Journal of Natural Products
Vol. 53, No. 4, pp. 816-820, Jul-Aug 1990

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 demethylnimbionol, 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-trihydroxypodocarpa-8,11,13-trien-7-one [**8**], respectively, on the basis of chemical and spectral studies.

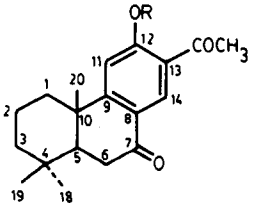
The isolation and structure elucidation of various terpenoidal constituents from fresh fruits, leaves, twigs, and root and stem bark of *Azadirachta indica* A. Juss (Meliaceae), "neem," has been communicated earlier by various groups of workers (1,2). The present paper deals with the studies undertaken in the stem bark resulting in the isolation of three new tricyclic diterpenoids, nimbosodione, nimbisonol, and demethylnimbionol, 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

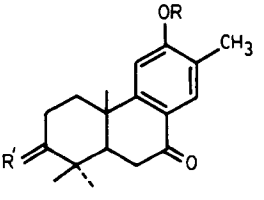
The residue obtained from the EtOH extract of the stem bark was divided into acidic and neutral fractions. The acidic fraction furnished nimbisonol [**4**] and demethylnimbionol [**8**], while the neutral fraction gave nimbosodione [**1**] as described in the Experimental section.

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.

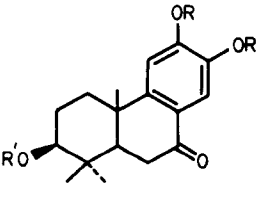
The ^{1}H-nmr spectrum showed three singlets of three protons each at δ 0.92 (H-18), 0.97 (H-19), and 1.20 (H-20). Two one-proton singlets were observed at δ 6.72 and 7.82 attributable to protons at C-11 and C-14, respectively. A three-proton singlet at δ 2.22 was assigned to the acetyl group, which 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
COMe	32.59	—
COMe	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 derivative **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 carbonyl function has to be accommodated in ring A or ring B because the molecular formula 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 resolved and was therefore inconclusive in this respect. However, in the ^{1}H-nmr spectrum of the acetyl derivative **7**, H-6β gave a clearly resolved double doublet at δ 2.79 (J_{gem} = 17.4, $J_{6\beta,5}$ = 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 observations 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 structure 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 spectrum. The downfield chemical shift of H-14 (δ 7.82) indicated that the carbonyl function 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 ($J_{3\alpha,2\beta}$ = 13.0, $J_{3\alpha,2}$ = 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 operating 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_2O_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, University of Karachi.

EXTRACTION AND ISOLATION.—The stem bark was repeatedly extracted with MeOH at room temperature, 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_2O-soluble and Et_2O-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_2O and poured into a flask containing Si gel, which was successively eluted with Et_2O, EtOAc, and MeOH. The Et_2O 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 ($C_{17}H_{21}O_2$); ^1H 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_2O was added an ethereal solution of freshly prepared CH_2N_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; ^1H 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_2O (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; ^1H 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 ($C_{18}H_{24}O_3$), $[M-C_3H_5O]^+$ 231; ^1H 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_2O was treated with freshly prepared CH_2N_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; ^1H 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_2O 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 nm; ir ν max 2900 (C-H), 1720 (C=O), 1600 (C=C), 1150 cm^{-1} (C-O); eims *m/z* $[M]^+$ 372; ^1H 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 ($C_{17}H_{22}O_4$); ^1H 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_2O 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, $[M-2\times Ac]^+$ 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.

Received 16 October 1989