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TETRANORTRITERPENOIDS FROM *AZADIRACHTA INDICA**

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Abstract—Two new tetranortriterpenoids, an aldehyde named nimbanal and the 3-acetyl derivative of salannol, were isolated from the seed extract of neem, *Azadirachta indica*. The structure of nimbanal was established by spectral data while that of salannol-3-acetate was proved by its correlation with salannin.

INTRODUCTION

The famous Indian neem tree, *Azadirachta indica* A. Juss, has attracted the attention of many chemists and biologists during the past decade due to its plethora of biological activities [1, 2]. A number of tetranortriterpenoids [3] have been isolated from different parts of the neem tree and the correct structure for the most potent insect growth regulator, azadirachtin, has been assigned recently by three groups of workers [4–6]. During our developmental work on the azadirachtin-rich fraction [7, 8] from neem for commercial exploitation as a biodegradable pesticide, we have isolated from the seed extract two tetranortriterpenoids possessing the nimbin skeleton. One is an aldehyde which we have named nimbanal while the other one is the 3-acetyl derivative of salannol. So far only three tetranortriterpenoids [9–11] possessing a formyl group have been isolated from the related species, *Melia azedarach* and this is the first report of the occurrence of a triterpene aldehyde in neem.

RESULTS AND DISCUSSION

The triterpene fraction of the ethanol extract of the neem seeds on repeated chromatography gave compound **1**, mp 195–197°, $C_{29}H_{34}O_8$ (M^+ at m/z 510), which showed in its UV spectrum λ_{max} 210 nm (ε 12940) and a band in its IR spectrum at 1680 cm^{-1} indicating the presence of an α,β-unsaturated ketone. The presence of a formyl group was evident by the signal at δ9.24 (singlet) in its ^1H NMR spectrum (Table 1) which exhibited amongst other signals important ones at δ1.27, 1.32 and 1.38 (all singlets) due to tertiary methyl groups, a doublet at 1.68 assignable to a methyl on the double bond, and two singlets at 1.98 and 3.63 assignable to acetyl and carbomethoxy groups respectively. An AB quartet at δ6.00 and 6.12 ($J = 10.1$ Hz) could be assigned to the olefinic protons of the enone system and the characteristic signals at δ6.34, 7.24 and 7.33 revealed the presence of a β-substituted furan ring. A critical comparison of the spectral data of **1** with those of three triterpene aldehydes ohchinal [9], ohchinolal [10] and sendanal [11] isolated from *Melia azedarach* revealed a close resemblance with those of ohchinolal. The only difference between nimbanal and ohchinolal appeared to be the presence of an enone moeity in the ring A of nimbanal. One of the possible positions for the enone group in ring A such as $-_1CH={}_2CH-{}_3CO-$ is ruled out as nimbanal is not identical with a transformation (oxidation) product of ohchinolal [10]. Hence, the A ring of nimbanal should contain the system $-_3CH={}_2CH-{}_1CO-$ as in nimbin (**3**) [12, 13] which is supported by the close similarity of the spectral characteristics (^1H NMR, IR and UV) of the compounds.

Table 1. ^1H NMR data for compounds **1** and **4**

H	**1**	**4**
1	—	4.96, *t* (3.6, 3.1)
2	6.00, *d* (10.1)	—
3	6.12, *d* (10.1)	4.85, *t* (3.1, 2.6)
5	3.27, *d* (12.3)	2.72, *d* (12.8)
6	5.20, *dd* (12.3, 3.08)	3.97, *dd* (12.6, 3.3)
7	4.08, *d* (3.1)	4.15, *d* (3.1)
11a	2.84, *dd* (18.4, 4.8)	—
11b	3.05, *dd* (18.5, 5.3)	—
15	5.57, *m*	5.48, *m*
16a	2.20, *dd* (13.2, 6.6)	—
16b	2.11, *dd* (12.5, 8.5)	—
17	3.78, *d* (5.6)	3.69, *m*
18	1.69, *d* (1.3)	1.65, *d* (1.3)
19	1.31, *s*	0.94, *s*
21	7.24, *m*	7.34, *m*
22	6.34, *m*	6.34, *m*
23	7.33, *m*	7.29, *m*
28	9.25, *s*	3.61, *s*
29	1.27, *s*	1.21, *s*
30	1.38, *s*	1.30, *s*
COOMe	3.64, *s*	3.28, *s*
OCOMe	1.99, *s*	2.07, *s*
H-4′	—	1.00, *d* (6.6)
H-5′	—	

90 MHz, δ scale in ppm, TMS as int. standard. Figures in parentheses denote coupling constants.

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Further evidence in support of this comes from the disappearance of the AB quartet in 2,3-dihydronimbanal obtained by the selective hydrogenation of **1**. The ^{13}C NMR spectral data of **1** (Table 2) are comparable with those reported for ohchinolal [10] and the hitherto unreported data of nimbin [13]. From the close similarity of the chemical shifts and coupling constants due to protons at various chiral centres in the ^{1}H NMR spectrum of **1** with those of nimbin [12] and ohchinolal [10] the structure and stereochemistry of nimbanal are proposed as in **1**.

The second compound **4**, obtained from the more polar fraction than that containing **1**, had the molecular formula $C_{34}H_{36}O_9$ (M^+ at m/z 598). Its ^{1}H NMR spectrum clearly indicated it to be the 3-acetyl derivative of the known triterpene, salannol [13] and as expected the H-3 of salannol at δ 3.40 had moved downfield to 4.85 in **4**. Also, both H-28 protons in compound **4** appeared as a singlet probably due to the effect of the α-acetoxy group at C-3. However, mild hydrolysis of **4** gave a dihydroxy compound **7** whose ^{1}H NMR spectrum showed the chemical shifts and coupling constants for H-3 and H-28 identical with those reported for salannol. The same compound **7** was also obtained by mild hydrolysis of salannin [14] which correlates **4** with salannin.

EXPERIMENTAL

Mps: uncorr. Optical rotations were taken for solns in $CHCl_3$. UV spectra were recorded in EtOH. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$. TMS was used as the internal standard for the ^{1}H NMR spectra. IR spectra were recorded in $CHCl_3$. Mass spectra were recorded at 70 eV. Petrol used throughout the experimental refers to the fraction boiling between 60–80°.

Isolation of compounds. Neem seeds, collected during July 1985, near the NCL campus, Pune, India, were shade-dried and powdered. The powder (0.9 kg) was extracted with EtOH to yield the extract (225 g, 25%) which was chromatographed over silicic acid using Me_2CO–petrol as the elution gradient to collect five broad fractions A (125 g), B (12 g) C (40 g), D (10 g) and E (38 g).

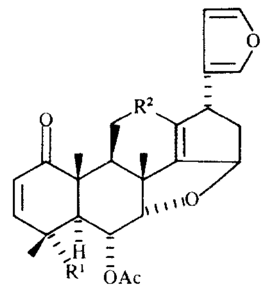
Nimbanal (**1**). Fraction C on further fractionation by column chromatography and repeated prep. TLC using EtOAc–benzene gave colourless crystals, 125 mg (0.014%), mp 195–197° (Me_2CO–petrol) $[\alpha]_D$ +46°. UV λ_{max} nm 210 (ε 12940). IR ν_{max}

Table 2. ^{13}C NMR spectral data of compounds, **1**, **3** and **4** (in $CDCl_3$ at 25.05 MHz, TMS as internal standard, values in δ)

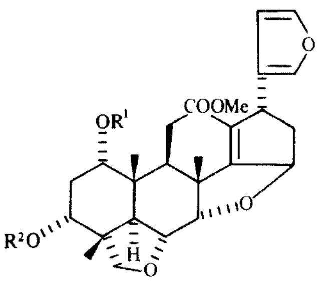
C	**1**	**3**	**4**
1	201.1 (*s*)	201.8 (*s*)	71.6 (*d*)
2	129.2 (*d*)	127.1 (*d*)	30.4 (*t*)
3	143.2 (*d*)	143.2 (*d*)	71.0 (*d*)
4	47.5 (*s*)	47.3 (*s*)	43.0 (*s*)
5	51.7 (*d*)	53.1 (*d*)	41.4 (*d*)
6	68.7 (*d*)	68.9 (*d*)	72.9 (*d*)
7	84.6 (*d*)	84.9 (*d*)	86.0 (*d*)
8	48.1 (*s*)	48.2 (*s*)	49.1 (*s*)
9	40.8 (*d*)	38.9 (*d*)	40.1 (*d*)
10	51.9 (*s*)	51.7 (*s*)	40.6 (*s*)
11	34.2 (*t*)	34.4 (*t*)	28.7 (*t*)
12	173.5 (*s*)	174.9 (*s*)	173.1 (*s*)
13	135.7 (*s*)	135.2 (*s*)	134.9 (*s*)
14	146.0 (*s*)	147.8 (*s*)	146.9 (*s*)
15	87.4 (*d*)	87.3 (*d*)	88.2 (*d*)
16	41.5 (*t*)	41.8 (*t*)	39.4 (*t*)
17	49.7 (*d*)	49.8 (*d*)	49.8 (*d*)
18	16.9 (*q*)	16.8 (*q*)	13.0 (*q*)
19	14.8 (*q*)	16.8 (*q*)	15.6 (*q*)
20	126.9 (*s*)	126.3 (*s*)	127.4 (*s*)
21	139.2 (*d*)	139.3 (*d*)	139.2 (*d*)
22	110.6 (*d*)	110.7 (*d*)	111.0 (*d*)
23	144.9 (*d*)	146.6 (*d*)	143.0 (*d*)
28	119.5 (*s*)	173.8 (*s*)	78.1 (*t*)
29	20.8 (*q*)	21.1 (*q*)	19.8 (*q*)
30	12.9 (*q*)	12.9 (*q*)	17.1 (*q*)
O$\underline{\text{CO}}$Me	170.3 (*s*)	170.8 (*s*)	170.7 (*s*)
OCO$\underline{\text{Me}}$	17.1 (*q*)	17.4 (*q*)	21.2 (*q*)
COO$\underline{\text{Me}}$ 31	—	41.8 (*q*)	—
COO$\underline{\text{Me}}$ 32	38.5 (*q*)	38.9 (*q*)	51.5 (*q*)
1′	—	—	172.7 (*s*)
2′	—	—	43.7 (*t*)
3′	—	—	25.2 (*d*)
4′	—	—	22.9 (*q*)
5′	—	—	22.8 (*q*)

Signal multiplication in the single frequency off resonance decoupled (SFOD) spectrum.

The signals are assigned tentatively by comparison with those reported for similar compounds.



1 R^1 = CHO, R^2 = COOMe
2 R^1 = COOMe, R^2 = CHO
3 R^1 = R^2 = COOMe



4 R^1 = $COCH_2CHMe_2$, R^2 = Ac
5 R^1 = $COCH_2CHMe_2$, R^2 = H
6 R^1 = COC(Me)=CH—Me, R^2 = Ac
7 R^1 = R^2 = H

cm^{-1}: 3010, 2800, 1730, 1680, 1635, 1500, 1440 1380, 1200, 1020, 950, 920, 860. ^{1}H NMR, Table 1. ^{13}C NMR, Table 2. MS m/z (rel. int): 510 $[\mathrm{M}]^+$ (3), 481 $[\mathrm{M}-29]^+$ (0.5), 451 $[\mathrm{M}-59]^+$ (2), 435 $[\mathrm{M}-60-15]^+$ (2), 422 (4), 335 (28), 273 (30), 231 (100), 215 (16), 199 (18), 185 (18.5), 173 (19), 159 (14), 147 (20), 135 (12).

Hydrogenation of **1**. Compound **1** (12 mg) was hydrogenated in EtOH (10 ml) in the presence of Pd/$CaCO_3$ (10 mg). The residue obtained after usual work-up was purified by prep. TLC to give 1,2-dihydronimbanal (3.5 mg), gum, UV, $\lambda_{\max}$ nm: 208.6 (ε 2018). IR $\nu_{\max}$ cm^{-1} 3000, 2900, 2820, 1725, 1430, 1370, 1210, 1020, 860. ^{1}H NMR (90 MHz): 1.27, (3H, *s*, H-29), 1.38 (3H, *s*, H-19), 1.41, (3H, *s*, H-30), 1.67, (3H, *d*, $J = 1.3$ Hz, H-18), 1.97, (3H, *s*, OCOMe), 2.16, (1H, *dd*, $J = 12.4$, 8.5 Hz, H-16a), 2.28, (1H, *dd*, $J = 13.2$, 6.6 Hz, H-16b), 3.06 (1H, *d*, $J = 10.14$ Hz, H-5), 3.58, (3H, *s*, OMe), 3.67 (1H, *br s*, H-17), 3.99, (1H, *d*, $J = 3$ Hz, H-7), 5.26, (1H, *dd*, $J = 13.3$ Hz, H-6), 5.58, (1H, *m*, H-15), 6.32, (1H, *br s*, H-22), 7.23, (1H, *br s*, H-21), 7.3, (1H, *m*, H-23), 9.2, (1H, *s*, H-28), MS m/z (rel. int.): 512 $[\mathrm{M}]^+$ (14), 484 $[\mathrm{M}-28]^+$ (3), 453 $[\mathrm{M}-59]^+$ (4), 424 $[453-29]^+$ (16), 282 (9), 231 (100), 215 (18), 173 (22), 105 (25), 91 (30), 55 (28).

Salannol-3-acetate **4**. Fraction D on further fractionation on CC and repeated prep. TLC using EtOAc–benzene gave compound **4**, 200 mg (0.022%) as a gum. $[\alpha]_D$ +118°. IR $\nu_{\max}$ cm^{-1}: 1730, 1500, 1230, 980, 940, 920, 860. ^{1}H NMR, Table 1. ^{13}C NMR, Table 2. MS m/z (rel. int.): 598 $[\mathrm{M}]^+$ (22), 580 $[\mathrm{M}-18]^+$ (12), 570 $[\mathrm{M}-28]^+$ (100), 513 $[\mathrm{M}-85]^+$ (12), 504 (16), 496 (12), 453 (5), 437 (12), 421 (83), 283 (40), 259 (34).

Hydrolysis of compound **4**. Compound **4** (80 mg) in 8 ml MeOH and 0.6 ml of satd $NaHCO_3$ was refluxed with stirring for 6 hr. After usual work-up the product obtained was esterified with CH_2N_2 and purified by prep TLC to yield **7**, 22 mg, mp 202–204° (Me_2CO–petrol). IR $\nu_{\max}$ cm^{-1}: 3400, 2980, 2880, 1720, 1480, 1420, 1380, 1300, 1280, 1200, 1140, 1050, 1020, 930, 850, 730, 650, 590. ^{1}H NMR (80 MHz): δ0.91 (3H, *s*, H-19), 1.1 (3H, *s*, H-29), 1.28 (3H, *s*, H-30) 1.72 (3H, *d*, $J = 1.2$ Hz, H-18), 2.7 (1H, *d*, $J = 12$ Hz, H-5), 3.6 (1H, *d*, $J = 8$ Hz, H-17), 3.6 (3H, *s*, OMe), 3.88 (1H, *m*, H-1) 4.1 (1H, *d*, $J = 3$ Hz, H-7), 4.3 (1H, *d*, $J = 3.2$ Hz, H-3), 5.52 (1H, *m*, H-15), 6.2 (1H, *m*, H-22), 7.2 (1H, *m*, H-21), 7.4 (1H, *m*, H-23). MS m/z (rel. int.): 472 $[\mathrm{M}]^+$ (60), 457 $[\mathrm{M}-15]^+$ (9), 441 $[\mathrm{M}-31]^+$ (4), 399 $[457-58]^+$ (22), 381 $[399-18]^+$ (10), 363 $[381-18]^+$ (3), 295 (18), 283 (79), 259 (40), 231 (36), 215 (36), 199 (42), 185 (50), 173 (60), 147 (65), 119 (50), 95 (56), 91 (100), 81 (58), 69 (40), 55 (34).

Hydrolysis of salannin **5**. Compound **5** (350 mg) was stirred with 5 ml of 6% methanolic NaOH for 6 hr. After usual work-up the product obtained was esterified with CH_2N_2 and purified by prep TLC to yield 115 mg of a compound which was identical with **7** (TLC, mp, mmp, IR ^{1}H NMR and MS).

^{1}H NMR spectrum of nimbin. As the ^{1}H NMR spectrum of numbin is not available in the literature, it is reported here for comparison purposes; (80 MHz) δ: 1.26, 1.13, 1.36, 1.66 (*d*), 2.04, 3.72, 3.78, 4.1 (*d*, H-7), 5.26 (*dd*, H-6), 5.60 (*m*, H-15), 5.88, 6.40 (AB *q*, H-2, H-3), 6.40 (*m*), 7.2 (*m*), 7.4 (*m*), 2.83 (*dd*, H-5), 2.80 (*s*, H-9), 2.34 (*d*, H-12), 2.15 (*dd*, H-16).

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