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NEPETIDONE AND NEPEDINOL, TWO NEW TRITERPENOIDS FROM *NEPETA HINDOSTANA*

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Nepeta hindostana (Roth) Haines, (Labiatae) is an important medicinal plant of the Indo-Pakistan subcontinent. In the Greco-Arab system of medicine, popularly practised in Pakistan, the plant is known as "Badranj-e-Boya," and its decoction is used as a cardiac tonic, in fevers, and as a gargle for sore throat (1). Its alcoholic extract has been shown to possess hypocholesteremic activity (2).

We have reported earlier the isolation of new triterpenes of the lup-20(29)-ene series from this plant (3-6). We now record the isolation and structure elucidation of a new nortriterpenoid ketone, nepetidone (**1**), and a new triterpenoid tetraol, nepedinol (**2**), from the same source.

Nepetidone (**1**) was eluted with C_6H_6-EtOAc (10:90) from the silica gel column, purified by repeated column chromatography and crystallized from MeOH as colorless crystals. It analyzed for $C_{29}H_{48}O_4$, and its uv spectrum in MeOH showed end absorption at 220 nm with a shoulder at 275 nm. The ir spectrum revealed the presence of hydroxyl (3400-3200 cm^{-1} br), and ketone (1700 cm^{-1}). In the eims, the molecular ion peak is absent; the highest peak at *m/z* 442 represents the M^+-H_2O peak. However, the field desorption (fd) and the fabms show strong M^+ and $M^+ + 1$ peaks at *m/z* 460 and 461, respectively. The eims shows further peaks at *m/z* 424, 370, 355, 327, 309, and 283 which are reminiscent of the ms of nepetidin, [1β,3β,11α-trihydroxy-lup-20(29)-ene] (**3**), a triterpenoid isolated by us from the same plant (4). Some of the peaks of nepetidone are 2 mu higher than the corresponding ones of **3**, and the hrms shows that this is due to the replacement of $C=CH_2$ of nepetidin with C=O in nepetidone.

The relationship between nepetidin and nepetidone is clearly indicated by ^{1}H- and ^{13}C-nmr spectroscopic studies. The ^{1}H-nmr spectrum (300 MHz) of **1** in C_5D_5N shows methyl singlets at δ 0.78, 1.03 (6H, 2×CH_3), 1.11, 1.25, 1.32, and 2.10, the last singlet being due to the $COCH_3$ group. The spectrum further shows carbinylic proton signals at δ 3.59 (dd, J=12, 3.8 Hz, H-3), δ 3.98 (dd, J=11, 4.7 Hz, H-1) and δ 4.12 (m, H-11). There is a sextet centered at δ 2.67 (J=11, 11, 5.7 Hz) which is assigned to H-19 adjacent to a carbonyl group.

On acetylation, **1** yields a 3,11-diacetate (**1a**), as was observed also in the case of **3**. The 1β-hydroxyl group, being sterically hindered, does not react with Ac_2O. In the ^{1}H-nmr spectrum of the diacetate recorded in $CDCl_3$, the signals due to H-3 and H-11 are shifted to δ 4.50 (dd, J=11.9 Hz, 4.1 Hz), δ 4.94 (ddd, J=9.5, 9.5, 8.5 Hz), respectively, whereas the H-1 signal is seen as a dd at δ 3.68 (J=10.9, 4.98 Hz). The slight variation in the chemical shifts of H-1 in **1** and **1a** is due to the difference in solvent in which their spectra were recorded.

Table 1 shows the chemical shifts and assignments in the ^{13}C-nmr spectra of compounds **1** and **3** recorded in the same solvent (C_5D_5N). The ^{13}C-nmr spectrum of 3β-hydroxy-30-norlupan-20-one (**4**) prepared from an authentic sample of lupeol by the OsO_4 method (7) is also included in Table 1 for comparison. The assignments were made on the basis of DEPT experiments as well as the known ^{13}C-nmr chemical shifts of lup-20(29)-ene derivative (3-6).

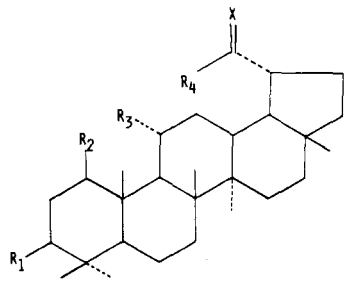
TABLE 1. ^{13}C-nmr Chemical Shifts (in ppm) of **1**, **2**, **3**, and **4** in C_5D_5N

Carbon No.	Compound			
	1	**2**	**3**	**4**
1	66.45	66.69	66.57	39.29[m]
2	35.29[a]	37.65[e]	36.24[i]	27.88[n]
3	75.06[b]	75.15[f]	75.12[j]	78.10
4	39.96	42.82	39.97	39.53
5	58.23	58.25	58.22	55.88
6	18.78	18.82	18.80	18.81
7	35.29[a]	35.80[e]	35.85[i]	34.66[o]
8	42.70[c]	43.00[g]	42.74[k]	41.08
9	52.19[d]	53.52	53.50	50.70[p]
10	46.33	46.40	46.34	37.34
11	76.50[b]	76.61[f]	76.54[j]	21.15
12	34.96[a]	35.12[e]	35.09[i]	27.56[n]
13	35.23	36.31	36.19	37.53
14	42.76[c]	43.27[g]	43.10[k]	42.41[q]
15	28.05	32.41	30.20	27.71[n]
16	38.01	38.06	37.95	35.30[o]
17	43.16[c]	43.27[g]	43.17[k]	43.22[q]
18	49.42	49.09	48.73	49.66
19	53.45[d]	44.01	48.19	52.51[p]
20	211.36	156.27	150.60	211.38
21	27.84	28.06	28.0	28.31[n]
22	39.96	40.04	40.10	40.18[m]
23	29.39	28.68	28.67	29.23
24	14.22	14.38[h]	14.37[l]	16.18
25	15.65	15.70	15.66	16.36
26	17.67	17.75	17.75	16.42
27	14.22	14.28[h]	14.25[l]	14.67
28	18.23	18.06	18.31	18.13
29	—	106.64	110.27	—
30	28.64	64.44	19.39	28.68

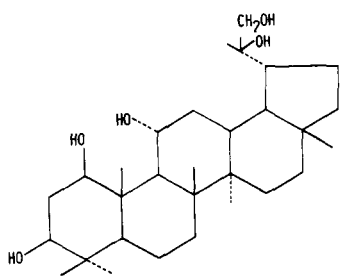
[a-p]Assignments may be reversed.

From the spectroscopic evidence, it is concluded that nepetidone has structure **1**. This is confirmed through chemical conversion of **3** into **1**; nepetidin (**3**) was treated with OsO_4, and the pentaol (**5**) so formed was cleaved with periodic acid yielding **1**.

Nepedinol (**2**) was isolated from the fractions eluted with EtOAc-MeOH (95:5) from the silica gel column, purified by repeated column chromatography and crystallized from MeOH as small colorless crystals. Its fabms showed $M^+ + H$ peak at *m/z* 475, corresponding to the formula $C_{30}H_{50}O_4$. In the eims, no molecular ion peak is observed, the highest peak at *m/z* 456.3603 ($C_{30}H_{48}O_3$) corresponding to the $M^+ - H_2O$. It shows further peaks at 438, 384, 341, 323, 271, 201, 135, and 107. Some of the peaks of nepedinol are 16 mu higher than the corresponding ones of **3**. Its ir spectrum in KBr revealed the presence of hydroxyl (3350 cm^{-1}), but no band due to carbonyl group is observed. The uv spectrum had a maximum at 202 nm (end absorption). The ^{1}H-nmr spectrum (C_5D_5N, 300 MHz) of **2** showed six tertiary methyl signals at δ 0.84, 1.05 (s, 6H, $2\times CH_3$), 1.13, 1.27, and 1.33. A singlet at δ 4.47 (2H) is due to CH_2OH, and nearby singlets with fine splitting at δ 5.12 and 5.41 can be assigned to the two H-29 protons. The secondary carbinylic proton signals are observed at δ 3.64 (m, H-3), δ 4.01 (dd, J=11, 4.7 Hz, H-1) and a distorted hextet at δ 4.13 (H-11). A



	R_1	R_2	R_3	R_4	X
1	OH	OH	OH	CH_3	O
1a	OAc	OH	OAc	CH_3	O
2	OH	OH	OH	CH_2OH	CH_2
2a	OAc	OH	OAc	CH_2OH	CH_2
3	OH	OH	OH	CH_3	CH_2
4	OH	H	H	CH_3	O



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broad signal at δ 4.47 is due to the two CH_2OH protons. Thus, it appears that nepedinol has a structure which is closely related to nepetidin (**3**). However, the absence of vinylic methyl, the presence of a CH_2OH group, only six tertiary methyl groups, and the relative downfield shift of H-29 signal, all indicate that C-30 contains a primary hydroxyl group.

All of the spectroscopic data cited above indicate that the structure of nepedinol is 1β,3β,11α,30-tetrahydroxy-lup-20(29)-ene. This proposed structure was also supported by the ^{13}C-nmr spectrum of **2** (Table 1) which shows that there are four carbon atoms bearing oxygen functions at δ 66.69 (C-1), 75.15 (C-3), 76.61 (C-11), and 64.44 ppm (C-30).

Acetylation of **2** with Ac_2O and pyridine furnished at 3,11,30-triacetate (**2a**). In the ^{1}H-nmr spectrum of the triacetate recorded in $CDCl_3$, the signal due to H-30 is shifted to δ 4.52 (brs) partly superimposed by a double doublet centered at δ 4.51 due to H-3. The H-11 multiplet at δ 4.95 is masked by two broad singlets at δ 4.90 and δ 4.97 due to the two H-29 protons. The H-1 multiplet is observed at δ 3.64.

On the basis of the above spectra, structure **2** is suggested for nepedinol. This structure was also supported by a comparison of the ^{13}C-nmr spectral data of **2** with those of **1** and **3**. (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Gallenkamp melting point apparatus. Uv spectra were measured in MeOH with a Shimadzu UC 240 Graphicord spectrometer. Ir spectra were prepared with a Jasco A-302 spectrometer. The ^{1}H-nmr (300.13 MHz) and ^{13}C-nmr (75.43 MHz) spectra were recorded on a Bruker AM-300 spectrometer in C_5D_5N. The DEPT experiments were carried out with θ=45°, 90°, and 135°; the quaternary carbons were determined by substraction of these spectra from the broad band ^{13}C-nmr spectrum. The ei, fd, and fabms spectra were recorded on a Finningan MAT 312 double focusing ms spectrometer coupled with PDP 11/34 computer system. Tlc was carried out on silica gel plates using the following solvent systems: $CHCL_3$-MeOH (17:3), (19:1); (8:2), and (9:1); spots were visualized by spraying with ceric sulfate solution in 10% H_2SO_4 followed by heating.

PLANT MATERIAL.—The plant material was purchased from the local market and identified by the Department of Pharmacognosy, University of Karachi. A voucher specimen has been deposited in the herbarium of the Department of Botany, University of Karachi.

EXTRACTION AND ISOLATION.—The plant material was extracted three times with *n*-hexane and then with EtOH. The combined ethanolic extract was evaporated under reduced pressure, leaving behind a greenish, syrupy residue which was then partitioned between EtOAc and H_2O. The EtOAc layer was evaporated under reduced pressure, yielding a green, gummy material which was then subjected to column chromatography on silica gel. Elution was carried out with a gradient of increasing polarity in the order of *n*-hexane, C_6H_6, EtOAc, and MeOH.

NEPETIDONE (**1**).—Compound **1** was eluted from the silica gel column with C_6H_6-EtOAc (10:90). It was further purified by repeated (three times) column chromatography on silica gel, and

crystallized from MeOH as colorless crystals (25 mg); mp 300° dec; $[\alpha]_D$ −28.13° (c=0.32, MeOH); uv λ max (MeOH) 220 nm (end absorption); ir ν max (KBr) 3200-3500 br (OH), 1700 (C=O), 1460, 1382, 1360, 1180, 1000 cm^{-1}; ^1H nmr (300 MHz, C_5D_5N) δ 0.78 (s, CH_3), 1.03 (s, 6H, 2×CH_3), 1.11 (s, CH_3), 1.25 (s, CH_3), 1.32 (s, CH_3), 2.10 (s, $COCH_3$), 2.67 (sext, J=11, 11, 5.7 Hz, H-19), 3.59 (dd, J=12, 3.8 Hz, H-3), 3.98 (dd, J=11, 4.7 Hz, H-1), 4.12 (m, H-11); ^{13}C nmr see Table 1; fdms 460 (M^+); fabms 461 (M^++1); eims M^+ absent, 442.344 (6, M^+-H_2O, calcd for $C_{29}H_{46}O_3$, 442.345), 424 (12, M^+-2H_2O), 370.322 (31, calcd. for $C_{26}H_{42}O$, 370.323), 327.268 (88, calcd for $C_{23}H_{35}O$, 327.269), 309.257 (26, calcd. for $C_{23}H_{33}$, 309.258), 257 (8), 283 (12), 231 (14), 205 (30), 163 (34), 135 (84), 107 (100), 95 (88). *Anal.* calcd for: $C_{29}H_{48}O_4$. C, 75.60; H, 10.50. Found: C, 73.67; H, 10.57.

ACETYLATION OF **1**.—Compound **1** (10 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (3 ml) at room temperature overnight. Ice was added to the reaction mixture which was extracted with EtOAc and H_2O. The EtOAc layer was evaporated to yield the diacetate (**1a**), which could not be crystallized. It, however, appeared pure by tlc. Ir ν max (KBr) 3500-3400 br (OH), 2850, 1750 ($OCOCH_3$), 1300, 1260-1160, 1000 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.76 (s, CH_3), 0.82 (s, CH_3), 0.85 (s, CH_3), 0.97 (s, CH_3), 1.00 (s, 6H, 2×CH_3), 1.94 (s, $OCOCH_3$), 2.04 (s, $OCOCH_3$), 2.14 (s, $COCH_3$), 2.61 (sext, J=11, 11, 5.67 Hz, H-19), 3.68 (dd, J=10.9, 4.98 Hz, H-1), 4.50 (dd, J=11.9, 4.1 Hz, H-3), 4.94 (ddd, J=9.5, 9.5, 8.5 Hz, H-11); eims *m/z* M^+ absent, 484 (2, M^+−AcOH), 466 (4, M^+−AcOH−H_2O), 424 (6, M^+−2AcOH), 406, (8, M^+−2AcOH-H_2O), 327 (100), 309 (26)], 283 (12), 231 (14), 205 (30), 163 (34), 135 (84), 107 (100), 95 (68).

CONVERSION OF **3** INTO **1**.—Compound **3** was treated with OsO_4 (8) in the presence of dioxane for 5 days to yield the pentaol (**5**); eims *m/z* M^+ absent, 456 (3, M^+-2H_2O), 438 (6), 384 (4), 341 (18), 283 (28), 161 (38), 135 (62), 107 (100), 95 (98). **5** was cleaved with periodic acid yielding **1**, which was identified through co-tlc, superimposible ir spectra and mixed melting points.

NEPEDINOL (**2**).—Compound **2** was eluted from the fractions eluted with $CHCl_3$-MeOH (19:1), purified and crystallized as described above in the case of **1**; mp 282° dec.; $[\alpha]_D$ −18.67° (c=0.75, C_5D_5N); uv λ max (MeOH) 202 nm (end absorption); ir ν max (KBr) 3350 (OH), 2950 cm^{-1}; ^1H nmr (300 MHz, C_5D_5N) 0.84 (s, CH_3), 1.05 (s, 6H, 2×CH_3), 1.13 (s, CH_3), 1.27 (s, CH_3), 1.33 (s, CH_3), 3.64 (m, H-3), 4.01 (dd, J=11, 4.7 Hz, H-1), 4.13 (distorted hext, H-11), 4.47 (s, CH_2OH), 5.12 (brs) and 5.41 (brs) (2×H-29); ^{13}C nmr see Table 1; fabms 475 (M^++1); eims *m/z* (M^+ absent), 456.361 (6, M^+-H_2O, calcd. for $C_{30}H_{48}O_3$, 456.360), 438.351 (8, M^+-2H_2O, calcd. for $C_{30}H_{46}O_2$, 438.349), 384.338 (12, calcd. for $C_{27}H_{44}O$, 384.339), 341.285 (32, calcd. for $C_{24}H_{37}O$, 371.284), 323.274 (34, calcd. for $C_{24}H_{35}$, 323.273), 271 (6), 201 (22), 135 (60), 107 (95), 95 (100).

ACETYLATION OF **2**.—Compound **2** was acetylated as **1** to yield **2a**. Ir ν max ($CDCl_3$) 3500 (OH), 2950, 1700 ($OCOCH_3$), 1240 (C-O stretching), 1020, 760 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.77 (s, CH_3), 0.82 (s, CH_3), 0.85 (s, CH_3), 0.97 (s, CH_3), 0.98 (s, CH_3), 1.02 (s, CH_3), 1.98 (s, $OCOCH_3$), 2.04 (s, $OCOCH_3$), 2.09 (s, $OCOCH_3$), 3.64 (m, H-1), 4.51 (dd, H-3), 4.52 (br.s, H-30), 4.90 and 4.97 (br.s, 2×H-29); eims *m/z* M^+ absent, 540 (4, M^+−AcOH), 480 (6, M^+−2AcOH), 462 (3, M^+−2AcOH-H_2O), 420 (4), 383 (31), 323 (100), 267 (7), 201 (28), 107 (80), 95 (74).

LITERATURE CITED

1. R.N. Chopra, S.L. Nayer, and I.C. Chopra, "Glossary of Indian Medicinal Plants," Council of Scientific and Industrial Research, New Delhi, 1956, p. 175.
2. H.M. Said, Ed., "Hamdard Pharmacopoeia of Eastern Medicine," Hamdard Foundation of Pakistan, Karachi, 1969, p. 446.
3. V.U. Ahmad, S. Bano, W. Voelter, and W. Fuchs, *Tetrahedron Lett.*, **22**, 175 (1981).
4. V.U. Ahmad, S. Bano, W. Voelter, and W. Fuchs, *Z. Naturforsch.*, **37b**, 1675 (1982).
5. V.U. Ahmad, S. Bano, and F.V. Mohammad, *Fitoterapia*, **55**, 244 (1984).
6. V.U. Ahmad, S. Bano, and F.V. Mohammad, *Planta Med.*, **6**, 521 (1985).
7. E.R.H. Jones and R.H. Meakins, *J. Chem. Soc. (London)*, **1**, 456 (1940).
8. R. Ruzicka and M. Brenner, *Helv. Chim. Acta*, **23**, 1328 (1940).

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