Constituents of *Nepeta caesarea*

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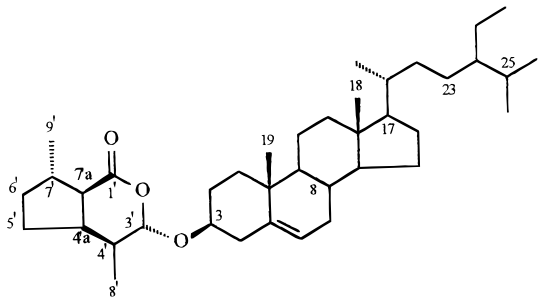
The acetone extract of *Nepeta caesarea* yielded four new nepetalic acid derivatives, 3′α-[β-sitosteryl-3β-oxy]dihydronepetalactone (**1**), 3′β-[5α-stigmast-7-ene-3β-oxy]dihydronepetalactone (**2**), 3′α-[olean-12-ene-28-oyl-3β-oxy]dihydronepetalactone (**3**), and 3′α-[lup-20(29)-ene-28-ol-3β-oxy]dihydronepetalactone (**4**). The structures were elucidated by NMR and MS techniques.

There are about 280 known species of the genus *Nepeta* (Lamiaceae).[1] In Turkey, there are 33 *Nepeta* species, half of them endemic.[2] Studies of essential oil compositions of four Turkish *Nepeta* species have been published.[3−5] We have also reported on the nonvolatile constituents of *Nepeta nuda* ssp. *albiflora*.[6,7] *Nepeta caesarea* Boiss. (Lamiaceae), an endemic species to Turkey, has folkloric uses in southern Anatolia and is used as a herbal tea to treat gastric disorders.[8] The essential oil composition of *N. caesarea* was previously investigated,[9] and the opioid analgesic activity of the main constituent of the essential oil, 4aα,7α,7aα-nepetalactone, was also investigated.[10] Another study of *N. caesarea* afforded 1,5,9-epideoxyloganic acid.[11] We now report the isolation of four new esters of nepetalic acid, **1**−**4**. In addition, the known compounds nepetalic acid (3α-hydroxy-4aα,7α,7aα-dihydronepetalactone) together with its β-methyl isomer, nepetonic acid [2β-[(1-methyl-2-al)-ethyl-5α-methylcyclopenta β-carboxylic acid] together with its 3β-epimer, caryophyllene oxide, lupeol, betulin, ursolic, oleanolic, betulinic, and virgatic acids, stigmasterol, β-sitosterol and its 3β-glucoside, rosmarinic acid, and 1,5,9-epideoxyloganic acid were isolated.

The HREIMS of compound **1** indicated molecular formula $C_{39}H_{64}O_3$ and eight double-bond equivalents. In the ^{1}H NMR spectrum, methyl signals were observed at δ 0.67, 0.83, 0.85, 0.88, 0.95, 1.02, 1.07, and 1.23. The methyl signal at the highest field was assigned to a C-18 methyl for Δ^5 steroids, and an olefinic proton signal at δ 5.38 was assigned to H-6. A carbinol proton signal at δ 3.63 with significant multiplicity was attributed to the H-3 α proton for a steroid. A signal at δ 5.01 was attributed to a methine hydrogen between two oxygens, a conclusion that was supported by the ^{13}C NMR signal at δ 104.7. The ^{13}C NMR spectrum displayed 39 signals assigned to 8 methyl, 13 methylene, 14 methine, and 4 quaternary carbon atoms. The carbonyl was considered to be a lactone carbonyl, as there was an IR absorption band at 1740 cm.$^{-1}$ The NMR spectra indicated that **1** consisted of a monoterpene and a steroid, and unambiguous assignments were made by HETCOR and COLOC experiments. Hydrolysis of **1** afforded 3α-hydroxy-4aα,7α,7aα-dihydronepetalactone (nepetalic acid) and β-sitosterol. A previous study, by Urones et al.,[12] yielded isopimarol derivatives having nepetalactone moieties from *Nepeta tuberosa* subsp. *reticulata*. Thus,



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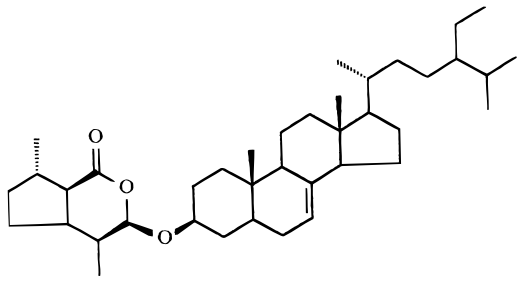

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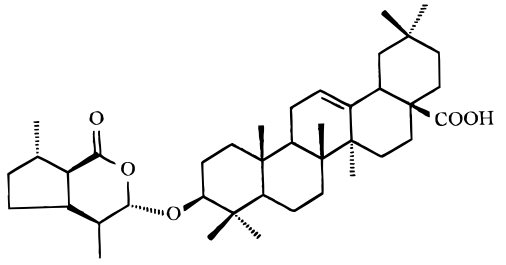

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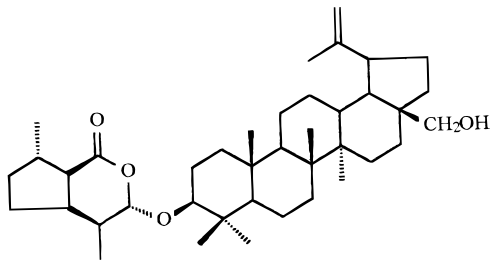

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compound **1** was identified as 3′α-[β-sitosteryl-3β-oxy]-dihydronepetalactone.

Compound **2** showed spectral features similar to those of compound **1**. The ^{1}H NMR of **2** was almost the same as that of compound **1**, but three signals appeared upfield relative to those of **1**: H-3 at δ 3.39 as a multiplet, a methyl singlet at δ 0.52, and an olefinic proton signal at δ 5.20. The latter two signals were assigned to a double bond between C-7 and C-8. The EIMS showed fragment ions at *m*/*z* 275 and 305, which supported placement of the double bond at Δ.[7] The HREIMS showed the same molecular formula ($C_{39}H_{64}O_3$) as **1**. The H-3′ signal, at δ 4.98, differed from that of **1**, indicating that **2** should be attached to the nepetalactone moiety with opposite stereochemistry at C-3. Therefore, compound **2** was identified as 3′β-[5α-stigmast-7-ene-3β-oxy]dihydronepetalactone.

The MS of compound **3** indicated a molecular formula of $C_{40}H_{62}O_5$. The fragment ion at *m*/*z* 576 indicated the presence of an acid group at C-17 of an olean-type triterpene, and fragments at *m*/*z* 454 and 439 indicated loss of the dihydronepetalactone moiety which was observed as the base peak at *m*/*z* 167. The ^{1}H NMR spectrum of **3** exhibited nine methyl signals, seven of which belonged to the triterpenic moiety (δ 0.76, 0.81, 0.92, 0.93, 0.94, 0.96, 1.12), and two methyl doublets belonging to the monoterpenic (dihydronepetalactone) part (δ 1.11 and 1.21). An olefinic proton at δ 5.28 (narrow triplet) and a doublet of doublets at δ 3.27 corresponded to H-12 and H-3, respectively, of the triterpene moiety, while a methine doublet at δ 4.97 was assigned to the C-3′ methine proton of the monoterpene moiety. The ^{13}C NMR spectrum showed 40 carbon signals consisting of 9 methyl, 10 methine, 12 methylene, and 9 quartenary carbon atoms. One of the quaternary carbons was a lactone carbonyl, and another was a free acid carbonyl. This conclusion was supported by IR absorption bands at 1740 and 1700 cm^{-1}, with a shoulder at 2500−2600 cm^{-1}. Thus, compound **3** was identified as 3′α-[olean-12-ene-28-oyl-3β-oxy]dihydronepetalactone.

Compound **4** showed a vinylic methyl at δ 1.68 and a pair of methylene protons at δ 4.68 and 4.59, consistent with a lupen skeleton. Methyl singlets were evident at δ 0.75, 0.83, 0.93, 0.98, 0.99, and 1.01. Two methyl doublets (δ 1.11 and 1.20) indicated dihydronepetalactone methyl protons. The H-3 signal of the triterpene part appeared at δ 3.14, while H-3′ of the dihydronepetalactone moiety was observed at δ 4.97. The presence of a hydroxymethylene group followed from a pair of signals at δ 3.50 and 3.72 (*J* = 10 Hz); its location was deduced to be at C-28, as in betulin.[13,14] The EIMS fragment ion at *m*/*z* 577 supported this conclusion. On the basis of the spectral data, **4** was identified as 3′α-[lup-20(29)-ene-28-ol-3β-oxy]dihydronepetalactone.

Experimental Section

General Experimental Procedures. Specific rotations were measured on an Optical Action Limited AA-S polarimeter in MeOH, and UV spectra on a Varian Techtron spectrophotometer in MeOH. IR spectra were run on a Perkin-Elmer 980 instrument in $CHCl_3$. ^{1}H and ^{13}C NMR were obtained 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 (VG-Mass) high-resolution mass spectrometer.

Plant Material. Whole plant material including roots of *N. caesarea* Boiss. (Lamiaceae) was collected from southern Turkey, İçel (Gözne), at an altitude of 1500 m, in June 1995. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, University of Ankara, AEF 19569, and identified by Prof. Dr. M. Vural (Departmentof Biology, Faculty of Arts and Sciences, University of Gazi, Ankara, Turkey).

Table 1. ^{13}C NMR Data (δ) for Compounds **1**, **3**, and **4** in $CDCl_3$

carbon	1	3	4
1	37.2	38.8	38.9
2	31.7	27.3	27.1
3	78.8	78.2	77.9
4	39.7	40.1	39.0
5	140.2	56.9	55.7
6	122.1	18.9	18.8
7	31.9	34.0	33.6
8	32.0	39.8	39.6
9	50.1	47.9	49.1
10	36.2	37.1	37.4
11	21.1	23.8	20.7
12	38.6	122.3	25.6
13	42.3	145.8	37.4
14	56.7	42.5	42.8
15	24.3	28.0	27.6
16	26.1	23.9	29.3
17	56.1	47.0	47.8
18	11.7	42.5	48.9
19	19.4	48.2	47.9
20	29.1	30.8	150.7
21	19.1	34.2	30.0
22	30.6	33.0	33.9
23	29.2	28.2	28.1
24	48.3	16.7	15.6
25	34.3	16.2	16.2
26	19.6	19.1	16.7
27	19.6	26.4	14.7
28	22.5	183.2	60.3
29	12.3	34.1	109.8
30		25.1	19.2
1′	173.5	174.2	174.0
3′	104.7	103.2	103.1
4a′	39.5	39.5	39.4
5′	39.5	39.9	39.7
6′	34.0	34.1	33.9
7′	36.8	36.8	37.0
7a′	48.7	49.1	49.4
8′	15.4	15.5	15.6
9′	19.8	20.1	20.2

Extraction and Isolation. Dried plant (4 kg) was extracted with acetone twice (3 days each), and the 167 g of dried extract obtained was then dewaxed with MeOH. Acetone solubles were concentrated by vacuum, and the 92 g of extract obtained was chromatographed on a silica gel vacuum column, using a solvent gradient (hexane, hexane/CH_2Cl_2, and CH_2Cl_2/MeOH). Thirty fractions were collected, and similar ones were then combined. Additional purifications were conducted using Sephadex LH-20 columns and preparative TLC. Compounds **1**−**4** were obtained from fractions 5−10 during hexane/CH_2Cl_2 (9:1) elution and purified by PTLC using benzene/CH_2Cl_2 (9:1). The following compounds were also isolated: lupeol (14 mg), caryophyllenoxide (9 mg), betulin (19 mg), nepetonic acid and its isomer (15 mg), nepetalic acid (9 mg), stigmasterol (20 mg), β-sitosterol (28 mg), oleanolic acid (22 mg), rosmarinic acid (26 mg), sitosterol 3-β-D-glucoside (28 mg), 1,5,9-epideoxyloganic acid (59 mg), **1** (67 mg), **2** (21 mg), **3** (14 mg), and **4** (16 mg).

The known compounds were identified by comparison of their spectral data (IR, ^{1}H NMR, and MS) with those in the literature, as well as by TLC comparison with previously obtained compounds.

3′α-[β-Sitosteryl-3β-oxy]dihydronepetalactone (1): white amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.12, MeOH); IR ($CHCl_3$, film) ν_{max} 1740 (lactone), 1620 (C=C); 1460, 1375, 1310, 1180, 1160, 990 cm^{-1}; ^{1}H NMR (200 MHz, in $CDCl_3$) δ 0.67 (3H, s), 0.83 (3H, t, *J* = 7 Hz), 0.85 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.5 Hz), 0.95 (3H, d, *J* = 7.0 Hz), 1.02 (3H, s), 1.07 (3H, d, *J* = 7 Hz, Me-9′) and 1.23 (3H,d, *J* = 6.5 Hz, Me-8′), 3.63 (m, H-3), 5.01 (d, *J* = 8.5, H-3′), 5.38 (d, *J* = 4.7 Hz); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 580.4 (M^+, 0.5), 412.4 ($[M - C_{10}H_{16}O_2]^+$, 11), 396.3 ($[M - C_{10}H_{15}O_3 + H]^+$, 100) 382.4 (28),

288.3 (8), 275.3 (13), 167.1 ($[C_{10}H_{15}O_2]^+$, 33), 81.1 (29), 69.1 (9); FABMS(+) 579.5 ($[M - 1]^+$) (1), 579.5 (4), 537.5 ([M − isopropyl]$^+$, 2), 521.4 (3), 397.3 ($[M - C_{10}H_{15}O_3]^+$, 100), 382.2 (12), 255.2(5), 185.1, 167.1 ($[M - C_{10}H_{15}O_2]^+$, 17), 109 (24); HRMS *m/z* 580.4845 (calcd 580.4855 for $C_{39}H_{64}O_3$).

3′*β*-[5α-Stigmast-7-ene-3*β*-oxy]dihydronepetalactone (2): white amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.08, MeOH) ; IR ($CHCl_3$, film) ν_{max} 1740 (lactone), 1625 (C=C); 1455, 1375, 1312, 1180, 1155, 990 cm^{-1}; ^{1}H NMR (200 MHz, in $CDCl_3$) δ 0.52 (3H, s), 0.66 (3H,d, J = 7.7 Hz), 0.69 (3H, d, J = 7.8 Hz), 0.77 (3H, d, J = 6.4 Hz), 0.87 (3H, t, J = 7.0 Hz), 1.11 (3H, s), 0.87 (3H, d, J = 6.8 Hz, Me-9′), 1.07 (3H, d, J = 6.5 Hz, Me-8′), 3.39 (m, H-3), 4.98 (d, J = 2.0 Hz, H-3′), 5.20 (br d, J = 5.0 Hz, H-6); EIMS (70 eV) *m/z* 580.5 (M^+,1), 412.4 ([M − $C_{10}H_{16}O_2]^+$, 4), 396.3 ([M − $C_{10}H_{15}O_3$+H]$^+$,100) 382.4 (43), 367.3 (7), 275.3 (25), 252.2 (32), 167.1 ($[C_{10}H_{15}O_2]^+$,49), 147.1 (32), 109.1 (24), 95.1 (30), 81.1 (67); HRMS (*m/z*) 580.4882 (calcd 580.4855 for $C_{39}H_{64}O_3$).

3′α-[Olean-12-ene-28-oyl-3*β*-oxy]dihydronepetalactone (3): white amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.06, MeOH); IR ($CHCl_3$, film) ν_{max} 2600 (shoulder) and 1705 (acid), 1742 (lactone), 1645 (C=C), 1100 cm^{-1}; ^{1}H NMR (200 MHz, in $CDCl_3$) δ 0.76 (3H,s), 0.81 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 0.96 (3H, s), 1.11 (3H, s), 1.21 (3H, d, J = 6.8 Hz, Me-9′), 1.21 (3H, d, J = 7.0 Hz, Me-8′), 3.27 (dd, J = 4.5 and 11.0 Hz, H-3), 4.97 (d, J = 7.5 Hz, H-3′), 5.28 (t, J = 2.5 Hz, H-12); EIMS (70 eV) *m/z* 622.6 ([M$^+$, 5), 576.5 ([M − COO]$^+$, 9), 454.4 ([M − $C_{10}H_{15}O_3]^+$, 72), 423.3 (24), 395.3 (27), 375.3 (39), 300.2 (11), 248.2 (90), 233.1 (17), 203.2 (80), 191.2 (100), 189.2 (37), 167.1 ($[C_{10}H_{15}O_2]^+$, 40), 147.1 (23), 133.1 (25), 121 (22), 109.1(15).

3′α-[Lup-20(29)-ene-28-ol-3*β*-oxy]dihydronepetalactone (4): pale yellow oily compound; IR ($CHCl_3$, film) ν_{max} 3410 (OH), 1742 (lactone)1660 and 1650 (C=C), 1455, 1380, 1175, 1155 cm^{-1}; ^{1}H NMR (200 MHz, in $CDCl_3$) δ 0.75 (3H,s), 0.83 (3H, s), 0.93 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.01 (3H, s), 1.68 (3H, br s), 1.11 (3H, d, J = 7.0 Hz, Me-9′), 1.20 (3H, d, J = 6.5 Hz, Me-8′), 3.14 (dd, J = 5.0 and 11.0 Hz, H-3), 3.50 (d, J = 10.0 Hz, H-28a), 3.72 (d, J = 10.0 Hz, H-28b), 4.59 (br s, H-29a), 4.68 (br s, H-29b) 4.97 (d, J = 8.0 Hz, H-3′); EIMS (70 eV) *m/z* 608 ((M)$^+$, 9), 590 ([M − H_2O]$^+$, 46), 577 ([M − CH_2-OH]$^+$, 34), 548 (18), 453 (7), 440 (12), 424 (24), 407 (46), 394 (23), 363 (10), 271 (8), 216 (25), 203 (45), 189 (60), 167 ([dihydronepetalactone], 100), 147 (23), 135 (35), 123 (45), 109 (40), 47 (95), 81 (97), 69 (34).

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