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NEPETADIOL, A NEW TRITERPENEDIOL FROM *Nepeta suavis*

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*Nepetadiol, a new tetracyclic triterpene diol (**1**), was isolated from the chloroform-soluble portion of the whole plant of* Nepeta suavis*. In addition, lawsonin (**2**) and lawsonic acid (**3**) have been isolated for the first time from this species. The structures of the isolated compounds were based on ^{1}H and ^{13}C NMR spectra, including two-dimensional NMR techniques like COSY, HMQC, and HMBC, and comparison with the literature data.*

Keywords: *Nepeta suavis*, Lamiaceae, triterpene diol.

The genus *Nepeta* L. (Lamiaceae) has over 250 species distributed in Europe, Asia, and a few areas of Africa [1, 2]. Many species of the genus have been investigated and found to contain monoterpenoids, diterpenoids, and triterpenoids [3, 4].

Among the various medicinal properties, *Nepeta* species are famous for treating cardiovascular complaints such as angina pectoris, cardiac thrombosis, tachycardia, and weakness of the heart [5]. Several Iranian *Nepeta* species have been of great interest for use in Iranian folk and traditional medicines, and are used in the treatment of various diseases [6], including *N. hindostana* for sore throat [7], and its decoction for fever and pain, including earache and toothache [8].

These medicinal properties prompted us to carry out phytochemical investigations on *Nepeta suavis*. Phytochemical investigations on this plant have led to the isolation of nepetadiol, a new triterpene diol (**1**), together with known compounds lawsonin (**2**) and lawsonic acid (**3**), which have been isolated for the first time from the title species.

The chloroform-soluble fraction of *Nepet*a suavis afforded compound **1** as a white powder. The EI-MS of **1** exhibited the $[M]^+$ at *m/z* 444.3162. The molecular formula was deduced from HR-EI-MS as $C_{30}H_{52}O_2$ corresponding to the mass *m/z* 444.3166 and was consistent with five degrees of unsaturation. The absorption at 3422, 1670, and 1610 cm^{-1} in the IR of **1** revealed the presence of hydroxy, carbonyl, and olefinic functions, respectively, in the molecule.

The ^{1}H NMR resonances (Table 1) of compound **1** exhibited eight methyl signals at δ 0.94, 0.93, 1.04, 1.18, 1.11, 1.68, and 1.69 (3H – 19, 29, 18, 28, 30, 26, 27), 1.10 (3H, d, J = 6.5 Hz, H-21), an olefinic proton at δ 5.23 (J = 7.0 Hz, H-24), and two oxygenated methines at δ 3.19 (dd, J = 4.0, 10.8 Hz, H-3) and 4.1 (m, H-16), suggesting that the molecule belongs to the tetracyclic triterpenoid skeleton.

The ^{13}C NMR spectrum (Table 1) of compound **1** corroborated the presence of 30 carbon signals, identified as eight methyls, nine methylenes, eight methines, and five quaternary carbons on the basis of DEPT experiment. The HMBC and NOESY experiments were very informative in determining the structure. The olefinic proton (1H, H-24) correlated with the C-24 (125.9) in the HMQC spectrum and showed long-range correlations with the vinylic methyl C-26 (23.6) and another methyl C-27 (21.2) in the HMBC spectrum, as shown in Fig. 1.

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TABLE 1. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz) and ^{13}C NMR Data (100 MHz, CD_3OD, δ) of Compound **1**

Position	δ_C	Position	δ_H	δ_C
1	40.1 (CH_2)	16	4.1 (m)	77.3 (CH)
2	27.8 (CH_2)	17	1.32 (dd, J = 7.5, 11.3)	55.2 (CH)
3*	79.0 (CH)	18	1.04 (3H, s)	17.8 (CH_3)
4	40.5 (C)	19	0.94 (3H, s)	17.6 (CH_3)
5	62.8 (CH)	20	0.98 (m)	39.8 (CH)
6	26.6 (CH_2)	21	1.10 (3H, d, J = 6.5)	25.3 (CH_3)
7	47.8 (CH_2)	22		27.7 (CH_2)
8	42.4 (C)	23		22.9 (CH_2)
9	50.8 (CH)	24	5.23 (t, J = 7)	125.9 (CH)
10	40.3 (C)	25		135.5 (C)
11	22.4 (CH_2)	26	1.68 (3H, s)	23.6 (CH_3)
12	27.7 (CH_2)	27	1.69 (3H, s)	21.2 (CH_3)
13	50.0 (C)	28	1.18 (3H, s)	31.4 (CH_3)
14	42.9 (CH)	29	0.93 (3H, s)	16.1 (CH_3)
15	42.3 (CH_2)	30	1.11 (3H, s)	18.1 (CH_3)

*For position 3: δ_H 3.19 (1H, dd, J = 4.0, 10.8, H-3).

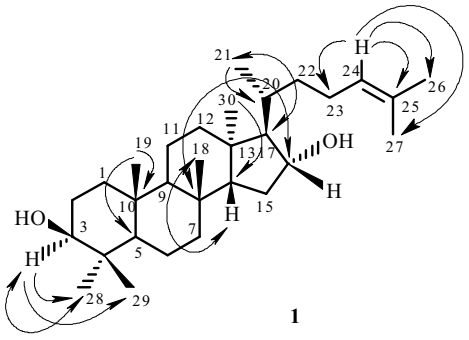

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Fig. 1. Significant correlation observed in HMBC (⟶) and NOESY (⟷) experiment of compound **1**.

This showed that both methyls are attached to the olefinic carbon and are located at the terminal of the side chain. Further interaction was also observed in the NOESY experiment between the vinylic proton at C-24 and the two terminal methyl protons at C-26 and C-27 of compound **1**. The presence of the hydroxyl group of compound **1** was confirmed at C-3 (79.0, CH). The *β*-orientation of the hydroxyl group was inferred from the chemical shift and coupling pattern of the C-3 proton [9]. On the other hand, the signal at δ 77.3 was due to the C-16 bearing a hydroxyl group, and the corresponding H-16 was absorbed at δ_H 4.1, which is in agreement with the reported compound having the hydroxyl group at C-16 [10]. This assignment was based on the multiplicity of the signals at H-15 and H-17, as well as the pronounced downfield shift of the C-17 signal to δ 55.2 as compared to the usual shifts of C-17 in the compound, which do not have the hydroxyl group [11, 12].

The stereochemistry of compound **1** was determined by the NOESY experiment by correlating the cross peaks observed between H_3-18/H-16, which confirms the *β*-position of H-16 at C-16 (Fig. 1). From these observations and from the comparison of spectral data with those reported in the literature, compound **1** was determined to be a lanostane-type triterpene diol [12].

EXPERIMENTAL

General Experimental Procedure. Optical rotation $[\alpha]_D$ was determined using a Jasco-DIP-360 digital polarimeter. UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-320-A spectrophotometers, respectively.

The ^1H and ^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 and 100 MHz, respectively, in CD_3OD using TMS as an internal standard. The chemical shift values are reported in ppm (δ units) and the coupling constants (J) are in Hz. EI, CI MS were recorded on a JMS-HX-110 spectrometer with a data acquisition system and on a JMS-DA 500 mass spectrometer. The sample was subjected to column chromatography using silica gel (Merck, Darmstadt, Germany) of 70–230 mesh size followed by flash column chromatography using silica gel 230–400 of mesh size. Thin layer chromatography was performed using pre-coated silica gel G-25-UV_{254} plates, while its detection was observed at 254 nm, and by the ceric sulfate reagent followed by heating.

Plant Material. The whole parts of *Nepeta suavis* (Lamiaceae) were collected in July 2006 at the Parachinar Kurram Agency NWFP and were identified by botanist Siraj Ahmad at the Department of Botany Post Graduate College, Jehanzeb Swat. A voucher specimen (No. GPGC. 507) has been deposited at the Herbarium of the department.

Extraction and Isolation. The air-dried ground, whole parts of *N. suavis* (4.0 kg) were initially extracted with (4.0 L) of MeOH at room temperature three times. The solvent was evaporated under reduced pressure to give a dark residue (120.0 g), which was partitioned between 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% and followed by methanol. Four fractions A, B, C, and D were collected. Fraction B of the first column was loaded on silica gel and eluted with hexane–chloroform (7:3) to give compound **1** (6 mg). Similarly the ethyl acetate soluble fraction was also frationated and four fractions A, B, C, and D were obtained.

Fraction C of the first column was also subjected to column chromatography and eluted with $CHCl_3$–MeOH (9:1) to give compound **2** (10.0 mg). Fraction D of the first column, which contained compound **3**, was loaded on a silica gel column using MeOH–$CHCl_3$ (2:8) to purify compound **3** (7.0 mg).

Compounds **2** and **3** were identified as lawsonin and lawsonic acid by comparison of their physical and spectral data with those reported in the literature [13].

Triterpenediol (1). White powder (11 mg); IR (KBr, cm^{-1}): 3422 (OH), 1725 (C=O), 1624 (C=C); $[\alpha]_D^{25}$ +19.37° (*c* 0.163 MeOH); UV (CH_3OH, λ_{max}, nm): 248; HR-EI-MS *m/z*: 444.3166 $[M]^+$ (calcd for $C_{30}H_{52}O_2$, 444.3162); EI-MS (70 e/v) *m/z* (rel. int. %): 444.3162 $[M^+]$ (8), 55 (35), 59 (38), 111 (42), 248 (100). For the ^1H and ^{13}C NMR spectral data, see Table 1.

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