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Farmanol: A New Dammarane Methoxytriterpenediol from *Nepeta suavis*

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Farmanol, a new dammarane methoxytriterpenediol (**1**), was isolated from the chloroform soluble portion of the whole plant of *Nepeta suavis* Stapf. In addition, the known pentacyclictriterpene, 2α-acetoxy-3β-hydroxy-olean-12-en-28-oic acid (**2**) and the diterpenenepetanoic acid (**3**) have been isolated for the first time from this species. The structure of the dammarane methoxytriterpenediol (**1**) was determined by comprehensive analysis of its 1D (^{1}H and ^{13}C NMR) and 2D NMR (COSY, HMQC and HMBC) spectra and HREIMSl data.

Keywords: *Nepeta suavis*, Labiatae, Dammarane methoxytriterpenediol.

INTRODUCTION

The genus *Nepeta* (Lamiaceae) comprises approximately 400 species, mainly which grow wild in central and southern Europe, North Africa and southern Asia. Several species of this genus are used in folk medicine for their antiseptic and astringent properties, as a tonic and for the treatment of children's cutaneous eruptions and for snake and scorpion bites. Orally, they are consumed for their anti-asthmatic, anti-tussive, anti-spasmodic, diureticand febrifuge properties[1,2]. Moreover, antibacterial, fungicidal and antiviral activities have been attributed to the nepetalactones and iridoids contained in several *Nepeta* species[3]. These medicinal properties prompted us to carry out a phytochemical investigation of *N. suavis* Stapf, which led to the isolation of three compounds **1-3**, farmanol (**1**), a new methoxytriterpenediol (Fig. 1), together with the known compounds 2α-acetoxy-3β-hydroxy-olean-12-en-28-oic acid (**2**) and nepetanoicacid (**3**), which have been isolated for the first time from this species.

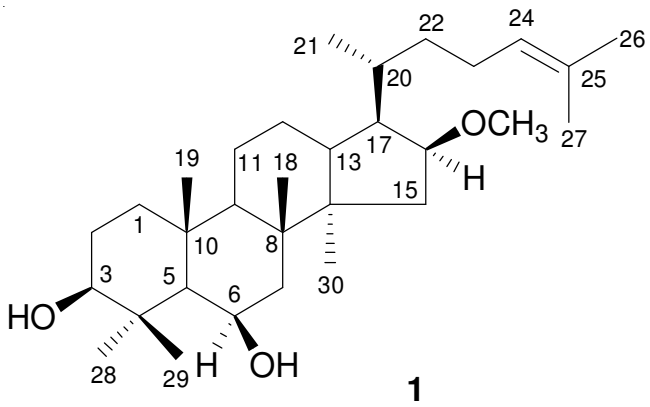

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Fig. 1. Compound **1** isolated from *Nepeta suavis* Stapf

EXPERIMENTAL

The air-dried, ground, whole parts of *N. suavis* Stapf. (4 kg) were initially extracted with (4 L) of MeOH at room temperature 3 times. The solvent was evaporated under reduced pressure to give a dark residue (120 g), which was partitioned between *n*-hexane (30 g), chloroform (60 g) and *n*-butanol (20 g) and water (10 g). The chloroform extract was subjected to silica gel CC using *n*-hexane with a gradient of chloroform up to 100 %, followed by methanol. Four fractions (A, B, C and D) were collected. Fraction B of the first column was loaded onto silica gel and eluted with *n*-hexane-chloroform (3:7) to give compound **1** (4 mg) (Fig. 1). Similarly, the ethyl acetate soluble fraction was also fractionated and 4 fractions were obtained (A, B, C and D). Fraction C of the first column was also subjected to CC and eluted with ethyl acetate:*n*-hexane (9:1) to give compound **2** (4 mg). Fraction D of the first column,

which contained compound **3**, was loaded onto a silica gel column and eluted with ethyl acetate:*n*-hexane (9:1) to purify compound **3** (6 mg).

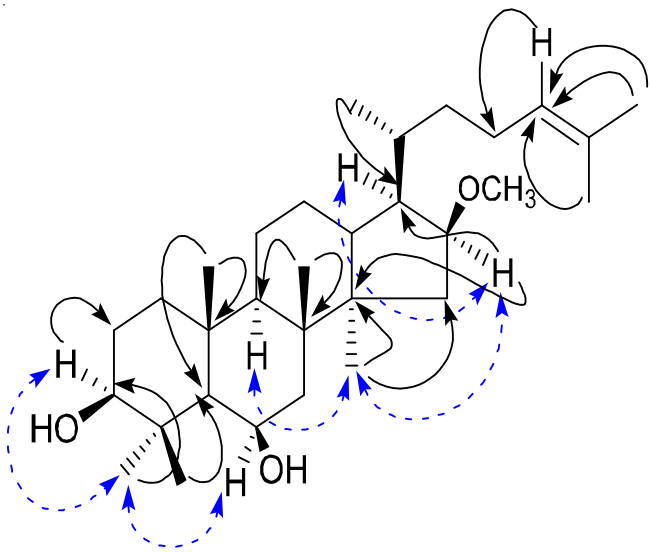
Characterization: Thin layer chromatography was performed using pre-coated silica gel G-25-UV_{254} plates; compounds were detected at 254 nm and by spraying with ceric sulfate reagent followed by heating. IR spectra were recorded with a NICOLET 510P FT-IR spectrometer and a Perkin-Elmer 241 polarimeter. EIMS and CIMS were recorded using JMS-HX-110, with a data acquisition system and JMS-DA 500 mass.

Spectrometers: ^{1}H, 2D ^{1}H-^{1}H COSY, ^{13}C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 400 MHz spectrometer. Chemical shifts are reported in ppm (δ) and are referenced to internal TMS (d = 0); coupling constants (J) are reported in Hz. The sample was subjected to column chromatography using silica gel (E. Merck, Darmstadt, Germany) having 70-230 mesh size, followed by flash column chromatography using silica gel (230-400 mesh). The whole plant of *N. suavis* Stapf was collected at Parachinar Kurram Agency, N.W.F.P, Pakistan, during July 2006 and identified by Mr. Siraj Ahmad (plant taxonomist) 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.

Farmanol (1): White solid 12 mg $[\alpha]_D^{20}$: + 19.37 (c 0.16, MeOH). IR (ν_{max}, cm^{-1}) ($CHCl_3$): 3422 (OH), 1610 (C=C stretch). ^{1}H NMR (400 MHz, CD_3OD): δ 1.23 (2H, t, *J* = 11.2, 8.1 Hz), 1. 62 (2H, dt, *J* = 11.2, , 4.4 Hz), 3.23 (1H, dd *J* = 4.4, 11.00 Hz, H-3), 1.27 (1H, d, *J* = 10.3 Hz), 3.18 (1H, dt, *J* = 6.4, H-6), 1.42 (1H, d, *J* = 12.2 Hz), 1.20 (1H, t, *J* = 9.7 Hz), 1.53 (2H, dt, *J* = 12.2, 10.6, 4.2 Hz), 1.50 (2H, dt, *J* = 10.2, 10.0, 3.6 Hz),1.50 (1H, dt, *J* = 13.2, 11.7, 4.5 Hz), 1.40 (2H, d, *J* = 12.3 Hz), 3.82 (1H, m, H-16), 1.80 (1H, dd, *J* = 11.5, 8.2 Hz), 1.00 (3H, s, H-18), 0.90 (3H, s, H-19), 0.98 (1H, m), 1.07 (3H, d, *J* = 7.4 Hz, H-21),1.47 (2H, dt, *J* = 12.0, 10.1, 5.2 Hz), 1.53 (2H, dt, *J* = 13.3 11.2, 6.4 Hz), 5.16 (1H, t, *J* = 8.3 Hz, H-24), 1.62 (3H, s, H-26), 1.64 (3H, s, H-27), 1.13 (3H, s, H-28), 0.95 (3H, s, H-29), 1.09 (3H, s, H-30), 2.70 (s, 3H, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 125.9 (C-24), 135.5 (C-25), 79.0 (C-3), 76.6 (C-6), 62.7 (C-5), 57.5 (-OCH_3), 55.2 (C-16), 51.0 (C-13), 50.8 (C-9), 50.4 (C-17), 47.8 (C-7), 42.9 (C-14), 42.4 (C-8), 42.3 (C-15), 40.5 (C-4), 40.3 (C-10), 40.1 (C-1), 33.4 (C-20), 31.4 (C-28), 27.8 (C-2), 27.7 (C-22), 27.7 (C-12), 25.3 (C-21) 23.6 (C-26), 22.9 (C-23), 22.4 (C-11), 21.2 (C-27),18.3 (C-30), 17.8 (C-18), 17.1 (C-19), 16.9 (C-29) (Table-1) EI-MS: *m/z* (rel. int. %) 474.3234 [M^+] (10), 248 (100), 111 (42), 59 (38), 55 (35). HR-EI-MS: *m/z* $[M]^+$ 474.3230 (Calcd. 444.3967 for $C_{31}H_{54}O_3$).

RESULTS AND DISCUSSION

Compound **1**, farmanol, was isolated as white powder, for which the IR spectrum showed absorption at 3422 and 1610 cm^{-1}, revealing the presence of hydroxyl and olefinic functions, respectively. The EIMS of **1** revealed a molecular weight of 474.3234, consistent with the molecular formula $C_{31}H_{54}O_3$, which was confirmed by HREIMS analysis. The ^{1}H NMR spectrum (section-1) showed nine methyl signals at δ 0.90 (3H, s, H-19), 0.95 (3H, s, H-29), 1.00 (3H, s, H-18), 1.07 (3H, d, *J* = 6.5 Hz, H-21), 1.13 (3H, s, H-28), 1.09 (3H, s, H-30), 1.62 (3H, s, H-26), 1.64(3H, s, H-27)) and 3.82 (1H, m, H-16), three of which were deshielded to δ 1.62,1.64 and 3.82. By homonuclear decoupling experiments, the deshielded-methyls were shown to couple allylically with a vinyl proton at δ 5.16 (t, *J* = 8.3 Hz), thus suggesting the presence of a terminal -CH_2-CH=C(CH_3)$_2$ group in the C-17 side chain[4,5]. This was confirmed by H-detected heteronuclear multiple quantum coherence (HMQC) experiments, whereby the two carbons at δ 125.9 (C-24) and 122.7 (C-25) represented a double bond and the former peak was linked to the proton at δ 5.16. The ^{13}C NMR spectrum of compound **1** corroborated the presence of 31 carbon signals, identified as nine methyls, eight methylenes, nine methines and five quaternary carbons on the basis of DEPT experiment (section-1). Therefore, compound **1** was suggested to be a dammarane methoxytriterpenoid derivative[6]. The oxymethine proton (H-3) resonated at δ 3.23 (dd, J = 4.4, 11.00 Hz), showing a *J* value, as well as chemical shift of H-3 consistent with an axial orientation[6]. On the other hand, the signal at d 55.2 is due to C-16 and the corresponding H-16 absorbsat δ 3.82. This assignment was based on the multiplicityof the signals at H-15 and H-17 and the pronounced downfield shift of the C-17 signal to δ 50.4, compared with the usual chemical shifts of C-17 in compounds that do not have the C-16 hydroxyl group (see section-1)[6-9]. The position of attachment for methoxy group was confirmed from the HMBC correlations: H-16 to C-14, C-15 and C-17. The detailed analysis of the HMBC and NOESY **(Scheme-I)** experiments permitted us to determine the structure. The stereochemistry was established by NOESY **(Scheme-I)**. The cross peaks observed between H_3-28/H-3, H_3-28/H-6, H-16/H-17 and between H_3-30/H-16 confirm the β disposition of the hydroxyl groups at C-3 and C-6. Consequently, the structure of farmanol (**1**) was elucidated as 3β, 6β dihydroxy-16-methoxy dammar-24(Z)-ene. The known constituents 2α-acetoxy-3β-hydroxy-olean-12-en-28-oic acid (**2**) and nepetanoicacid (**3**)[10,11] were identified by comparison of their spectral data with those published.



Scheme-I: Significant correlation observed in HMBC (→) and NOESY (↔) spectra

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