Nepetolide: A New Diterpene from *Nepeta suavis*

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A new tricyclic clerodane-type diterpene, nepetolide (**1**), has been isolated from *Nepeta suavis* along with three known compounds namely β-sitosterol, stigmasterol, and ursolic acid. The structure elucidation of the isolated compounds was based primarily on two-dimensional (2D)-NMR techniques including correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) experiments.

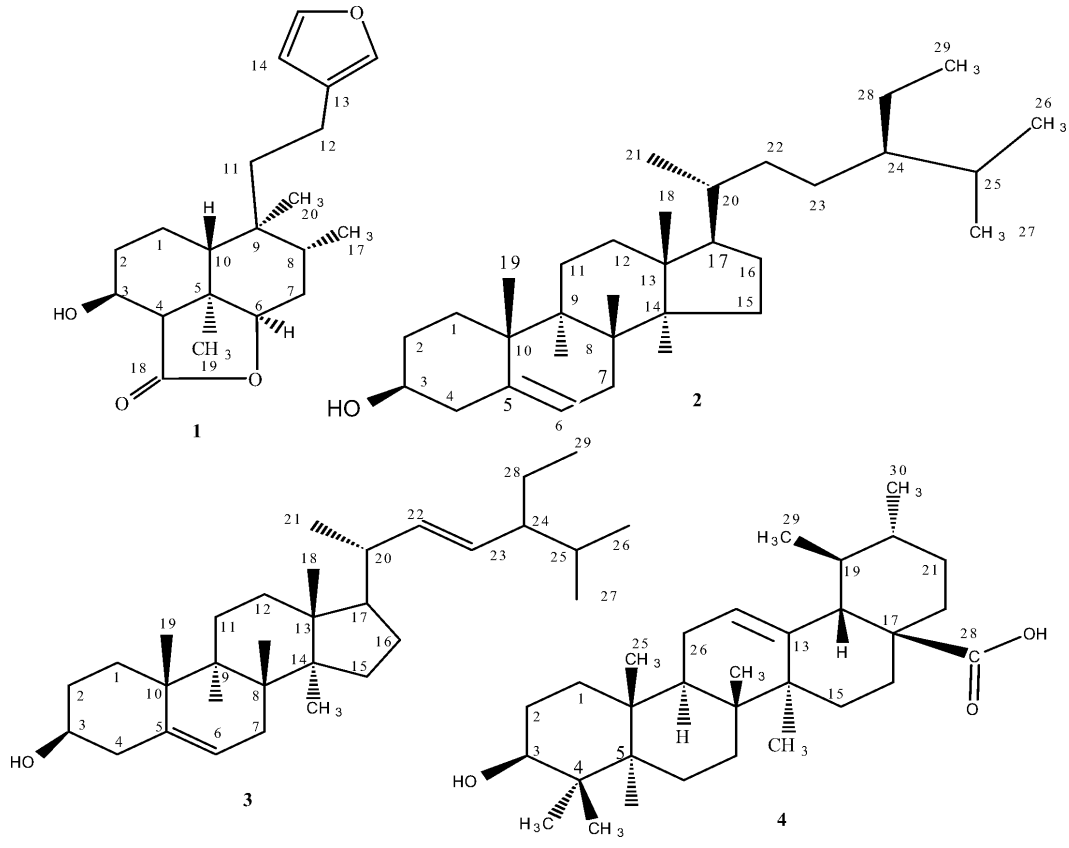
Key words: Nepeta suavis, Labiateae, Nepetolide, Clerodane Type Diterpene Lactone

Introduction

The genus *Nepeta* (also called *Glechoma* and *Cataria*) is named after the ancient Italian City of Nephi [1]. The multiregional genus *Nepeta* is one of the largest genus among the genera of Lamiaceae having approximately 250 species distributed mainly in South-West and Central Asia, Europe, North-Africa and North-America [2, 3]. According to Pojarkova, the widest variation of types and the greatest abundance of species within the genus *Nepeta* is found in two regions, namely South-West Asia (especially Iran) and the western Himalaya including the adjacent Hindukush mountains. About 67 species of the genus *Nepeta* 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 [4 – 6]. In many countries, several *Nepeta* species are used in traditional medicine *e. g.* as diuretic, diaphoretic, vulnerary, antispasmodic, antiasthmatic, tonic febrifuge and sedative agents [7 – 9]. Some of the Iranian *Nepeta* species are employed in Iranian folk and traditional medicine and are used in the treatment of various disorders, such as nervous, respiratory and gastrointestinal diseases [10]. A literature survey shows that members of the genus *Nepeta* are rich in fatty acids, flavones, flavone-glycosides, coumarins, steroids, iridoid-glycosides, monoterpenic lactones, eudesmane sesquiterpenoids, abietane diterpenoids, triterpenoids, and carbohydrates [11 – 15]. These medicinal properties prompted us to carry out phytochemical investigations on *Nepeta suavis*. Our current study has led to the isolation of one new clerodane-type diterpenoid nepetolide (**1**). In addition to the new compound **1**, some known constituents such as β-sitosterol (**2**), stigmasterol (**3**), and ursolic acid (**4**) have been isolated for the first time from this specie [16 – 17].

Result and Discussion

The $CHCl_3$ fraction of the air dried roots of *Nepeta suavis* was subjected to silica gel chromatography to give the new clerodane-type diterpene **1**. The compound was isolated in powder form, and its molecular formula $C_{20}H_{28}O_4$ was established by HREIMS. The peaks at *m/z* = 95, 94, and 81.1 in the EIMS suggested the presence of a furan ring with an alkyl chain in compound **1** [18], while the ion peaks at *m/z* = 219, 201, and 173 in the EIMS showed the presence of a diterpenoid skeleton. The IR spectrum of **1** showed the presence of a furan ring (1510 and 870 cm^{-1}) [21], while the UV spectrum revealed an absorption at λ_{max} = 212 nm. The ^1H NMR spectrum of **1** (Table 1) closely resembled those of *trans*-clerodanes, revealing the same substitution pattern in rings A and B [19, 20]. It exhibited signals for two tertiary methyls at δ = 0.95 and 1.20 (each 3H, s) and one secondary methyl at δ =



0.85 (d, J = 7.3 Hz). A β-monosubstituted furan ring was indicated by characteristic ^{1}H NMR resonances at δ = 6.25 (d, J = 1.8 Hz, H-14), 7.24 (br. s, H-16), and 7.34 (d, J = 1.8 Hz, H-15), and the corresponding ^{13}C resonances at δ = 110.7, 139.7 and 143.0, as revealed by the HMQC experiment [21]. The ^{13}C NMR spectrum (Table 1) corroborated the presence of three CH_3, five CH_2, eight CH and four quaternary C-atoms. The chemical shift of CH_3-19 was observed at δ = 16.6, while the α-positioned axial CH_3-20 appeared at δ = 22.9, and the α-positioned equatorial CH_3-17 resonated at δ = 22.3. These values reveal the *trans* configuration at the A/B ring junction in **1** [22]. The positions of the γ-lactone moiety and the furan ring in the molecule were confirmed by ^{1}H-^{1}H COSY experiments. The HMBC experiment was very informative in the structure elucidation of **1**.

The HMBC of **1** revealed that the α-oriented CH_3-19 at δ = 1.20 was correlated to the carbon atoms at δ = 28.3 (C-4), 75.4 (C-6), and 32.2 (C-10), which established the presence of a lactone moiety joining the A/B rings of *trans*-clerodane through the carbon atoms C-4 and C-6 [20, 23, 24]. The A/B pattern centered at δ = 1.50 and 2.05 could be assigned to CH_2-11, the HMBC of which showed correlations to the carbons at δ = 32.2 (C-10), 39.3 (C-9), 44.8 (C-8), 22.9 (C-20), and 28.3 (C-12), confirming the attachment of the alkyl chain at C-9.

The NOE experiment carried out on **1** established NOEs between CH_3-19 and CH_3-20, and between CH_3-17 and CH_3-20, consistent with a *cis*-relationship between these CH_3 groups. These results and the fact that irradiation of H-10 did not cause any increase in the intensities of either the CH_3-19 or CH_3-20 signals, confirmed the *trans* configuration of the A and B rings of the decalin system of **1** [19]. NOEs were also observed between H-6 and CH_3-19, indicating their *cis* relationship, and establishing the β-configuration and axial configuration of the lactone at C-6. This was also confirmed by the coupling constant of H-6 and the inspection of the model. Based on the foregoing evidence the structure was thus established as nepetolide (**1**). β-Sitosterol (**2**), stigmasterol (**3**) and ursolic acid (**4**) were identified by comparison with published data [16 – 17].

Experimental Section

General

Column chromatography (CC): silica gel, 70 – 230 mesh. Flash chromatography (FC): silica gel 230 – 400 mesh. TLC: pre-coated silica gel G-25-UV_{254} plates, detection at 254 nm, and by the ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-320-A spectrophotometer, respectively. ^{1}H and ^{13}C NMR, COSY, HMQC and HMBC spectra: Bruker spectrometers operating at 500 and 400 MHz; chemical shifts δ in ppm and coupling constants in Hz. EI-, CI MS: JMS-HX-110 with a data acquisition system.

Plant Material

The whole parts of *Nepeta suavis* (labiateae) were collected in July 2006 at the Parachinar Kurram Agency NWFP and were identified by Mr. Siraj Botanist at the Department of Botany Post Graduate College Jehanzeb Swat. A voucher specimen has been deposited at the herbarium of the department.

Extraction and Purification

The air-dried ground, whole parts of *N. suavis* (4.0 kg) were initially extracted with MeOH (4.0 L) at r. t. three times. The solvent was evaporated under reduced pressure to give a dark residue (120.0 g), which was partitioned between *n*-hexane (30.0 g), chloroform (60.0 g), butanol (20.0 g) and water (10.0 g). The chloroform extract was subjected to silica gel chromatography using hexane with a gradient on chloroform up to 100 %, followed by methanol. Fifteen fractions were collected. Fraction 5 (6.8 g) of the first column was loaded on silica gel and eluted with ethyl acetate-hexane (4 : 6) to give compound **1** (15 mg). Fraction 10 (5.7 g) was subjected to column chromatography and eluted with ethyl acetate-hexane (3 : 7) to give compound **2**. Fraction 9 of the first column which contains compounds **3** and **4** was loaded on a silica gel column using hexane : ethyl acetate (50 : 50) and hexane : ethyl acetate (60 : 40) to purify compounds **3** (83.0 mg) and **4** (10.2 mg), respectively.

β-Sitosterol (**2**), stigmasterol (**3**) and ursolic acid (**4**) were identified by comparison with published data [16 – 17].

*Nepetolide (**1**)*

White powder. – ^{1}H and ^{13}C NMR ($CDCl_3$) data see Table 1. – $[\alpha]_D^{23} = -3.28$ ($c = 0.12$, $CHCl_3$). – IR: ν = ($CHCl_3$) = 1763, 1510, 870 cm^{-1}. – UV (MeOH): λ = 214 (5.2) nm. – EIMS: *m/z* (%) = 332 (11) $[M]^+$, 249.1 (6), 191 (4), 165.1 (9), 135.1 (24), 109 (59), 81.1 (100). – HREIMS: *m/z* = 332.1975 (calcd. 332.1980 for $C_{20}H_{28}O_4$, $[M]^+$).

*β-Sitosterol (**2**)*

Colorless solid. – ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.32 (1H, m, H-6), 3.36 (1H, m, H-3α), 0.92 (3H, s, CH_3-19), 0.88 (3H, d, $J_{21,29}$ = 6.5 Hz, CH_3-21), 0.83 (3H, d, $J_{26,25}$ = 6.5 Hz, CH_3-26), 0.31 (3H, d, $J_{27,25}$ = 6.5 Hz, CH_3–27), 0.77 (3H, t, $J_{29,28}$ = 7.0 Hz, CH_3-29), 0.63 (3H, s, CH_3-18). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 141.0 (C-5), 121.1 (C-6), 71.9 (C-3), 56.9 (C-14), 56.6 (C-17), 50.5 (C-24), 50.4 (C-9), 43.8 (C-4), 43.0 (C-13), 40.8 (C-12), 39.5 (C-22), 38.7 (C-1), 37.2 (C-20), 37.1 (C-10), 33.0 (C-7), 32.9 (C-8), 29.9 (C-2), 29.7 (C-16), 29.5 (C-23), 35.9 (C-25), 25.8 (C-15), 23.7 (C-28), 21.5 (C-11), 19.7 (C-21), 20.1 (C-27), 19.5 (C-19), 13.2 (C-26), 12.0 (C-18), 11.9 (C-29). – EIMS: *m/z* (%) = 414 (100), 399 (15), 396 (19), 381 (22), 320 (25), 303 (21), 273 (12), 213 (18), 161 (14), 145 (19), 135 (9), 119 (10), 107 (18), 95 (21). – HREIMS: *m/z* = 414.3857 (calcd. 414.3861 for $C_{29}H_{50}O$, $[M]^+$).

Table 1. NMR spectral data of compound **1** (δ in ppm).

Carbon atom	δ C	δ H
1	31.5	1.56 m, 1.73 m
2	35.1	2.09 m
3	76.2	4.71 t (J = 3.4 Hz)
4	28.3	3.12 d (J = 3.6 Hz)
5	43.7	–
6	75.4	2.50 dt (J = 3.8, 7.9 Hz)
7	18.1	1.73 m
8	44.8	1.80 m
9	39.3	–
10	32.2	1.44 m
11	20.9	1.51 m
12	28.3	2.06 m
13	125.0	–
14	110.7	6.25 d (J = 1.8 Hz)
15	143.0	7.24 br s
16	139.7	7.34 d (J = 1.8 Hz)
17	22.3	0.85 d (J = 7.3 Hz)
18	183	–
19	16.6	1.03 s
20	22.9	0.75 s

*Stigmasterol (**3**)*

Colorless powder. – ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.32 (1H, t, $J_{6,7a}$ = 5.4 Hz, H-6), 5.11 (1H, dd, J_{trans} = 15.5 Hz, $J_{22,21}$ = 8.5 Hz, H-22), 4.98 (1H, dd, J_{trans} = 15.5 Hz, $J_{22,24\beta}$ = 8.5 Hz, H-23), 3.46 (1H, m, H-3α), 1.00 (3H, d, $J_{21\alpha,20\beta}$ = 6.5 Hz, CH_3-21), 0.98 (3H, s, CH_3-19), 0.82 (6H, d, $J_{26,27,25}$ = 6.5 Hz = 6.5 Hz, CH_3-26/CH_3-27), 0.78 (3H, t, $J_{29,28}$ = 7.5 Hz, CH_3-29), 6.3 (3H, s, CH_3-18). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 140.9 (C-5), 138.4 (C-22), 129.4 (C-23), 121.7 (C-6), 71.9 (C-3), 57.0 (C-14), 56.0 (C-17), 51.3 (C-24), 50.3 (C-9), 42.5 (C-13), 42.2 (C-4), 40.5 (C-20), 39.7 (C-12), 37.5 (C-1), 36.6 (C-10), 32.2 (C-8), 32.0 (C-25), 310.9 (C-7), 31.8 (C-2), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-27), 21.1 (C-21), 21.0 (C-11), 19.4 (C-19), 19.0 (C-26), 12.4 (C-18), 12.0 (C-29). – EIMS: *m/z* (%) = 412 (100), 397 (16), 369 (7), 368 (15), 340 (12), 301 (10), 272 (7), 273 (21), 220 (31), 164 (15), 139 (12), 138 (11), 111 (15). – HREIMS: *m/z* = 412.3712

(calcd. 412.3709 for $C_{29}H_{48}O$), 273.2203, (calcd. 273.2218 for $C_{19}H_{29}O$).

*Ursolic acid (**4**)*

Colorless needles. – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3α), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.91 (3H, d, J = 6.6 Hz, Me-30), 0.86 (3H, s, Me-26), 0.81 (3H, s, Me-24), 0.80 (3H, J = 6.8 Hz, Me-29). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 176.2 (s, C-28), 138.7 (s, C-13), 125.8 (d, C-12), 79.1 (d, C-3), 52.4 (s, C-5), 55.2 (d, C-18), 47.9 (s, C-17), 47.4 (d, C-9), 42.0 (s, C-14), 39.6 (s, C-8), 38.5 (t, C-1), 37.0 (t, C-22), 37.1 (s, C-10), 33.2 (t, C-7), 30.5 (d, C-19), 30.3 (d, C-20), 29.4 (t, C-15), 27.5 (t, C-21), 24.5 (q, C-27), 27.4 (t, C-2), 24.0 (t, C-23, C-30), 23.9 (t, C-11), 23.5 (t, C-16), 22.4 (q, C-29), 18.3 (t, C-6), 17.2 (q, C-26), 15.9 (q, C-25), 15.4 (q, C-24). – EIMS: *m/z* (%) = 456 (16) $[M]^+$, 438 (19) $[M–H_2O]^+$, 411 (11) $[M–COOH]^+$, 248 (18), 203 (100), 189 (16).

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