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Nepetalactones and Other Constituents of *Nepeta nuda* ssp. *albiflora*

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The hexane extract of *Nepeta nuda* ssp. *albiflora* led to the isolation of six known monoterpene nepetalactones, 4aα, 7α,7aα-nepetalactone (1), 4aα, 7α,7aβ-nepetalactone (2), 4aβ,7α,7aβ-nepetalactone (3), 4aβ,7α,7aα-nepetalactone (4), 4aα,7β,7aβ-nepetalactone (5), 3α-hydroxy-4aα,7α,7aα-dihydronepetalactone (=nepetalic acid) (6) and a dimeric 5,9-dehydronepetalactone **(7)** along with two known steroids β-sitosterol and sitosterol 3-one and a triterpene betulinic acid. The acetone extract of the same plant afforded compound 7 in a higher yield in addition to compounds **2** and **6**, and sesquiterpene caryophyllene oxide, the iridoid glucoside 1,5,9-epideoxyloganic acid, the triterpenoids oleanolic, ursolic and betulinic acids, and sitosterol 3β-glucoside.

Keywords: *Nepeta nuda* ssp. *albiflora*; Lamiaceae; nepetalactones; dimeric 5,9-dehydronepetalactone; sesquiterpene; triterpenoids; steroids.

1. Introduction

There are more than 250 species ($\sim$ 280) of the genus Nepeta (Lamiaceae) throughout the world[1]. In Turkey, 33 Nepeta species are present, half of them being endemic[2]. In our previous work, we have investigated the essential oil of *Nepeta nuda* L. ssp. *albiflora* (Boiss.) Gams which is one of the most common species to Anatolia[2]. The main components of its essential oil were 4aα,7α,7aα-nepetalactone (37.5 %) and 4aα,7α,7aβ-nepetalactone (37.6 %) while 4aα,7α,7aβ-nepetalactone and 5,9-dehydronepetalactone were present in small percentages, 2.1 % and 0.2 %, respectively[3]. Nepetalactones have previously been isolated from some other Nepeta species which have feline attractant and insect repellant effects[1,4] in addition to some other effects[5–6]. Some Nepeta species are used in folk medicine particularly due to their diuretic and bacteriostatic activities[1,4].

We report here the chemical investigations of the hexane and acetone extracts of *N. nuda* ssp. *albiflora*. While our study was in progress, two other studies were published involving the same species collected from different locations, the methanol extracts of which afforded new iridoid glucosides[7,8].

2. Experimental

2.1. General

The spectra were recorded with the following instruments: Ultraviolet spectra were measured on a Varian Techtron 685 spectrophotometer in MeOH. Infrared spectra were run on a Perkin Elmer 980 instrument in $CHCl_3$. ^1H and ^{13}C NMR spectra were measured on a Bruker AC 200 L spectrometer, 200 and 50.32 MHz, respectively, with TMS as an internal standard. Mass spectra were run on a ZabSpec (Micromass) high resolution GC-MS spectrometer.

Plant material

The aerial parts of *Nepeta nuda* ssp. *albiflora* (Boiss.) Gams were collected from İçel (Mersin, Arslanköy, by Dr. Gamze Kökdil), at an altitude 1700 m in July 1994. The plant was identified by Prof. Dr. Mecit Vural (Gazi University, Dept. of Biology, Faculty of Arts and Science, Ankara), and a voucher specimen (AEF 19147) is deposited in the Herbarium of Faculty of Pharmacy, University of Ankara.

Extraction and Isolation The aerial parts of the powdered plant (2 kg) were first extracted with hexane and then acetone. The extracts were evaporated *in vacuo* to give 20 g and 38 g of the residues, respectively.

The hexane extract (20 g) was fractionated on a Si-gel column (5.5x 70 cm) and eluted with petrol, followed by a gradient of $CHCl_3$ up to 100 % and MeOH. During elution with 80 % CHCl3 sitosterone (12 mg), with 90 % $CHCl_3$, nepeta-lactones **1** (16 mg) and **2** (26 mg) were obtained. Subsequently, compounds **3** (6 mg), and (**4** + **5**) were isolated as a mixture (22 mg). Elution with $CHCl_3$, β-sitosterol (25 mg), and with 2 % MeOH, betulinic acid (12 mg), with 3 % MeOH, dimeric 5,9-dehydronepetalactone (**7**) (7 mg) and 5 % MeOH, nepetalic acid (**6**) (56mg) were obtained. The acetone extract (38 g) was subjected to flash-column chromatography on silica gel and elution was started with hexane, and continued with gradient chloroform, acetone and finally with methanol. For purification of the compounds, small Si-gel columns, and if necessary, prep TLC plates were used. The following compounds were obtained successively: 4aα,7α,7aβ-nepetalactone (**2**) (9 mg), caryophyllene oxide (10 mg), dimeric 5,9-dehydronepetalactone (**7**) (42 mg), nepetalic acid (**6**) (23 mg) betulinic acid (17 mg), oleanolic and ursolic acids (21 mg), sitosterol 3β-glucoside (14 mg) and iridoid glucoside 1,5,9-*epi*-deoxyloganic acid (32 mg).

Dimeric 5,9-dehydronepetalactone (7). IR ν $CHCl_3$: 2920, 2880, 1745, 1475, 1460, 1380, 1285, 1255, 1195, 1180, 1095, 1050, 995, 980, 950, 920, 880, 760, 665 cm^{-1}. UV λ^{MeOH} 241 ($\in$ 4.9). ^1H NMR (in C_6D_6) see Table 2. HRCIMS *m/z* (rel. int.): 329.3268 $[M+1]^+$ (95), 311.2895 $[(329\text{-}H_2O)]^+$ (23), 285.3040 (42), 267.2915 (63), 241.3031 (7), 205.2031 (14), 179.1698 (15), 165.2102 $[M/2+1]^+$ (100), 137.1933 (6), 109.2582 (5), 97.4700 (8), 79.8404 (41). EIMS *m/z* 328.2 $[M]^+$ (1), 165.1 $[M/2+1]^+$ (100), 164 .1 $[M/2]^+$ (94), 149.1 $[M/2\text{-Me}]^+$ (95), 136.1 (55), 121.1 (26), 107.0 (18), 91.0 (38), 77.0 (37), 65.1 (13). FABMS (+) *m/z* 329.3 [M+1]+ (4), 311.3 $[329\text{-}H_2O]^+$ (1), 283.3 (2), 267.3 (3), 165.2 $[M/2+1]^+$ (100), 149.2 (12).

3. Results and Discussion

From the hexane extract of *Nepeta nuda*, six known nepetalactones 4aα,7α,7aα-nepetalactone (**1**), 4aα,7α,7aβ-nepetalactone (**2**), 4aβ,7α,7aβ-nepetalactone (**3**)[1,9,10], 4aβ,7α,7aα-nepetalactone (**4**)[10], 4aα,7β,7aβ-nepetalactone

(**5**)[10], 3α-hydroxy-4aα,7α,7aα-dihydronepetalactone (nepetalic acid) (6) [11] and dimeric 5,9-dehydronepetalactone (**7**)[12] were isolated, in addition to steroids β-sitosterol, sitosterone and a triterpene betulinic acid. The acetone extract of the same plant afforded compound 7 in a higher yield in addition to the known compounds, 4aα,7α,7aβ-nepetalactone (**2**) and nepetalic acid (**6**), betulinic, ursolic and oleanolic acids, β-sitosterol glucoside and caryophyllene oxide, and the iridoid glucoside 1,5,9-epideoxyloganic acid[13]. In this study, dimeric 5,9-dehydronepetalactone was isolated for the second time while betulinic acid was isolated for the first time from *Nepeta* species.

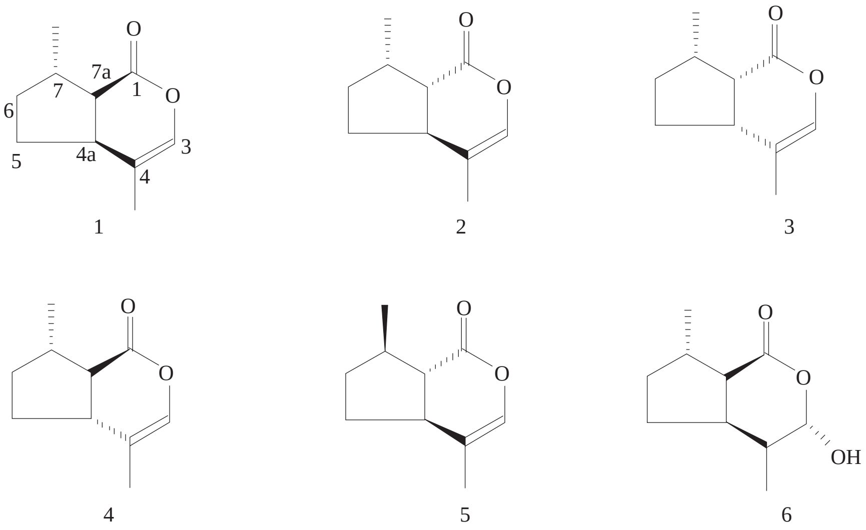


Figure 1. Formula of nepetalactones **1-6**

The ^{1}H NMR shifts of the characteristic protons of the isolated monoterpene lactones (**1-6**) are given in Table 1.

Table 1. ^{1}H NMR data of nepetalactones **1-6***

H	**1**	**2**	**3**	**4 (5)**^{x}	**6**
3	6.15 nm**	6.24 nm	6.17 nm	6.24 nm (6.23)	5.44 d (2)
8	1.64 brs	1.71 t (1)	1.70 t (1)	1.70 t (1.70)	1.01 d (7)
9	1.20 d (6)	1.11 d (6)	0.96 d (7)	0.98 d (0.97)	1.22 d (7)

* J values are given in parantheses as Hz.

^{x} J values are the same as those of compound **3**, **nm=narrow multiplet.

The ^{13}C NMR data are given in Table 2, and which are comparable with the literature data[9,10]. The five nepetalactones (**1-5**) had the same molecular ion peak at *m/z* 166, corresponding to the molecular formula $C_{10}H_{14}O_2$, while the EIMS of compound **6** yielded a molecular ion peak at m/z 184 analyzing for $C_{10}H_{16}O_3$. Its high resolution mass spectrum gave a [M+1]+ ion peak at *m/z* 185.1181 (calc. 185.1177) analyzing for $C_{10}H_{16}O_3$. The characteristic vinylic Me signal at around δ 1.6-1.7 for compounds (**1-5**) was missing in compound **6**. Instead, there was a second doublet methyl signal at δ 1.01 and an anomeric proton signal at δ 5.44 (H-3) situated between a hydroxyl and a lactone moiety. In all nepetalactones (**1-6**), C-7 methyl signal was present as a doublet.

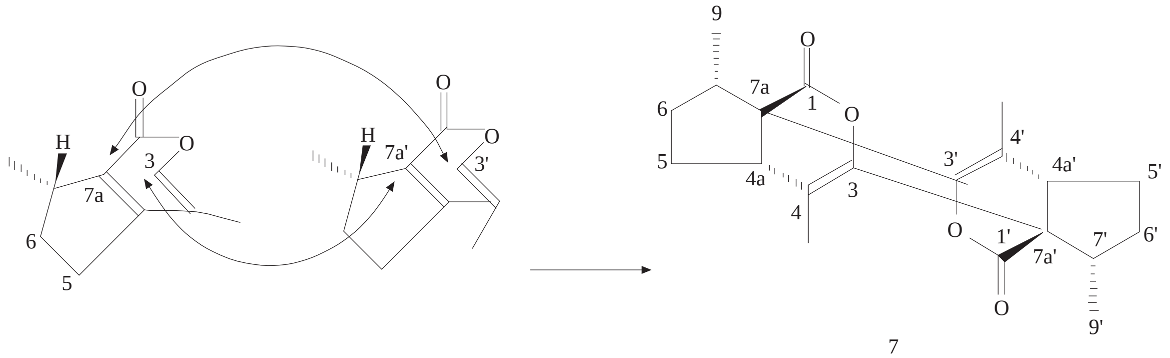
Table 2. ^{13}C NMR data of nepetalactones **1-6***

C	**1**	**2**	**3****	**4 (5)**	**6**
1	170.49	170.19	169.8	168.80 (167.70)	177.01
2	133.00	135.84	134.0	130.92 (128.84)	99.03
3	114.94	120.52	115.4	120.92 (120.65)	39.02
4a	40.15	37.33	39.4	37.29 (37.29)	38.48
5	30.49	26.11	30.4	30.57 (26.11)	33.87
6	32.98	32.08	32.7	32.05 (32.05)	34.40
7	39.58	29.99	38.3	49.07 (29.95)	37.72
7a	49.15	49.06	46.2	14.28 (50.02)	49.06
8	15.40	14.25	14.7	17.61 (13.74)	15.04
9	20.21	17.58	17.2	20.40 (17.61)	20.40

*The assignments were determined based on APT experiments.

** The data are taken from the lit 9.

The EIMS of compound **7** yielded a molecular ion peak at m/z 328.2 corresponding to molecular formula $C_{20}H_{24}O_4$. HRCIMS yielded an [M+1] ion peak at *m/z* 329.3268. The ^{1}H NMR spectrum ($CDCl_3$) (Table 2) exhibited a pair of signals with a small shift differences for some protons; two methyl doublets with J values of 7 Hz at δ 1.06 and 1.33, two vinylic methyl singlets at δ 1.79 and 1.80, and two oxygenated methine singlet signals at δ 4.12 and 4.46. The ^{1}H NMR spectrum run in C_6D_6 also exhibited a pair of signals for oxygenated methine protons at δ 4.22 and 3.77 as broadened singlets as well as methyl doublets at δ 1.03 and 1.12 and methyl singlets at δ 1.54 and 1.56 indicating the presence of either two isomers or a dimeric compound. The ^{13}C NMR spectrum (Table 3) by BB and APT experiments displayed twenty carbon signals as two sets of signals, attributable to the presence of two isomers in a 1:1 ratio or a dimeric structure. Two carbonyl carbons were observed at δ 172.13 and 174.28 indicating the presence of two lactone groups supported by the IR absorption at 1745 cm^{-1}. The signals at δ 79.09 and δ 87.70 were assigned to the carbons (C-3 and C-3') adjacent to oxygen functionality. A pair of quaternary carbon signals found at δ 65.17 and 67.60 were identified as C-7a and C-7a'. Pairs of methylene carbon signals were present at δ 25.67 and 26.65 and at δ 30.80 and 31.37.



7

Figure 2. Formation of dimeric nepetalactone (**7**) via 4+4 adduct between two molecules of 5,9-dehydronepetalactone

Table 3. ^{1}H and ^{13}C NMR data of compound **7**

Position	$^{13}C(C_6D_6)$	$^{1}H(C_6D_6)$	$^{1}H(CDCl_3)$	$^{13}C(CDCl_3)$
1	172.13 s			172.68
1’	174.28s			174.73
3	79.09 d	4.22br s	4.46br s	79.08
3’	87.70 d	3.77br s	4.12 br s	87.66
4	141.04 s			140.83
4’	142.76 s			142.53
4a	132.36 s			132.27
4a’	132.63 s			132.32
5(a,b)	30.80 t	1.64 m	2.40 m	30.57
5’(a,b)	31.37 t	1.34 br dd (6,13)	1.45 ddd (2, 4 ,13)	31.37
6(a,b)	25.67 t	1.96 m	1.89 dd (7, 13)	25.69
6’(a,b)	26.65 t	1.66 m, 1.96 m	2.15 m, 2.32 m	26.72
7	41.62 d	2.08 heks(7)	2.31 m	41.33
7’	43.76 d	2.16 heks(7)	2.34m	43.46
7a	65.17 s			64.97
7a’	67.60 s			67.31
8	15.90 q	1.54 br s	1.79 br s	15.99
8’	16.50 q	1.56 br s	1.80 br s	16.56
9	13.93 q	1.03 d(7)	1.33 d(7)	13.99
9’	16.68 q	1.12 d(7)	1.06 d(7)	16.77

*J values are given in parantheses as Hz.

Olefinic quaternary pairs showed very small shift differences observing at δ 141.04 and 142.76, and at δ 132.36 and 132.63. Spin decoupling experiments aided in the assignment of the proton signals and HETCOR experiments provided direct proton-carbon correlations. The compound could not be acetylated, even under conditions: therefore, the possibility of a hydroxyl group was eliminated. The IR spectrum supported this finding. The carbon signals for quaternary carbons at δ 65.17 and 67.60 and for the oxygenated methine carbons at δ 79.09 and 87.70 are unusual for monomeric lactones closed via 7a and 3. Upon consideration of the model, a symmetric dimer is only possible when epimers are combined. The asymmetric centre of each monomer leads to a dimer with no element of point group symmetry resulting in a doubling of all carbon signals. The literature survey showed that this compound has previously been isolated from another Nepeta species[12], *Nepeta tuberosa*. According to that study, the dimerization adduct should be asymmetric, so the possibilities were through cyclo addition reactions (2+2), (2+4) or Diels-Alder and (4+4)[14–16]. In terms of hyperenergetic conditions and the presence of two allylic hydrogens geminal to oxygen and also two vinylic methyl groups with an allylic coupling suggested that compound **7** is a (4+4) adduct of 5,9-dehydronepetalactone.

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