Nepehinal: A New Triterpenoidal Aldehyde from *Nepeta hindostana*

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Abstract

A new triterpenoidal aldehyde, nepehinal (**1**), has been isolated from the alcoholic extract of the whole plant of *Nepeta hindostana*. Its structure was established as 1β,3β,11α-trihydroxy-lup-20(29)-en-30-al through chemical and spectroscopic studies including two dimensional NMR. ^{13}C-NMR spectral assignments of nepeticin (**2**) have also been revised.

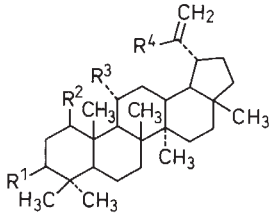
Key words

Nepeta hindostana, Labiatae, triterpenoid, structure elucidation.

Introduction

Nepeta hindostana (Roth) Haines [syn. *Nepeta ruderalis* Hamilt] (Labiatae) is a drug of repute in the Unani system of medicine known as "Badranjboya". It is used as a sedative, tonic, resolvent, stomachic, hepatic tonic, against cardiac asthma and syncope (1), for sore throat, and also as an antipyretic (2). Its extract is reported to lower the blood cholesterol level in animals (3).

Previous investigations on this plant resulted in the isolation and structural elucidation of new triterpenes of the lup-20(29)-ene series (4, 5). As a result of our continuing interest in the chemical constituents of *N. hindostana*, we have isolated a new pentacyclic triterpenoidal aldehyde, nepehinal (**1**). The structure was assigned on the basis of spectroscopic studies (UV, IR, FAB-mass, ^{1}H-NMR, ^{1}H-^{1}H COSY, 2D *J*-resolved, NOESY, HOHAHA experiments, broad-band ^{13}C-NMR, DEPT, ^{1}H-^{13}C COSY, HMQC, and HMBC experiments). ^{13}C-NMR spectral assignments of nepeticin (**2**) have also been revised.



	R^1	R^2	R^3	R^4
1	HO	HO	HO	O=CH–
1a	AcO	HO	AcO	O=CH–
2	HO	H	HO	H_3C–
3	HO	HO	HO	HO–CH_2–

Materials and Methods

General experimental procedures

Melting points were recorded in glass capillary tubes on a Gallenkamp melting point apparatus and are uncorrected. Optical rotation was measured on a Schmidt + Haensch Polartronic D polarimeter. UV spectra were scanned in MeOH on a Shimadzu UV-240 Graphicord spectrometer. IR spectra were recorded in KBr or $CHCl_3$ on a JASCO A-302 infrared spectrophotometer. EI-MS were determined on a Finningan MAT-312 Varian MAT-112 double focusing mass spectrometer connected to a DEC PDP 11/34 computer system. The negative ion FAB mass spectra were recorded on a JEOL JMS-HX 110 spectrometer coupled with a PDP 11/73 computer system. ^{1}H-NMR spectra were obtained from a Bruker AM-300 (300 MHz) spectrometer using both C_5D_5N and $CDCl_3$ as solvents and TMS as an internal standard (s, singlet; d, doublet; q, quartet; m, multiplet). ^{1}H chemical shifts are in the δ scale and reported from TMS and coupling constants are in Hz. ^{13}C-NMR spectra were measured in CD_3OD and C_5D_5N at 75.43 MHz and TMS as an internal standard using the Bruker AM-300 NMR spectrometer. The DEPT experiments were carried out with θ = 45°C, 90°C, and 135°C. The column chromatography was performed on Merck silica gel 60 (70–230 mesh) and silica gel 60 (230–400 mesh, Merck) is used for flash column chromatography.

The two dimensional ^{1}H-^{1}H COSY experiments were performed at 300 MHz with sweep width of 3030 Hz (1K data points) in ω_2 and 1515 Hz (256 t_1 value 1K) in ω_1. A 1.5 sec relaxation delay was used. The HMQC experiments were carried out at 400 MHz with sweep width of 4032 Hz (2K data points) in ω_2 and 8064 Hz (128 t_1 values zero-filled in 1K) in ω_1. A relaxation delay of 1.0 sec was used and 32 transients were performed for each t_1 value in both the 2D experiments. The purity of the samples was checked on DC-Micro cards SIF (Riedel de Haën, cat. no. 37341) using the following solvent systems: $CHCl_3$: MeOH (17 : 3), (19 : 1). The chromatograms were sprayed with 0.1 % $Ce(SO_4)_2$ in 2 N H_2SO_4 and heated at 80°C for 5 min.

Plant material

The dried aerial parts of *N. hindostana* (40 kg) were purchased from the local market of Karachi and identification was carried out by Dr. Shahida Siddiqui, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. A voucher specimen (KU 022) is available for inspection at the herbarium of the Department of Pharmacognosy, University of Karachi.

Extraction and isolation

Plant material (40 kg) was chopped into small pieces and extracted with petroleum ether and then with cold EtOH (4 times each) at room temperature. The combined EtOH extract was evaporated under reduced pressure. The residue was further subjected to partitioning between EtOAc and H_2O; the EtOAc soluble fraction was evaporated to dryness in vacuum and subjected to column chromatography over silica gel. The elution was carried out with a solvent gradient of increasing polarity in the order of *n*-hexane, C_6H_6, EtOAc, and MeOH.

Nepehinal (1)

The eluate obtained (C_6H_6 : EtOAc, 5 : 95) was further purified through repeated column chromatography over silica gel using C_6H_6-EtOAc (10 : 90) to yield compound **1**. Final purification of **1** was accomplished by flash column chromatography whereby fine needles of **1** (65 mg) were obtained, m.p. 212 °C (dec.); $[\alpha]_D^{24}$: + 28.0 °C (*c* 0.5, C_5D_5N); UV: λ_{max} (MeOH) 208, 230 nm; IR ν_{max} (KBr) cm^{-1}: 3470, 3200 br. (OH), 1690, 1675 (α,β-unsaturated carbonyl group); ^{1}H-NMR (C_5D_5N, 300.13 MHz): δ = 0.82, 0.98, 1.03, 1.10, 1.25 and 1.29 (s, 3H, 6 × Me), 2.67 (sext, *J* = 11.12, 11.12, 5.62 Hz, H-19), 3.57 (dd, *J* = 12.03, 4.45 Hz, H-3), 3.98 (dd, *J* = 10.59, 5.04 Hz, H-1), 4.09 (m, H-11), 5.78 and 6.29 (each s, 2 × H-29), 9.50 (s, H-30); ^{13}C-NMR (C_5D_5N, 75.43 MHz): see Table **1**; EI-MS *m/z* (rel. int.): $[M]^+$ absent, 454 $[M - H_2O]^+$ (4), 436 $[M - 2H_2O]^+$ (4), 424 (6), 370 (10), 355 (6), 339 (12), 327 (16), 309 (10), 283 (8), 255 (6), 231 (8), 205 (12), 189 (14), 135 (46), 95 (86); negative ion FAB-MS: *m/z* 563 [M + glycerol − H]$^-$; positive ion FAB-MS: *m/z* 565 [M + glycerol + H]$^+$, 587 (M + glycerol + Na]$^+$.

Acetylation of 1

Compound **1** (10 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (5 ml) at room temperature overnight. Usual workup provided the diacetate **1a** (7 mg), which could not be crystallized. It, however, appeared pure by TLC, IR ν_{max} ($CHCl_3$): 3500 (OH), 2950, 1730 ($OCOCH_3$), 1250 (C-O stretch.) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ = 0.80, 0.83, 0.94, 0.98, 1.23, 1.36 (s, 3H, 6 × Me), 2.02 (s, $OCOCH_3$), 2.13 (s, $OCOCH_3$), 2.60 (sext, *J* = 10.96, 10.96, 5.5 Hz, H-19), 3.65 (t-like, H-1), 4.48 (dd, *J* = 8.37, 3.69 Hz, H-3), 4.90 (m, H-11), 5.84 and 6.23 (each s, 2 × H-29), 9.47 (s, H-30); EI-MS *m/z* (rel. int.) $[M]^+$ absent, 496 $[M - AcOH]^+$ (5), 478 $[M - AcOH - H_2O]^+$ (2), 436 $[M - 2AcOH]^+$ (5), 418 $[M - 2AcOH - H_2O]^+$ (2), 327 (38), 309 (8), 217 (20), 163 (22), 149 (30), 135 (50), 121 (60), 107 (100), 95 (90).

Reduction of 1 *with* $NaBH_4$

To a stirred solution of **1** (15 mg) in MeOH (2 ml), $NaBH_4$ (10 mg) was slowly added. After 25 minutes the reaction mixture was poured into water (5 ml) and neutralized with 2N HCl, extracted with Et_2O, dried over Na_2SO_4 and the solvent evaporated. After flash chromatography on a silica gel column eluted with $CHCl_3$: MeOH (9.5 : 0.5), 2.5 mg of **3** was obtained, m.p. 280 °C (dec.), (lit. m.p. 282 °C dec.) (5); ^{1}H-NMR (C_5D_5N, 300.13 MHz) (5); negative ion FAB-MS: *m/z* = 473 $[M - H]^-$, 565 [M + glycerol − H]$^-$.

Results and Discussion

The pure triterpene, nepehinal (**1**), $C_{30}H_{48}O_4$, was isolated as described in the Materials and Methods section. Recrystallization from MeOH furnished colorless needles, m.p. 212 °C (dec.), $[\alpha]_D^{24}$: 28.0 °C (*c* 0.5, C_5D_5N). Its UV spectrum in MeOH showed only an end absorption. The IR spectrum (KBr) showed intense absorptions at 3200 and 3470 cm^{-1} indicating the presence of hydroxy groups. Other absorptions, observed at 1675 with a shoulder at 1690 cm^{-1}, were attributed to an α,β-unsaturated carbonyl group.

The ^{1}H-NMR spectrum of nepehinal (**1**) (C_5D_5N, 300.13 MHz) showed six tertiary methyl singlets at δ = 0.82, 0.98, 1.03, 1.10, 1.25, and 1.29. The spectrum also showed methine proton signals at δ = 3.57 (dd, *J* = 12.03, 4.45 Hz, H-3), 3.98 (dd, *J* = 10.59, 5.04 Hz, H-1) and 4.09 (m, H-11). This was confirmed by ^{13}C-NMR which showed CH signals at δ = 66.31, 74.98, and 76.49 characteristic of carbons bearing hydroxy groups. The exocyclic methylene group, H-19, appeared as sextet centered at δ = 2.67 (*J* = 11.12, 11.12, 5.62 Hz). The UV absorptions, coupled with the downfield chemical shift (singlets at δ = 5.78 and 6.29) of vinylic protons (H-29) in the ^{1}H-NMR spectrum of compound **1** was in agreement with the presence of aldehyde function on the vinylic carbon. A sharp one proton singlet at δ = 9.50 together with IR bands at ν_{max} 1675 and 1690 cm^{-1} coupled with ^{13}C-NMR carbon signal at δ = 195.13 proved that compound **1** carried an α,β-unsaturated aldehyde function.

Broad band decoupled ^{13}C-NMR (C_5D_5N, 75.43 MHz) of **1** indicated the presence of 30 carbons in agreement with molecular formula $C_{30}H_{48}O_4$. The DEPT experiment revealed six methyl, nine methylene and nine methine carbons. Six tertiary methyl groups were evident by signals at δ = 14.11 (C-24, C-27), 15.60, 17.57, 17.97, and 28.57. Assignments were determined using BB, DEPT, two dimensional direct CH chemical shift correlation spectra (6, 7) as well as known ^{13}C-NMR chemical shifts of lupeol and related compounds (4, 5). The revised ^{13}C-NMR chemical shifts of nepeticin (**2**) were further confirmed by DEPT experiments (6) and are summarized in Table **1**.

Table 1 ^{13}C-NMR chemical shifts (δ_c/ppm) of triterpenoids **1** and **2** in C_5D_5N.

C	1	2	C	1	2
C- 1	76.49	42.02	C-16	38.28	35.93[b]
C- 2	37.92	28.82	C-17	43.37	43.27[c]
C- 3	74.98	78.08	C-18	53.35	48.36[d]
C- 4	39.88	39.69	C-19	58.02	48.00[d]
C- 5	53.35	56.19	C-20	131.50	150.56
C- 6	18.69	18.76	C-21	29.93	30.30
C- 7	35.56	35.87[b]	C-22	39.88	40.13
C- 8	42.48[a]	42.78[c]	C-23	28.57	29.06
C- 9	58.02	56.48	C-24	14.11	16.59[e]
C-10	46.14	39.69	C-25	15.60	16.85[e]
C-11	66.31	69.67	C-26	17.57	17.56
C-12	34.91	38.03	C-27	14.11	14.76
C-13	35.87	37.46	C-28	17.97	18.31
C-14	42.77[a]	42.96[c]	C-29	129.29	110.02
C-15	27.77	27.87	C-30	195.13	19.59

[a–e] These values are interchangeable.

The EI-mass spectrum of **1** does not show a molecular ion peak. The highest peak at m/z = 454 represents the $M^+ - H_2O$ peak. However, negative ion FAB-MS of **1** afforded a M^+ + glycerol − H peak at m/z = 563, corresponding to the molecular formula $C_{30}H_{48}O_4$ and indicating seven degrees of unsaturation in the molecule. Positive FAB mass spectrum showed M^+ + glycerol + H (m/z = 565) and M^+ + glycerol + Na (m/z = 587). Other peaks were seen in the EI-MS at m/z = 424, 370, 355, 327, 309, and 283. Some of peaks of nepehinal were 2 m.u. less than the corresponding peaks of **3** due to replacement of C-CH_2OH of nepedinol with C-CHO in nepehinal.

Acetylation of **1** furnished a 3,11-diacetate (**1a**) because the 1β-hydroxy group is sterically hindered. The ^{1}H-NMR spectrum of **1a** shows six tertiary methyl singlets at δ = 0.80, 0.83, 0.94, 0.98, 1.23, and 1.36. Two OAc groups were confirmed by singlets at δ = 2.02 and 2.13. The carbinylic protons, C-3 and C-11, were shifted to δ = 4.48 (dd, J = 8.37, 3.69 Hz) and 4.90 (m, H-11), respectively, in the diacetyl derivative. Thus, acetylation occurred only at C-3 and C-11. A sextet centered at δ = 2.60 (J = 10.96, 10.96, 5.5 Hz) was assigned to H-19 and a sharp singlet at δ = 9.47 to the -CHO group. In the EI-MS of the diacetate, the highest peak, at m/z = 496, represents M^+ − AcOH.

Structure **1** was supported by extensive two dimensional NMR experiments. The ^{1}H-^{13}C COSY and HMQC spectra (7) established the direct (one bond) C-H connectivities providing further insights into the structure. The CH signals of C-1, C-3, and C-11 of carbon bearing hydroxy groups at δ = 76.49, 74.98, and 66.31 could easily be correlated with chemical shifts of H-1, H-3, and H-11 at δ = 3.98, 3.57, and 4.09. The C-19, C-29, and C-30 carbons were coupled to the protons resonating at δ = 2.67 (H-19), 5.78, and 6.29 (2 × H-29), and 9.50 (H-30). The tertiary methyl carbons resonated at δ = 28.57 (C-23), 14.11 (C-24), 15.60 (C-25), 17.57 (C-26), 14.11 (C-27), and 17.97 (C-28), respectively. These carbons are coupled with protons at δ = 1.25, 1.03, 1.10, 0.98, 1.29, and 0.82, respectively, in the ^{1}H-^{13}C COSY and HMQC spectra. Thus, all the carbons and their attached protons were identified as expected from the molecular formula. These interactions were further confirmed from the ^{1}H-detected (inverse) ^{1}H/^{13}C correlation experiments (HMBC) (7, 8) and HOHAHA experiments.

The structure of compound **1** was confirmed by two dimensional ^{1}H-^{1}H homonuclear chemical shift correlation spectroscopy (^{1}H-^{1}H COSY) (7) which showed the connectivity of H-2 (δ = 2.30) to both H-1 (δ = 3.98) and H-3 (δ = 3.57). On the other hand, H-11 (δ = 4.09) showed cross peak for H-9 (δ = 1.70). Another set of COSY interactions was observed between the C-29 methylenic protons at δ 5.78 and 6.29 (2 × H-29). Finally, H-5 (δ 0.87) showed a cross peak for only H-6 as C-4 and C-10 are substituted with tertiary methyl groups. The position of the aldehyde group at C-30 was finally confirmed by NOESY spectrum which showed the connectivity of H-29 (δ = 5.78 and 6.29) to H-30 (δ = 9.50). The structure **1** was also confirmed through reduction of **1** to nepedinol **3** (5).

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