Diterpenoids from the Root Bark of *Azadirachta indica*

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From the root bark of *Azadirachta indica* A. Juss (neem) two new diterpenes nimbilicin and nimbocidin have been isolated and their structures established through chemical and spectroscopic methods.

Introduction

Azadirachta indica A. Juss (Meliaceae) commonly known as "neem" is widely distributed in Asia, Africa and other tropical parts of the world. Its different parts are highly reputed in folklore and traditional systems of medicine for the treatment of a variety of human ailments [1–2]. In view of the therapeutic [1, 3] and pesticidal [4–5] importance attributed to neem, comprehensive investigation on different parts of the tree has been carried out by various groups of workers leading to the isolation and structure elucidation of a series of terpenoidal constituents [6–9]. Present studies undertaken on the root bark have resulted in the isolation of two new diterpenes nimbilicin and nimbocidin.

Results and Discussion

Nimbilicin **1,** $C_{20}H_{24}O_3$ (M^+ 312.1727) $[\alpha]_D$ +25, exhibits in its IR spectrum peaks at 2900 (C–H), 1710 (C=O, ketone and ester), 1610 (C=C), 1380 (gem. dimethyl) and 1100 cm^{-1} (C–O). It has ultraviolet absorptions at 206, 230, 270 and 296 nm. The diterpenoidal nature of **1** was indicated by the molecular formula and presence in the ^1H NMR spectrum (Table I) of three three-proton singlets at δ = 0.87, 1.13 and 1.67 assignable to H-18, H-19 and H-20. The appearance of the aromatic protons as two singlets at δ = 6.76 and δ = 7.86 attributable to H-11 and H-14 respectively suggested two substituents at C-12 and C-13. The NMR spectra suggested that one of these substituents was CH_3 (δ_H = 2.03, δ_C = 21.2) while the other was an acetoxy function (δ_H = 2.25, δ_C = 23.4 and 169.8) which were also confirmed by fragments at 253 ($M-OCOCH_3$) and 238 ($M-OCOCH_3-CH_3$) in the mass spectrum. The ^{13}C NMR spectrum further showed that **1** has one more ketonic carbonyl conjugated with an aromatic ring (δ_C = 199.7), which was placed at C-7 in the light of chemical shifts of H-11, H-14 and C-7, are comparable with those reported for sugiol [10–11]. The assignment of position of acetoxy and methyl groups at C-12 and C-13 respectively could be done through the chemical shifts of H-11 and H-14 which are more comparable with the values calculated [12] with this arrangement as against those of its isomer. In addition to this, the ^1H NMR spectrum revealed the presence of one olefinic proton (δ_H = 5.11, dd, J = 7.48 and 3.00, δ_C = 135.2) and a vinylic methyl (δ = 1.67). Absence of H-5 and appearance of H-6 as two

Table I. ^1H NMR chemical shifts of **1** and **2.**

Assignment	**1**	**2**
H-1α	2.01 (m)	–
H-1β	2.20 (m)	–
H-2α	2.28 (m)	–
H-2β	2.30 (m)	–
H-3	5.11 (dd) $J_{3,2\alpha}$ 7.48 $J_{3,2\beta}$ 3.00	–
H-5	–	1.82 (m)
H-6a	2.75 (d) J_{gem} 14.00	–
H-6b	2.67 (d) J_{gem} 14.00	–
H-11	6.76 (s)	–
H-12	–	6.21
H-14	7.86	–
H-15	–	3.10 h J = 6.92
H-16	–	1.28 (d)
H-17	–	J = 6.92
H-18	1.67 (s)	1.21 (s)
H-19	1.13 (s)	1.23 (s)
H-20	0.87 (s)	1.40 (s)
CH_3	2.03 (s)	–
$COCH_3$	2.25 (s)	–

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AB doublets (δ_H = 2.67, 1H, d, J = 14.0 and 2.75, 1H, d, J = 14.0 Hz) led to accommodate 18 and 19 methyls at C-5 and C-4 respectively with the double bond at C-3. These substitutions were finally confirmed through COSY-45 plot which showed the connectivities of H-6α with H-6β, H-3 with H-2α and H-2β, H-2α with H-2β, H-1α, and H-1β and H-1α with H-1β. The 2D n.O.e (NOESY) plot showed spatial connectivities of H-18 with H-19 and aromatic CH_3 with acetoxy methyl. These data led to the assignment of structure **1** for nimbilicin.

Nimbocidin **2** has molecular formula $C_{20}H_{30}O_2$ (through peak matching of molecular ion) showing six double bond equivalents. IR spectrum of nimbocidin displayed peaks at 3400 (OH), 1600 (aromatic ring) and 1380 (gem. dimethyl) and 1100 cm^{-1} (C–O) while in the UV spectrum maxima were observed at 205, 228 and 285 nm.

The molecular formula of **2** and presence in the ^1H NMR spectrum of three three-proton upfield singlets at δ = 0.85, 0.89 and 0.91 indicated that **2** also belongs to the diterpenoids. The ^{13}C NMR spectrum (Table II) showed that **2** has one tertiary, five quaternary olefinic carbons (of aromatic ring), five methyls, five methylenes, two methines and two sp^3 quaternary carbons. The appearance of only one downfield singlet (aromatic proton) at δ = 6.21 ppm and only one tertiary aromatic carbon (δ_C = 118.1) exhibited three substituents in ring C. The ^1H NMR spectrum indicated that one of these substituents is isopropyl (δ = 1.28, 6H, d, J = 6.92 Hz; H-16 and H-17; δ = 3.10, 1H, m, H-15) which was further supported by the fragment at m/z = 259.1697 ($C_{17}H_{23}O_2$) in the mass spectrum resulting from the loss of C_3H_7. These data indicated that **2** has a tricyclic abietane type of skeleton. Formation of the dimethyl (**3**, δ OCH_3 = 3.98, 4.09) derivative on reaction of **2** with CH_2N_2 demonstrated that the remaining two substituents of the aromatic ring are hydroxyl groups. The appearance of H-20 at δ = 1.40 led to place one of the hydroxyl groups at C-11 in analogy with the earlier observations in the case of 11-hydroxy derivatives [13–15]. On the other hand, the chemical shift of the aromatic proton (δ = 6.21) which is more close to the calculated value [12] of this proton in a 11,14-dihydroxy derivative (δ = 6.59) as against δ = 6.8 for an 11,12-dihydroxy derivative decided the position of the second hydroxyl function at C-14. In the light of these observations structure **2** has been assigned to nimbocidin.

Tab. II. ^{13}C NMR chemical shifts (δ/ppm) of **1** and **2** (75 MHz) $CDCl_3$.

Carbon	**1**	**2**
1	26.4	38.5
2	22.7	14.1
3	135.2	39.5
4	134.0	33.4
5	35.2	49.1
6	32.2	21.6
7	199.7	29.3
8	122.2	127.3
9	156.1	145.9
10	36.9	35.0
11	121.6	150.0
12	144.1	118.1
13	131.5	132.5
14	125.1	151.5
15	–	31.9
16	–	22.7
17	–	22.7
18	29.3	34.0
19	14.1	24.5
20	23.4	28.1
CH_3	21.2	–
$CO\underline{CH_3}$	23.4	–
$\underline{CO}CH_3$	169.8	–

Material and Methods

Melting points were recorded on an air bath type melting point apparatus and are uncorrected. IR ($CHCl_3$) and UV (MeOH) spectra were measured on Jasco-IRA-I and Pye-Unicam SP-800 spectrometer respectively. Mass spectra were recorded on Finnigan MAT 311A double focussing mass spectrometer. ^1H and ^{13}C NMR (broad band and dept) spectra were recorded in $CDCl_3$ on Bruker Aspect AM 400 spectrometer operating at 400 MHz and on Bruker Aspect 300 spectrometer operating at 75 MHz respectively. The assignments of ^{13}C NMR chemical shifts have been made through chemical shift rules [16], gaspe spectrum and comparison with sugiol [11] and other similar compounds [17]. Merck Kieselgel 60 PF_{254} coated on glass plates was used for analytical (thin layer) and preparative (thick layer) chromatography. A voucher specimen (No. NM-1) has been deposited in the Herbarium of Botany Department, University of Karachi.

Extraction and isolation

Neem root bark (28 kg) collected in June 1987 from Karachi region was successively extracted out with petroleum ether and methylene chloride at room temperature. The methylene chloride extract

was concentrated under reduced pressure and shaken out with ethyl acetate and water. The ethyl acetate layer was repeatedly extracted out with 4% aqueous Na_2CO_3 solution to separate acidic and neutral fractions.

The Na_2CO_3 phase was acidified with dil HCl and extracted out with ethyl acetate which was washed, dried (anhydrous Na_2SO_4) and charcoaled. The residue obtained on removal of the solvent was successively treated with petroleum ether, ether and ethyl acetate. The ethyl acetate soluble portion was concentrated, taken in a flask containing silica gel (E. Merck, 7719) and eluted with benzene and benzene–ethyl acetate in the order of increasing polarity. Fractions obtained with solvent systems benzene–ethyl acetate (9:1–6:4) were combined, concentrated and again subjected to flask chromatography (silica gel, benzene–ethyl acetate; fractionally increasing the polarity of solvent system). The benzene–ethyl acetate (8:2–7:3) eluates were combined, freed of the solvent and subjected to flash column chromatography (silica gel E. Merck 9385; petroleum ether, petroleum ether–ethyl acetate) when nimbilicin (**1**) was obtained as a pure product with solvent system – petroleum ether–ethyl acetate 9.15:0.85, respectively.

The residue from the hexane extract of neem root bark referred to above was divided into petroleum ether soluble and insoluble fractions. The latter was taken in a small quantity of ethyl acetate, adsorbed on silica gel contained in a flask and eluted with petroleum ether and petroleum ether–benzene, successively increasing the ratio of benzene. Nimbolicidin was obtained with some allied impurities in the fractions eluted with the solvent system hexane–benzene (7.5:2.5). Purification through thin layer chromatography (silica gel, hexane–ethyl acetate; 9:1) yielded **2** as a uniform component.

Nimbilicin **1**

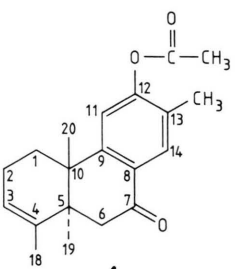
It crystallized from chloroform as needles, 7 mg, $[\alpha]_D^{24}$ +25, m.p. 78–79 °C. EIMS *m/z* (%): Found M^+, 312.1727 (**6**) (calcd for $C_{20}H_{24}O_3$ 312.1725); 216.1145 (22) ($C_{14}H_{16}O_2$), 203.1068 (18) ($C_{13}H_{15}O_2$), 175.0759 (60) ($C_{11}H_{11}O_2$) and 57 (100).

Nimbocidin **2**

Needles (petroleum ether), 7.8 mg, m.p. 132–133 °C. EIMS *m/z* (%): Found M^+, 302.2257 (24) (calcd for $C_{20}H_{30}O_2$ 302.2245), 287.2000 ($M-CH_3$) (23) and 259.1704 ($M-C_3H_7$) (28).

Methylation of **2**

A solution of nimbocidin (3.3 mg) in ether was treated with freshly prepared diazomethane at room temperature for 3–4 h. The reaction mixture was evaporated to dryness when chromatographically pure **3** was obtained as irregular plates (3.1 mg) m.p. 116–117 °C, UV λ_{max}(MeOH) nm 205, 228, 285, IR ν_{max}($CHCl_3$) cm^{-1} 1600 (C=C), 1380 (gem. dimethyl) and 1100 (C–O). EIMS *m/z* (%): 330 (M^+) (6); ^1H NMR δ: 0.87 (3H, s), 0.88 (3H, s), 0.91 (3H, s), 3.98, 4.09 (6H, s, 2×OCH_3) and 6.21 (1H, s, H-11).



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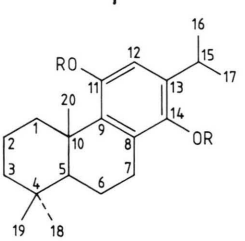

2: R = H

3: R = CH_3

[1] W. Dymock, C. J. H. Warden, and D. Hooper, Pharmacographia Indica **2,** 322 (1890). The Institute of Health and Tibbi Research republished under the auspices of Hamdard National Foundation of Pakistan.
[2] R. N. Chopra, S. L. Nayar, I. C. Chopra, Glossary of Indian Medicinal Plants, p. 31, C.S.I.R. Publication, New Delhi (1956).
[3] N. R. Pillai and G. Santhakumari, Planta Medica **50,** 143 and 146 (1984).
[4] H. Schmutterer, K. R. S. Ascher, and H. Rembold, Proceedings of the 1st International Neem Conference, F.R.G. Rottach-Egern, Ger. Agency Tech. Coop., Eschborn (1981).
[5] H. Schmutterer, K. R. S. Ascher, and H. Rembold, Proceedings of the 2nd International Neem Conference, F.R.G. Rottach-Egern, Ger. Agency Tech. Coop., Eschborn (1984).
[6] S. Siddiqui, B. S. Siddiqui, S. Faizi, and T. Mahmood, J. Nat. Prod. **51,** 30 (1988).
[7] S. Siddiqui, I. Ara, S. Faizi, T. Mahmood, and B. S. Siddiqui, Phytochemistry **27,** 3903 (1988).
[8] P. L. Majumder, D. C. Maiti, W. Kraus, and M. Bokel, Phytochemistry **26,** 3021 (1987).
[9] S. M. Lee, J. I. Olsen, M. P. Schweizer, and J. A. Klocke, Phytochemistry **27,** 2773 (1988).
[10] W. L. Meyer, G. B. Clemans, and R. A. Mannigan, J. Org. Chem. **40,** 3686 (1975).
[11] E. Wenkert, B. L. Buckwalter, I. R. Burfitt, M. J. Gasic, H. E. Gottlieb, E. W. Hagaman, F. M. Schell, and P. M. Wovkulich, in G. C. Levy (ed.): Topics in carbon-13 NMR spectroscopy, Wiley-Interscience, New York **2,** 81 (1976).
[12] D. H. Williams and I. Fleming, "Spectroscopic Methods in Organic Chemistry", McGraw-Hill Book Company (UK) Limited 135 (1973).
[13] T. Miyase, P. Ruedi, and C. H. Eugster, Helv. Chim. Acta **60,** 2770 (1977).
[14] J. A. Hueso-Rodriguez, M. L. Jimeno, B. Rodriguez, G. Savona, and M. Bruno, Phytochemistry **22,** 2005 (1983).
[15] R. V. Stevens and G. S. Bisacchi, J. Org. Chem. **17,** 2398 (1982).
[16] T. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York (1972).
[17] F. W. Wehrli and T. Nishida, in W. Herz, H. Grisebach, and G. W. Kirby (eds): Progress in the Chemistry of Organic Natural Products, Wien, SpringerVerlag, New York **36,** 1 (1979).