7.68 (*dd*,  $J = 6.0, 2.0$ , H-C(3), H-C(6)); 7.51 (*dd*,  $J = 6.0, 2.0$ , H-C(4), H-C(5)); 5.86 (*s*, H-C(8'), H-C(8'')); 4.17–4.21 (*m*,  $CH_2(1')$ ,  $CH_2(1'')$ ); 3.80 (*s*, Me(10'), Me(10'')); 1.93–1.96 (*m*,  $CH_2(13')$ ,  $CH_2(13'')$ ); 1.63–1.67 (*m*, H-C(2'), H-C(2'')); 1.39–1.41 (*m*,  $CH_2(4')$ ,  $CH_2(4'')$ ); 1.21–1.24 (*m*,  $CH_2C(3')$ ,  $CH_2(5')$ ,  $CH_2(6')$ ,  $CH_2(11')$ ;  $CH_2(14')$ ,  $CH_2(3'')$  to  $CH_2(6'')$ ,  $CH_2(11'')$ ,  $CH_2(14'')$ ); 0.86 (*t*,  $J = 6.0$ , Me(12'), Me(12'')); 0.89 (*t*,  $J = 6.0$  Hz, Me(15'), Me(15'')).  $^{13}C$ -NMR: 178.3 (C(9'), C(9'')); 167.3 (C(1), C(8)); 157.4 (C(7'), C(7'')); 131.5 (C(2), (C(7)); 130.9 (C(4), C(5)); 128.8 (C(3), C(6)); 107.4 (C(8'), C(8'')); 68.2 (C(1'), C(1'')); 56.5 (C(10'), C(10'')); 38.7 (C(2'), C(2'')); 32.1 (C(13'), C(13''), C(6'), C(6'')); 29.0 (C(14'), C(14'')); 28.9 (C(5'), C(5'')); 23.7 (C(3'), C(3'')); 22.7 (C(11'), C(11'')); 22.9 (C(4'), C(4'')); 14.1 (C(15'), C(15'')); 10.9 (C(12'), C(12'')). HR-EI-MS: 642.4130 ( $M^+$ ,  $C_{38}H_{58}O_8^+$ ; calc. 642.4132).

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