Three New Diterpenoids from the Stem Bark of Azadirachta indica

4 AUTHORS, INCLUDING:

Bina S Siddiqui
University of Karachi

308 PUBLICATIONS **4,468** CITATIONS

Shaheen Faizi

120 PUBLICATIONS **1,753** CITATIONS

THREE NEW DITERPENOIDS FROM THE STEM BARK OF *AZADIRACHTA INDICA*

IFFAT ARA, BINA S. SIDDIQUI,* SHAHEEN FAIZI, and SALIMUZZAMAN SIDDIQUI

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan

ABSTRACT.—Three new tricyclic diterpenoids, nimbosodione, nimbisonol, and de-methylnimbionol, have been isolated from the stem bark of *Azadirachta indica*. These compounds have been assigned the structures 12-hydroxy-13-acetylpodocarpa-8,11,13-trien-7-one [1], 3β,12-dihydroxy-13-methylpodocarpa-8,11,13-trien-7-one [4], and 3β,12,13-trihy-droxypodocarpa-8,11,13-trien-7-one [8], respectively, on the basis of chemical and spectral studies.

The isolation and structure elucidation of various terpenoidal constituents from fresh fruits, leaves, twigs, and root and stem bark of *Azadirachta indica* A. Juss (Meliaceae), "neem," has been communicated earlier by various groups of workers (1,2). The present paper deals with the studies undertaken in the stem bark resulting in the isolation of three new tricyclic diterpenoids, nimbosodione, nimbisonol, and de-methylnimbionol, the structures of which have been elucidated as 12-hydroxy-13-acetylpodocarpa-8,11,13-trien-7-one [1], 3β,12-dihydroxy-13-methylpodocarpa-8,11,13-trien-7-one [4], and 3β,12,13-trihydroxypodocarpa-8,11,13-trien-7-one [8], respectively, through chemical transformations and spectroscopic methods.

RESULTS AND DISCUSSION

The residue obtained from the EtOH extract of the stem bark was divided into acidic and neutral fractions. The acidic fraction furnished nimbisonol [4] and de-methylnimbionol [8], while the neutral fraction gave nimbosodione [1] as described in the Experimental section.

Nimbosodione [1] has the molecular formula $C_{19}H_{24}O_3$ (through peak matching of the molecular ion and ^{13}C-nmr spectroscopy) indicating the presence of eight double-bond equivalents in the molecule. The uv spectrum showed absorption characteristic of an aromatic ring, and its ir spectrum displayed peaks for hydroxyl and carbonyl groups.

The ^1H-nmr spectrum showed three singlets of three protons each at δ 0.92 (H-18), 0.97 (H-19), and 1.20 (H-20). Two one-proton singlets were observed at δ 6.72 and 7.82 attributable to protons at C-11 and C-14, respectively. A three-proton singlet at δ 2.22 was assigned to the acetyl group, which was also confirmed by a fragment at 257.1541 $[M - Ac]^+$ in the mass spectrum and by ^{13}C-nmr spectra (broad band and DEPT) (Ac, δC = 198.62 and 32.59). The ^{13}C-nmr spectrum further showed that 1

1	R=H
2	R=Me
3	R=Ac
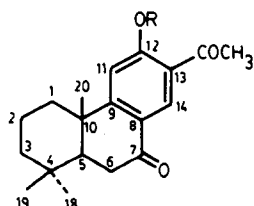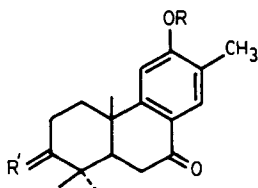
4	R=H, R'=β-OH, H
5	R=Me, R'=β-OH, H
6	R=Me, R'=O
7	R=Ac, R'=β-OAc, H
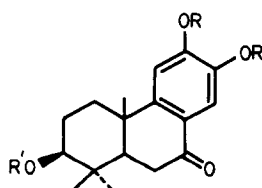
8	R,R'=H
9	R=Me, R'=H
10	R,R'=Ac

has one more ketonic carbonyl conjugated with the aromatic ring (δ C = 198.60), two tertiary and four quaternary olefinic carbons (of the aromatic ring), three methyls, four methylenes, one methine, and two saturated quaternary carbons. These data, along with the eight double-bond equivalents, indicated that **1** is a diterpenoid with a tricyclic dehydropodocarpane type of skeleton. The carbonyl function was placed at C-7 in the light of the chemical shifts of H-11, H-14, and C-7 (δ C = 198.60), which are comparable with those reported for sugiol (3,4) (Table 1). This placement was further corroborated by the chemical shifts and multiplicities of H-6α (δ 2.69, dd, J = 18.0 and 4.6 Hz) and H-6β (δ 2.58, dd, J = 18.0 and 13.0 Hz). The singlets at δ 6.72 and 7.82 for H-11 and H-14 showed that C-12 and C-13 are substituted. One of these substituents was taken for an acetyl group, while the other was identified as a hydroxyl function by the ir (3600 cm^{-1}) and ^{13}C-nmr (δ C = 159.15) spectra, acetylation to the acetyl derivative **3** (δ Ac = 2.16 and 2.30), and methylation with CH_2N_2 to the monomethyl derivative **2** (δ CH_3O = 4.07).

TABLE 1. ^{13}C-nmr Chemical Shifts (ppm) of Nimbosodione [**1**] and Sugiol.

Carbon	Compound	
	1	Sugiol[a]
C-1	37.95	37.3
C-2	18.90	18.4
C-3	41.37	40.9
C-4	33.31	32.7
C-5	49.58	49.1
C-6	36.03	35.4
C-7	198.60	199.2
C-8	157.08	122.4
C-9	159.08	156.3
C-10	33.31	37.4
C-11	109.62	109.0
C-12	159.15	160.3
C-13	157.42	133.0
C-14	130.78	125.6
C-15	—	26.1
C-16	—	21.8
C-17	—	21.6
C-18	15.10	20.7
C-19	23.22	31.7
C-20	21.31	22.5
CO*Me*	32.59	—
C*O*Me	198.62	—

[a]Values in this column are from Wenkert *et al.* (4).

Placement of the OH and acetyl groups at C-12 and C-13, respectively, and A/B trans ring junction in **1** was established through 2D nOe (NOESY) spectral analysis. Thus, the NOESY spectrum showed spatial connectivities between Ac and H-14, between H-19 (δ 0.97) and H-20 (δ 1.20), and between H-18 (δ 0.92) and H-5 (δ 1.83). In the light of these observations the structure of nimbosodione was determined to be **1**.

Nimbisonol [**4**] has molecular formula $C_{18}H_{24}O_3$ (through peak matching of molecular ion 288.1725). Three three-proton singlets at δ 1.21, 1.04, and 0.95 and two downfield one-proton singlets at δ 7.90 and 6.84 in the ^{1}H-nmr spectrum indicated that **4** also belongs to the tricyclic diterpenoids with the dehydropodocarpane

skeleton and substitution at both C-12 and C-13. Methylation of **4** to the methyl de-
rivative **5** ([M]$^+$ 302) on reaction with freshly prepared CH_2N_2 showed that one of
these substituents is a hydroxyl group, while a three-proton singlet at δ 2.22 suggested
that the other substituent is either an acetyl or a methyl group. In the latter case a car-
bonyl function has to be accommodated in ring A or ring B because the molecular for-
mula showed seven double bond equivalents. In the ^{1}H-nmr spectrum of **4** the region
corresponding to the methylene protons adjacent to the carbonyl group was not fully re-
solved and was therefore inconclusive in this respect. However, in the ^{1}H-nmr spec-
trum of the acetyl derivative **7**, H-6β gave a clearly resolved double doublet at δ 2.79
($J_{gem} = 17.4, J_{6β,5} = 14.0$ Hz), thus leading to the conclusion that one of the aromatic
substituents is a methyl group and that the carbonyl function is at C-7. These observa-
tions left one oxygen function to be accounted for; it could be assigned as a hydroxyl
group because the ^{1}H-nmr spectrum showed a one-proton doublet of doublets at δ 3.35
($J = 13.0$ and 6.0 Hz). This was placed at C-3 in the light of the multiplicity of H-3
and a downfield shift of H-19 (1.04) as compared to that of **1** (δ 0.97), thus leading to
the assignment of structure **4** to nimbisonol. Conclusive evidence in favor of this struc-
ture was obtained through oxidation (CrO_3/pyridine) of methylnimbisonol [**5**] to
methylnimbinone [**6**] (5).

That demethylnimbionol [**8**] has a tricyclic diterpenoid skeleton similar to those of
1 and **4** was indicated by the presence in the ^{1}H-nmr spectrum of three three-proton
singlets at δ 0.95 (H-18), 1.04 (H-19), and 1.20 (H-20) and appearance of two one-
proton singlets at δ 6.81 (H-11) and 7.82 (H-14). The molecular formula $C_{17}H_{22}O_4$
(290.1517) and seven double-bond equivalents exhibited that **8** has three hydroxyl and
one carbonyl substituents, because no olefinic proton was observed in the ^{1}H-nmr spec-
trum. The downfield chemical shift of H-14 (δ 7.82) indicated that the carbonyl func-
tion in **8** is also at C-7. Two of the hydroxyl groups were placed at C-12 and C-13 and
the third at C-3, as H-3 gave a double doublet at δ 3.34 ($J_{3α,2β} = 13.0, J_{3α,2} = 5.0$
Hz). These functionalities were supported by acetylation of **8** to the triacetyl derivative
10 ([M]$^+$ 416) and methylation (CH_2N_2) to the dimethyl derivative **9** ([M]$^+$ 318). A
co-tlc of **9** with methylnimbionol showed them to be identical, thus establishing the
structure of **8** as demethylnimbionol (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's (uncorrected) were recorded on an air bath type
melting point apparatus. Mass spectra were determined on Finnigan MAT 311A double focussing mass
spectrometers. ^{1}H-nmr spectra were recorded in $CDCl_3$ on a Bruker Aspect AM 300 spectrometer operat-
ing at 300 MHz, and ^{13}C-nmr spectra (broad band and DEPT) were recorded in $CDCl_3$ on Bruker Aspect-
300 spectrometer operating at 75 MHz. Ir spectra (in $CHCl_3$) were recorded on JASCO IRA-I, and uv
spectra (in MeOH) on Pye-Unicam sp-800 spectrometer.

The assignment of ^{13}C-nmr chemical shifts is based on chemical shift rules (7) and on comparison
with those of similar compounds (4). Merck Kieselgel 60 PF$_{254}$ and Al$_2$O$_3$ 60 PF$_{254}$ coated on glass plates
were used for analytical (thin layer) and preparative (thick layer) chromatography.

PLANT MATERIAL.—"Neem" stem bark (20 kg) was collected from the Karachi region in the month
of June 1987, and identified by Professor S.I. Ali, Department of Botany, University of Karachi. A
voucher specimen (No. NM-1) has been deposited in the Herbarium of the Botany Department, Univer-
sity of Karachi.

EXTRACTION AND ISOLATION.—The stem bark was repeatedly extracted with MeOH at room tem-
perature, and the combined extracts were concentrated under reduced pressure giving a dark brown
gummy residue that was partitioned between EtOAc and H_2O. The former was shaken out with 4%
Na_2CO_3 solution to separate the acidic from neutral constituents. The EtOAc phase was washed, dried
(anhydrous Na_2SO_4), and freed of the solvent under reduced pressure, furnishing the neutral fraction. The
aqueous Na_2CO_3 phase was acidified (30% HCl) and shaken out with EtOAc. The EtOAc phase containing
the acidic compounds was washed with H_2O, dried (Na_2SO_4), and treated with charcoal. The residue ob-

tained on removal of the solvent was divided into hexane-soluble and hexane-insoluble fractions. The hexane-insoluble portion was again divided into Et$_2$O-soluble and Et$_2$O-insoluble fractions. The former was concentrated and treated with an excess of hexane, which precipitated an insoluble material that was filtered and subjected to thick layer chromatography [Si gel, CHCl$_3$-MeOH (9.5:0.5)], ultimately yielding compounds **4** and **8**. The neutral fraction was treated with hexane, and the hexane-soluble portion was shaken out with 80% MeOH. The residue obtained on usual workup of the MeOH phase was dissolved in a small quantity of EtOAc and treated with an excess of hexane, furnishing hexane/EtOAc-soluble and hexane/EtOAc-insoluble fractions. The latter fraction was redissolved in Et$_2$O and poured into a flask containing Si gel, which was successively eluted with Et$_2$O, EtOAc, and MeOH. The Et$_2$O and EtOAc eluates were combined and subjected to thick layer chromatography (Si gel, CHCl$_3$), furnishing **1**.

NIMBOSODIONE [**1**].—Compound **1** crystallized from hexane as irregular plates (12.2 mg): mp 134–135°; uv λ max 209, 230, 280, 300 nm; ir ν max 3600–3200, 2900, 1680, 1600, 1200; eims *m/z* 300.1725 (C$_{19}$H$_{24}$O$_3$), 272.1776 (C$_{18}$H$_{24}$O$_2$), [M − Ac]$^+$ 257.1541 (C$_{17}$H$_{21}$O$_2$); ^{1}H nmr (CDCl$_3$, 300 MHz) δ 0.92 (s, H-18), 0.97 (s, H-19), 1.20 (s, H-20), 1.42 (ddd, J = 13.4, 9.9, 6.4 Hz, H-3α), 1.52 (ddd, J = 13.4, 6.4, 3.2 Hz, H-3β), 1.61–1.70 (m, H-1α and H-2α), 1.83 (dd, J = 13.0, 4.6, H-5), 1.94–1.99 (m, H-2β), 2.22 (s, COCH$_3$), 2.54–2.58 (m, H-1β), 2.58 (dd, J = 18.0, 13.0, H-6β), 2.69 (dd, J = 18.0, 4.6, H-6α), 6.72 (s, H-11), 7.82 (s, H-14); ^{13}C nmr see Table 1.

METHYLATION OF NIMBOSODIONE [**1**].—To a solution of compound **1** (4.5 mg) in Et$_2$O was added an ethereal solution of freshly prepared CH$_2$N$_2$, and the reaction mixture was kept for 4–5 h at room temperature. Its workup in the usual manner afforded **2** as needles: mp 84–85°; uv λ max 208, 218, 298, 302 nm, ir ν max 2900 (C-H), 1700 (C=O), 1600 (aromatic double bond), 1100 (C-O); eims *m/z* [M]$^+$ 314; ^{1}H nmr (CDCl$_3$, 300 MHz) δ 0.93 (s, H-18), 0.99 (s, H-19), 1.23 (s, H-20), 1.85 (dd, J = 14.0, 4.0, H-5), 2.22 (s, Ac), 2.55 (dd, J = 18.0, 4.0), 2.63 (dd, J = 18.0, 14.0, H-6β), 4.07 (s, OMe), 6.72 (s, H-11), 7.81 (s, H-14).

ACETYLATION OF NIMBOSODIONE [**1**].—To a solution of **1** (5.5 mg) in pyridine (1 ml) Ac$_2$O (2 ml) was added, and reaction mixture was kept overnight at room temperature. The acetylated product [**3**] (3.5 mg) was obtained on usual workup as rods: mp 98–99°; uv λ max 208, 258, 300; ir ν max 2900 (C-H), 1720 (C=O), 1600 (C=C), 1150 (C-O); eims *m/z* 342; ^{1}H nmr (CDCl$_3$, 300 MHz) δ 0.93 (s, H-18), 0.98 (s, H-19), 1.23 (s, H-20), 1.88 (dd, J = 13.5, 4.5, H-5), 2.16 (s, Ac), 2.30 (s, Ac), 2.61 (dd, J = 18.3, 13.5, H-6β), 2.70 (dd, J = 18.3, 4.5, H-6α), 6.99 (s, H-11), 7.88 (s, H-14).

NIMBISONOL [**4**].—Compound **4** crystallized from hexane as plates (7.6 mg): mp 178–179°; uv λ max 208, 229, 280, 305 nm, ir ν max 3400, 2800, 1710, 1600, 1150, 1050; eims *m/z* 288.1725 (C$_{18}$H$_{24}$O$_3$), [M − C$_3$H$_5$O]$^+$ 231; ^{1}H nmr (CDCl$_3$, 300 MHz) δ 0.95 (s, H-18), 1.04 (s, H-19), 1.21 (s, H-20), 1.83 (dd, J = 13.0, 5.7, H-5), 2.22 (s, Me), 3.35 (dd, J = 13.0, 6.0, H-3α), 6.84 (s, H-11), 7.90 (s, H-14).

METHYLATION OF NIMBISONOL [**4**].—A solution of compound **4** (3.3 mg) in Et$_2$O was treated with freshly prepared CH$_2$N$_2$ at room temperature for 6 h and evaporated to dryness under reduced pressure. The methylated product **5** crystallized from MeOH as irregular plates (2.9 mg): mp 162–163°; ir 3000 (OH), 2800 (C-H), 1700 (C=O), 1620 (C=C), 1200 cm^{-1} (C-O); uv 210, 223, 290 nm; eims *m/z* [M]$^+$ 302; ^{1}H nmr (CDCl$_3$, 300 MHz) δ 4.05 (s, OMe).

OXIDATION OF METHYLNIMBISONOL [**5**].—Compound **5** (2 mg) was stirred with CrO$_3$ and pyridine at room temperature for 24 h. The keto derivative **6**, mp 106–107°, obtained after usual workup, showed a single spot on tlc and gave [M]$^+$ peak at 300. A co-tlc and undepressed mmp of **6** with methylnimbinone showed them to be identical.

ACETYLATION OF NIMBISONOL [**4**].—Compound **4** was taken up in pyridine and Ac$_2$O was added; the solution was kept at room temperature overnight. On usual workup, the acetylated product **7** was obtained as plates: mp 113–114°; uv λ max 210, 255, 300; ir ν max 2900 (C-H), 1720 (C=O), 1600 (C=C), 1150 cm^{-1} (C-O); eims *m/z* [M]$^+$ 372; ^{1}H nmr (CDCl$_3$, 300 MHz) δ 1.03 (s, H-18), 1.14 (s, H-19), 1.20 (s, H-20), 1.85 (m, H-5), 2.07, 2.29, 2.30 (each 3Hs, 2 × Ac and Me), 2.68 (m, H-6α), 2.79 (dd, J = 17.4, 14.0, H-6β), 4.56 (dd, J = 10.0, 4.0, H-3), 7.19 (s, H-11), 7.83 (s, H-14).

DEMETHYLNIMBIONOL [**8**].—Compound **8** crystallized from hexane as irregular plates (8.5 mg): mp 132–133°; uv λ max 208, 230, 280, 304, 390 nm; ir ν max 3600–3200, 2850, 1735, 1600, 1150; eims *m/z* [M]$^+$ 290.1517 (C$_{17}$H$_{22}$O$_4$); ^{1}H nmr (CDCl$_3$, 300 MHz) δ 0.95 (s, H-18), 1.04 (s, H-19), 1.20 (s, H-20), 1.85 (dd, J = 11.3, 4.0 Hz, H-5), 3.34 (dd, J = 13.0, 5.0, H-3α), 6.81 (s, H-11), 7.82 (s, H-14).

METHYLATION OF DEMETHYLNIMBIONOL [8].—Freshly prepared CH_2N_2 was added to an ethereal solution of compound 8 (2.5 mg), and the reaction mixture was kept overnight at room temperature and evaporated to dryness. The methylated product 9 (2.7 mg) crystallized as rods: mp 103–104°; uv λ max 208, 230, 275, 312 nm; ir ν max 3500 (OH), 2900 (C-H), 1708 (C=O), 1600 (C=C), 1150 (C-O); eims m/z [M]$^+$ 318, [M − Me]$^+$ 303; ^1H nmr (CDCl$_3$, 300 MHz) δ 4.06 (s, 2 × OMe).

ACETYLATION OF DEMETHYLNIMBIONOL [8].—To a solution of compound 8 in pyridine, Ac$_2$O was added. The reaction mixture was kept overnight at room temperature. On usual workup it yielded pure 10: mp 89–90°; ir ν max 2900 (C-H), 1720 (C=O), 1600 (aromatic double bond), 1350 (C-O); uv λ max 203, 230, 271, 330 nm; eims m/z [M]$^+$ 416, [M − 43]$^+$ 373, [M − 2 × Ac]$^+$ 332.

LITERATURE CITED

1. S. Siddiqui, B.S. Siddiqui, S. Faizi, and T. Mahmood, *J. Nat. Prod.*, **50**, 30 (1988).
2. P.L. Majumder, D.C. Maiti, W. Kraus, and M. Bokel, *Phytochemistry*, **26**, 3021 (1987).
3. W.L. Meyer, G.B. Clemans, and R.A. Manning, *J. Org. Chem.*, **40**, 3686 (1975).
4. E. Wenkert, B.L. Buckwalter, I.R. Burfitt, M.J. Casic, H.E. Gottlieb, E.W. Hagaman, F.M. Schell, and P.M. Wovkulich, in: "Topics in Carbon-13 NMR Spectroscopy." Ed. by G.C. Levy, Wiley-Interscience, New York, 1976, Vol. 2, p. 81.
5. I. Ara, B.S. Siddiqui, S. Faizi, and S. Siddiqui, *Phytochemistry*, **27**, 1801 (1988).
6. S. Siddiqui, I. Ara, S. Faizi, T. Mahmood, and B.S. Siddiqui, *Phytochemistry*, **27**, 3903 (1988).
7. J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.