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PHENOLIC TRICYCLIC DITERPENOIDS FROM THE BARK OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; Meliaceae; neem stem bark; nimbionone; nimbionol; diterpenoids; 2D-NMR; antibacterial activity.

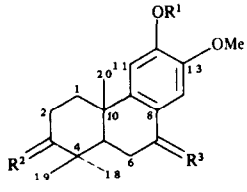
Abstract—Two new diterpenoids nimbionone and nimbionol have been isolated from the acidic fraction of the bark of *Azadirachta indica* (neem) and their structures determined as 12-hydroxy-13-methoxypodocarpa-8,11,13-trien-3,7-dione and 3,12-dihydroxy-13-methoxypodocarpa-8,11,13-trien-7-one, respectively, through chemical and spectral studies. The mother fraction of **1** and **2** showed significant antibacterial activity.

INTRODUCTION

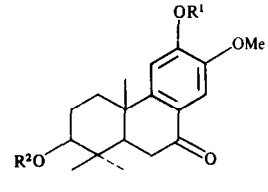
Azadirachta indica A. Juss (neem) belongs to the family Meliaceae and is indigenous to the Indo-Pakistan subcontinent. Its various parts are reputed as therapeutic agents [1, 2] and also possess significant pesticidal activity. Recent studies have revealed that various factors derived from neem possess diverse biological effects against insects like repellence, phagodeterrence, reduced growth, abnormal development and reduced oviposition [3, 4]. Moreover, it has been found that polysaccharides isolated from neem bark have strong anti-inflammatory [5] and antitumour action [5, 6]. More recently, an antineoplastic drug has also been obtained from the bark [7].

In view of the therapeutic importance attributed to neem, comprehensive investigations on its different parts have been carried out by various groups of workers [3, 4, 8–10]. As a result of studies on fresh, undried, uncrushed fruits, leaves and twigs, Siddiqui and co-workers reported the isolation and structure elucidation of a number of new triterpenoids [10]. Extension of these studies to the stem bark has resulted in the isolation of two new phenolic diterpenoids nimbionone and nimbionol, the structures of which have been established, through detailed ^1H and ^{13}C NMR spectroscopy and chemical transformations, as 12-hydroxy-13-methoxy podocarpa-8,11,13-trien-3,7-dione (**1**) and 3,12-dihydroxy-13-methoxypodocarpa-8,11,13-trien-7-one (**2**), respectively.

Preliminary studies on the mother fraction containing **1**, **2** and the diterpenoids nimbione and nimbinone reported earlier [11], showed significant antibacterial activity against the gram positive organisms, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, and also against the gram negative organism *Klebsiella ozaenae*. Some inhibitory effects were also observed against *Staphylococcus citreus*, *Streptococcus lactis* and *Acinetobacter calcoaceticus*. However, nimbionone (**1**) was found to be ineffective against the organisms noted below; gram positive: *B. subtilis*, *S. citreus*, *S. epidermidis*, *S. aureus*, *C. hoffmanni*, *S. pyogenes*, *C. diphtheriae*, *S. faecalis* and *S. lactis*. Gram negative: *K. ozaenae*, *P. vulgaris*, *A. calcoaceticus*, *S. typhi*, *S. typhi para A*, *S. typhimurium*, *A. aerogenes*, *E. coloacae*, *S. sonnei*, *C. freundii*, *K. pneumoniae*, *E. coli* and *S. marcescens*.



	R^1	R^2	R^3
1	H	O	O
1a	Me	O	O
1b	Ac	O	O
1c	Ac	β-OH	α-OH
		α-H	β-H



	R^1	R^2
2	H	H
2a	Me	H
2b	Me	Ac

Further, the potential biological significance of **1** and **2** is evident from the fact that the diterpenes embrace diverse biological effects including antitumour [12], antileukaemic [13], antibiotic [14] and insecticidal activity [3, 4] as well as plant cell expansion and cell division inhibition [15, 16].

RESULTS AND DISCUSSION

The ethanolic extract of neem bark was separated into acidic and neutral fractions and after usual work-up, the acidic fraction was subjected to solvent fractionation followed by purification through preparative TLC on silica gel, ultimately yielding nimbionone (**1**) and nimbionol (**2**).

Nimbionone (**1**) had the molecular formula $C_{18}H_{22}O_4$. Its UV spectrum showed absorption at 203, 234, 280 and 310 nm with a bathochromic shift on addition of alkali while the IR spectrum showed peaks at 3550 (OH), 2850 (C–H), 1720–1680 *br* (six membered and α,β-unsaturated

ketone) 1660–1560 (aromatic ring) and 1280 cm^{-1} (C–O). The molecular formula showed eight double bond equivalents in the molecule, four of which can be accounted for by an aromatic ring, two by the carbonyl functions and two by the remaining two rings of the skeleton.

The terpenoidal nature of **1** was indicated by the presence in the ^1H NMR spectrum (Table 1) of three three-proton singlets at δ1.18 (H-19), 1.13 (H-18) and 1.40 (H-20). The appearance of the aromatic protons as singlets at δ7.51 (H-14) and 6.85 (H-11) suggested two substituents at C-12 and C-13. The NMR spectra indicated that one of these substituents is OMe (δ3.92, δ56.0) while the nature of the other could be identified as a hydroxy function from the bathochromic shift of the UV absorption at basic pH, methylation with diazomethane and the ^{13}C NMR spectrum (δ145.5).

In the ^1H NMR spectrum of the methyl derivative (**1a**), the signals of H-11 and H-14 resonated at δ6.75 and 7.51, respectively, along with the appearance of another singlet for *O*-methyl protons at δ3.94. Acetylation of **1** yielded

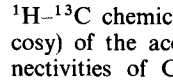
Table 1. ^1H NMR spectral data for compounds **1** and **2** and their derivatives

Assignment	1	1a	1b	1c	2	2a	2b
H-1α	2.01 (*ddd*) $J^{13.5}_{gem}$ $J^{13.5}_{1\alpha,2\beta}$ $J^{5}_{1\alpha,2\alpha}$	2.02 (*ddd*) $J^{12.9}_{gem}$ $J^{12.4}_{1\alpha,2\beta}$ $J^{5.6}_{1\alpha,2\alpha}$	2.01 (*ddd*) $J^{13.3}_{gem}$ $J^{13.3}_{1\alpha,2\beta}$ $J^{5.3}_{1\alpha,2\alpha}$	1.95–2.55 (*m*)	1.68 (*ddd*) $J^{13.4}_{gem}$ $J^{13.4}_{1\alpha,2\beta}$ $J^{4.5}_{1\alpha,2\alpha}$	1.82 (*m*)	1.70 (*m*)
H-1β	2.72 (*ddd*) $J^{13.5}_{gem}$ $J^{7.5}_{1\beta,2\beta}$ $J^{4}_{1\beta,2\alpha}$	2.73 (*ddd*) $J^{12.9}_{gem}$ $J^{7.5}_{1\beta,2\alpha}$ $J^{6.4}_{1\beta,2\beta}$	2.76 (*ddd*) $J^{13.3}_{gem}$ $J^{7.5}_{1\beta,2\alpha}$ $J^{4}_{1\beta,2\beta}$	2.61–2.75 (*m*)	2.67 (*m*)	2.80 (*m*)	2.20–2.65 (*m*)
H-2α	2.53 (*ddd*) $J^{16.5}_{gem}$ $J^{5}_{2\alpha,1\alpha}$ $J^{4}_{2\alpha,1\beta}$	2.57 (*ddd*) $J^{16.1}_{gem}$ $J^{7.5}_{2\alpha,1\beta}$ $J^{5.6}_{2\alpha,1\alpha}$	2.55 (*ddd*) $J^{15.5}_{gem}$ $J^{7.5}_{2\alpha,1\beta}$ $J^{5.3}_{2\alpha,1\alpha}$	2.61–2.75 (*m*)	1.71–1.78 (*m*)	2.30 (*m*)	2.31 (*m*)
H-2β	2.85 (*ddd*) $J^{16.5}_{gem}$ $J^{13.5}_{2\beta,1\alpha}$ $J^{7.5}_{2\beta,1\beta}$	2.84 (*ddd*) $J^{16.1}_{gem}$ $J^{12.4}_{2\beta,1\alpha}$ $J^{5.4}_{2\beta,1\beta}$	2.86 (*ddd*) $J^{15.5}_{gem}$ $J^{13.3}_{2\beta,1\alpha}$ $J^{4}_{2\beta,1\beta}$	1.95–2.55 (*m*)	2.28 (*dddd*) J^{16}_{gem} $J^{11.3}_{2\beta,3\alpha}$ $J^{13.4}_{2\beta,1\alpha}$ $J^{4.5}_{2\beta,1\beta}$	1.76 (*m*)	1.90–2.11 (*m*)
H-3α	—	—	—	3.28 (*dd*) $J^{11.4}_{3\alpha,2\beta}$ $J^{4.8}_{3\alpha,2\alpha}$	3.34 (*dd*) $J^{11.3}_{3\alpha,2\beta}$ $J^{4.3}_{3\alpha,2\alpha}$	3.30 (*m*)	3.32 (*m*)
H-5	2.30 (*dd*) $J^{14}_{5,6\beta}$ $J^{4}_{5,6\alpha}$	2.34 (*dd*) $J^{13.7}_{5,6\beta}$ $J^{3.9}_{5,6\alpha}$	2.34 (*dd*) $J^{13.9}_{5,6\beta}$ $J^{3.8}_{5,6\alpha}$	1.95–2.55 (*m*)	1.83 (*dd*) $J^{12.0}_{5,6\beta}$ $J^{5.2}_{5,6\alpha}$	2.34 (*m*)	2.11 (*dd*) $J^{9.5}_{5,6\beta}$ $J^{4.5}_{5,6\alpha}$
H-6β	2.75 (*dd*) J^{18}_{gem} $J^{14}_{6\beta,5}$	2.77 (*dd*) $J^{17.6}_{gem}$ $J^{13.7}_{6\beta,5}$	2.78 (*dd*) $J^{17.5}_{gem}$ $J^{13.9}_{6\beta,5}$	2.61–2.75 (*m*)	2.70 (*dd*) $J^{12.5}_{gem}$ $J^{12.0}_{6\beta,5}$	2.70 (*m*)	2.20–2.65 (*m*)
H-6α	2.62 (*dd*) J^{18}_{gem} $J^{4}_{6\alpha,5}$	2.63 (*dd*) $J^{17.6}_{gem}$ $J^{3.9}_{6\alpha,5}$	2.68 (*dd*) $J^{17.5}_{gem}$ $J^{3.8}_{6\alpha,5}$	2.61–2.75 (*m*)	2.72 (*m*)	2.70 (*m*)	2.68 (*dd*) $J^{15.6}_{gem}$ $J^{4.5}_{6\alpha,5}$
H-7β	—	—	—	4.28 (*t*) $J^{6.0}_{7\beta,6\alpha}$ $J^{6.0}_{7\beta,6\beta}$	—	—	—
H-11	6.85 (*s*)	6.75 (*s*)	7.02 (*s*)	6.85 (*s*)	6.87 (*s*)	6.75 (*s*)	6.75 (*s*)
H-14	7.51 (*s*)	7.52 (*s*)	7.60 (*s*)	7.50 (*s*)	7.51 (*s*)	7.48 (*s*)	7.49 (*s*)
H-18	1.13 (*s*)	1.14 (*s*)	1.14 (*s*)	0.95 (*s*)	0.96 (*s*)	0.96 (*s*)	0.97 (*s*)
H-19	1.18 (*s*)	1.19 (*s*)	1.19 (*s*)	1.04 (*s*)	1.04 (*s*)	1.05 (*s*)	1.04 (*s*)
H-20	1.40 (*s*)	1.41 (*s*)	1.41 (*s*)	1.22 (*s*)	1.21 (*s*)	1.24 (*s*)	1.17 (*s*)
OMe	3.92 (*s*)	3.92 (*s*), 3.94 (*s*)	3.86 (*s*)	3.92 (*s*)	3.92 (*s*)	3.93 (*s*)	3.93 (*s*)
O ‖ OC-Me	—	—	2.32 (*s*)	2.32 (*s*)	—	3.89 (*s*)	3.90 (*s*) 2.24 (*s*)

the monoacetyl derivative (**1b**) in the ^{1}H NMR spectrum of which signals of H-11 and H-14 shifted to δ7.02 and 7.60, respectively, and a singlet for the methyl protons of the acetoxy function appeared at 2.32. The two carbonyl groups indicated by the IR spectrum and double bond equivalents were chemically confirmed through reduction ($NaBH_4$) of **1b** to **1c**. The respective position of the hydroxy and the methoxy groups could be deduced through NOE difference spectroscopy of **1** when irradiation of the *O*-methoxy signal (δ3.92) gave the signal for H-14 (δ7.51) and *vice versa* while the signal of H-11 remained unaffected. One of the carbonyl functions was placed at C-7 in the light of the chemical shifts of H-11 and H-14, whereas the position of the second at C-3 could be inferred from the significant fragment at *m/z* 125.0965 ($C_8H_{13}O$) in the mass spectrum [17] and the downfield appearance of H-20 (δ1.40) [18], C-2 (δ34.6) and C-4 (δ47.3) as compared to δ18.4 and 32.7, respectively, observed for those of sugiol [19].

These substitutions were confirmed by the multiplicities of ring A/B protons observed in the ^{1}H NMR spectrum, ^{1}H–^{1}H decoupling and COSY-45 experiments. Thus irradiation at δ 2.85 (H-2β) collapsed the doublets of double doublets at 2.53 (J = 16.5, 5, 4 Hz), 2.01 (J = 13.5, 13.5, 5 Hz) and 2.72 (J = 13.5, 7.5, 4 Hz) each into a double doublet with J = 5, 4 Hz; J = 13.5, 5 Hz; and J = 13.5, 4 Hz, respectively, and irradiation at δ2.53 (H-2α) resulted in the collapse of the doublets of double doublets at 2.85 (J = 16.5, 13.5, 7.5 Hz), 2.01 and 2.72 each into a double doublet with coupling constants of J = 13.5, 7.5 Hz; J = 13.5, 13.5 Hz; and J = 13.5, 7.5 Hz, respectively. In the reverse experiments irradiation at δ2.72 (H-1β) changed the doublets of double doublets at δ2.01, 2.53 and 2.85 into double doublets with J = 13.5, 5 Hz; J = 16.5, 5 Hz; and J = 16.5, 13.5 Hz, respectively, while on irradiation at δ2.01 (H-1α) doublets of double doublets at 2.72, 2.53 and 2.85 were converted into each double doublet with coupling constants of J = 7.5, 4 Hz; J = 16.5, 4 Hz; and J = 16.5, 7.5 Hz, respectively. On the other hand, irradiation at δ2.30 (H-5) collapsed the double doublets at δ2.75 (J = 18, 14 Hz) and 2.62 (J = 18, 4 Hz) into each doublet with the same coupling constant of J = 18 Hz. When the signal at δ2.75 (H-6β) was irradiated the sets of double doublets at 2.30 (J = 14, 4 Hz) and 2.62 were transformed into each doublet with J = 4 Hz while irradiation at δ2.62 (H-6α) collapsed the double doublets at 2.30 and 2.75 into doublets with the same coupling constant of J = 14 Hz. These observations suggested that there are two proton systems in the molecule apart from the aromatic system. These correlations were further supported by the proton connectivity pattern observed in the COSY-45 plot.

The assignment of ^{13}C NMR chemical shifts is based on chemical shift rules [20] and a two dimensional ^{1}H–^{13}C chemical shift correlation experiment (heterocosy) of the acetyl derivative (**1b**) which showed connectivities of C-11 (δ120.5) with H-11 (δ7.02), C-14 (δ111.0) with H-14 (δ7.60), O–C–Me (δ21.0) with the singlet at δ2.32 of OAc, and OMe (δ56.0) with the protons at δ3.86; C-5 (δ50.0) with H-5 (δ 2.30), C-18 (δ25.0) with the singlet at δ1.13, C-19 (δ29.5) with δ1.18 and C-20 (δ22.0) with δ1.40.



The assignment of the C-methyl protons, placement of hydroxyl and methoxyl groups at C-12 and C-13, respectively, and the stereochemistry of various centres of nimbionone (**1**) was established through NOE difference and 2D NOE (NOESY) spectral analyses. Thus, irradiation at δ1.13 (H-18) influenced the signals of H-5 (δ2.30) and H-2α (δ2.53). This observation showed that H-18, H-5 and H-2α are oriented in the same plane. Irradiation at δ1.18 (H-19) affected the signal at H-20 (δ1.40) and H-6β (δ2.75) showing spatial proximity of H-19 with H-20 and H-6β. Similarly, irradiation at δ1.40 (H-20) affected the signal of H-19 (δ1.18), H-6β (δ2.75) and H-11 (δ6.85). Irradiation at δ3.92 (OMe) affected the signal of H-14 (δ7.51) and *vice versa*. In the same way irradiation at δ6.85 (H-11) influenced the signal at H-20 (δ1.40). These observations showed that OMe is located on the carbon adjacent to C-14 and the A/B ring junction is *trans*. These results were confirmed by a 2D NOE (NOESY) experiment which showed the spatial connectivities of OMe with H-14; H-2α with H-18, H-1α; H-1β with H-11, H-19; H-2β with H-20, H-11; H-1α with H-2α, H-11; H-11 with H-20, and H-19 with H-2β.

Nimbionol (**2**) had the molecular formula $C_{18}H_{24}O_4$ (high resolution mass 304.1668). The UV spectrum showed maximum absorptions at 218, 241, 280 and 312 nm. The molecular formula showed seven double bond equivalents, four of which have been accounted for by the aromatic ring; one by ketonic function and two by the remaining two rings of the skeleton. The IR spectrum exhibited peaks at 3500–3100 (OH), 2820 (C-H), 1680 (α,β-unsaturated ketone) 1600–1580 (aromatic ring) and 1260 cm^{-1} (C–O). The ^{1}H NMR spectrum (Table 1) showed three singlets at δ0.96, 1.04 and 1.21 attributable to H-18, H-19 and H-20, respectively. A three-proton singlet appeared at δ3.92 (OMe) and two sharp singlets were observed at δ6.87 and 7.51 due to H-11 and H-14, respectively.

The spectral data recorded so far suggested that compound **2** has the same skeleton as **1** with an increment of two mass units. The chemical shifts of H-11 and H-14 and the singlet at δ3.92 in the ^{1}H NMR spectrum of **2** indicated that the aromatic ring and the carbonyl substituent at C-7 are identical in compounds **1** and **2**. The NOESY spectrum showed that the respective positions of methoxy and hydroxy groups were also the same as observed in **1**.

The increment in the mass could be justified by a hydroxyl function at C-3 instead of the carbonyl group in the case of compound **1**. This was corroborated by a one-proton double doublet at δ3.34 (J = 11.3, 4.3 Hz) and formation of only the monomethyl derivative (**2a**), with diazomethane, which on reaction with acetic anhydride–pyridine gave the monomethyl-mono acetyl derivative (**2b**). In the light of these observations structure **2** has been assigned to nimbionol.

The assignment of the methyl protons and the location of the methoxyl group at C-13 was decided from the 2D NOE (NOESY) spectrum which showed the spatial connectivity of OMe with H-14; H-18 with H-6α; H-19 with H-20, and H-20 with H-2β. The upfield shift of H-20 (δ1.21) as against δ1.40 in compound **1**, and the double resonance experiments, are also in keeping with this structure. Thus, irradiation at δ2.28 (H-2β) collapsed the double doublet at 3.34 (J = 11.3, 4.3 Hz) into a doublet (J = 4.3 Hz), the doublet of double doublets at δ1.68 (J = 13.4, 13.4, 4.5 Hz) of H-1α into a doublet (J = 13.4, 4.5 Hz) while the multiplets at 2.67 (H-1β) and 1.71–1.78 (H-2α) were simplified. On irradiation at δ3.34 the signal

at 2.28 ($J = 16$, 13.4, 11.3, 4.5 Hz) of H-2β changed to a doublet of double doublets ($J = 16$, 13.4, 4.5 Hz) while the multiplet of H-2α was also simplified. The irradiation results of other centres and a COSY-45 plot were in accordance with the observations made in the case of compound **1**.

EXPERIMENTAL

Mps: uncorr. IR (in $CHCl_3$) and UV (in MeOH). NMR spectra were recorded in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C nuclei. Chemical shifts are reported in ppm (δ) and delay time for heterocosy was 36 msec. Optical rotations were measured at 24° in $CHCl_3$. Merck Kieselgel 60 PF 254 coated glass plates were used for analytical (thin layer) and preparative (thick layer) chromatography.

The ethanolic extract of neem bark (1.7 kg) collected in February 1985 from Karachi region was separated into acidic and neutral fractions. The residue obtained on usual work-up of the acidic fraction was divided into petrol soluble and insoluble portions and the former was shaken with 90% aq. MeOH. The residue (840 mg) obtained on usual work-up of the 90% MeOH phase was subjected to prep. TLC (silica gel; $CHCl_3$–MeOH, 39:1) yielding nimbionone (**1**) as a uniform component. The remaining diffused portion was purified through chromatography on precoated thin layer plates (silica gel, $CHCl_3$–MeOH, 39:1) when the second major constituent nimbionol (**2**) was isolated.

Nimbionone (**1**). Irregular plates (260 mg, 0.015% on the wt of the bark) mp 78–79° (from $CHCl_3$), $[\alpha]_D = 0.03°$ ($CHCl_3$; *c* 0.036). Found, M^+ at *m/z* 302.1528 (through peak matching of the molecular ion); ($C_{18}H_{22}O_4$ requires M^+ 302.1518; UV λ_{max} nm (ε): 203 (76100), 234 (85150), 280 (48700), 310 (38000); UV $\lambda_{max}^{MeOH-NaOH}$: 205 (57600); 254 (34428); 350 (76100); $\lambda_{max}^{MeOH-HCl}$; 203 (70668), 234 (79003), 280 (48924), 310 (34428) nm. HRMS *m/z* (rel. int.): 302.1528 $[M]^+$ (42), 287.1290 $[M-Me]^+$ (19), 269.1175 $[M-Me-H_2O]^+$ (11), 203.0710 $[M-C_6H_{11}O]^+$ (26) and 125.0965 $[M-C_{10}H_9O_3]^+$ (37); ^{13}C NMR: δ37.4 (C-1), 34.6 (C-2), 214.0 (C-3), 47.3 (C-4), 49.8 (C-5), 39.1 (C-6), 196.7 (C-7), 123.8 (C-8), 151.4 (C-9), 37.4 (C-10), 108.8 (C-11), 145.5 (C-12), 149.4 (C-13), 110.1 (C-14), 25.1 (C-18), 29.7 (C-19), 21.0 (C-20) and 56.0 (Me).

Methylation of nimbionone (**1**). A soln of nimbionone (**1**) (3.8 mg) in Et_2O was treated with freshly prepared CH_2N_2 at room temp for 4 hr. The reaction mixture was evapd to dryness under red. pres. and purified by TLC (silica gel $CHCl_3$–MeOH, 39:1) to give the methylated product **1a**, fine needles (2.6 mg 68.4%) mp 116–119° (from MeOH), $[\alpha]_D = 10.0°$ ($CHCl_3$; *c* 0.005); UV λ_{max} nm: 206, 232, 278, 308; IR ν_{max} cm^{-1}: 1725–1700 (carbonyls). (Found, M^+ 316.1650; $C_{19}H_{24}O_4$ requires M^+ 316.1674). EIMS *m/z* (rel. int.): 316 $[M]^+$ (68), 301 $[M-Me]^+$ (49), 283 $[M-Me-H_2O]^+$ (38), 273 $[M-C_2H_3O]^+$ (40) and 259 $[M-C_3H_5O]^+$ (45).

Acetylation of nimbionone (**1**). To a soln of **1** (24.6 mg) in pyridine (1 ml) Ac_2O (2 ml) was added and the reaction mixture kept overnight at room temp. The acetylated product (**1b**) obtained on usual work-up of the reaction mixture crystallized from MeOH as plates (24.2 mg 98.3%) mp 148–150° (from MeOH), $[\alpha]_D = 10.0°$ ($CHCl_3$; *c* 0.002); UV λ_{max} nm: 209, 220, 255, 310; IR ν_{max} cm^{-1}: 1700–1680 (carbonyls), 1480–1590 (aromatic ring); EIMS *m/z* (rel. int.): 344 $[M]^+$ (24), 302 $[M-C_2H_2O]^+$ (100), 245 $[M-C_6H_{11}O]^+$ (35), 203 $[M-C_6H_{11}O]^+$ (33) and 125 $[C_8H_{13}O]^+$ (65).

Reduction of acetyl nimbionone (**1b**) *with sodium borohydride.* The acetyl derivative of nimbionone (**1b**) was dissolved in MeOH and treated with excess of $NaBH_4$. The reaction mixture was kept stirring for 5 hr, worked-up in the usual manner, and subjected to purification on prep. TLC (silica gel, $CHCl_3$) affording the reduction product (**1c**) as needles, mp 92–93° (from petrol); UV λ_{max} nm: 210, 225, 280 and 312; IR ν_{max} cm^{-1}: 3500–3100 (OH); 1680 (ester carbonyl) and 1500–1600 (aromatic ring). MS *m/z* (rel. int.): 348 $[M]^+$ (0.2) and 288 $[M-C_2H_4O_2]^+$ (31).

Nimbionol (**2**). Needles (39 mg, 0.002% on the wt of the bark) mp 127–129° (from $CHCl_3$), $[\alpha]_D = 10.0°$ ($CHCl_3$; *c* 0.001). (Found, M^+ 304.1668; $C_{18}H_{24}O_4$ requires M^+ 304.1674); UV-λ_{max} nm: 218, 241, 280 and 312; IR ν_{max} cm^{-1}: 3550–3400, 2850, 1680, 1660–1560 and 1280; HRMS *m/z* (rel. int.): 304.1668 $[M]^+$ (9), 202.0944 $[M-C_5H_{10}O_2]^+$ (11), 148.0864 $[M-C_8H_{12}O_3]^+$ (18) and 148.1231 $[M-C_7H_8O_4]^+$ (18).

Methylation of nimbionol (**2**). A soln of nimbionol (**2**) (2.5 mg) in Et_2O was treated with freshly prepared CH_2N_2 at room temp for 4 hr. The reaction mixture was dried under red. pres. when the methylated product (**2a**) was obtained as irregular plates (1.9 mg 76%), UV λ_{max} nm: 210, 232, 275 and 312; IR ν_{max} cm^{-1}: 1700 (carbonyl); EIMS *m/z* (rel. int.): 318 $[M]^+$ (100), 303 $[M-Me]^+$ (10), 285 $[M-Me-H_2O]^+$ (41) and 217 $[M-C_6H_{13}O]^+$ (82).

Acetylation of **2a**. To a soln of **2a** (1.9 mg) in pyridine (1 ml), Ac_2O (2 ml) was added and the reaction mixture kept overnight at room temp. The acetylated product obtained after usual work-up crystallized from MeOH as irregular plates (1.4 mg 73.6%) mp 52–53° (from MeOH), $[\alpha]_D = -0.07$ ($CHCl_3$; *c* 0.027); UV λ_{max} nm^{-1}: 208, 230, 273 and 312; IR ν_{max} cm^{-1}: 1700 *br* (carbonyls) 1400–1590 (aromatic ring); EIMS *m/z* (rel. int.): 360 $[M]^+$ (47.8) and 285 $[M-C_3H_7O_2]^+$ (52).

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