Triterpenoids of the Fruit Coats of *Azadirachta indica*

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Three new triterpenoids, azadironolide [24,25,26,27-tetranorapoeupha-7α-acetoxy-23ξ-hydroxy-21,23-epoxy-1,14,20(22)-trien-3,21-dione] (**1**), isoazadironolide [24,25,26,27-tetranorapoeupha-7α-acetoxy-21ξ-hydroxy-21,23-epoxy-1,14,20(22)-trien-3,23-dione] (**2**), and azadiradionolide [24,25,26,27-tetranorapoeupha-7α-acetoxy-21,23-epoxy-1,14,20(22)-trien-3,16,21-trione] (**3**), were isolated from the fresh fruit coats of *Azadirachta indica*. Their structures have been elucidated through spectral analysis.

In continuation of studies on the terpenoidal constituents of fresh fruit coats of *Azadirachta indica* A. Juss[1,2] (Meliaceae) three new triterpenoids, azadironolide, isoazadironolide, and azadiradionolide, have been isolated in the present study. The structures of these new constituents have been deduced as **1**−**3**, respectively, through spectral data interpretation.

Compounds **1**−**3** (Chart 1). were isolated from the neutral fraction of the uncrushed ripe fruit coats through solvent separation followed by purification using vacuum-liquid chromatography, flash column chromatography, successive preparative TLC on silica gel, and HPLC.

Azadironolide (**1**) was assigned the molecular formula $C_{28}H_{36}O_6$ by HREIMS. The UV and IR spectra showed absorption bands for an α,β-unsaturated γ-lactone, a conjugated cyclohexenone, a trisubstituted double bond, and geminal methyls (λ_{max} 227 nm; ν_{max} 1760, 1660, 800, and 1375 cm^{-1}). The ^1H NMR spectrum (Table 1) indicated the triterpenoid nature of **1** by the presence of five quaternary methyls and also showed a pair of AB doublets at δ 7.12 (J = 10.2 Hz, H-1) and 5.87 (J = 10.2 Hz, H-2), while the ^{13}C NMR spectrum (Table 2) exhibited signals at δ 158.1 (C-1), 125.5 (C-2), and 204.0 (C-3), characteristic of the ring A 1-en-3-one system of the known azadirone[1,3] and its analogues.[4,5] The ^1H NMR spectrum further showed four one-proton double doublets at δ 2.09 (J = 13.0, 2.7 Hz, H-5), 2.41 (J = 11.7, 4.6 Hz; H-9), 5.26 (J = 3.1, 3.1 Hz, H-7), and 5.38 (J = 3.1, 2.7 Hz, H-15). The chemical shifts of H-7 and H-15 clearly indicated the presence of an acetoxy group (δH 1.94 s) at C-7 and a double bond at C-14 (δ 156.9). The corresponding signals of these functionalities were also observed in the ^{13}C NMR spectrum at δ 46.2 (C-5), 38.6 (C-9), 71.6 (C-7), 156.9 (C-14), 119.9 (C-15), 170.0 (O*C*OMe), and 21.2 (OCO*M*e). Along with the above data, fragment peaks appeared at *m*/*z* 137.0970 ($C_9H_{13}O$, ion **a**), 150.1059 ($C_{10}H_{14}O$, ion **b**), and 369.2443 ($C_{24}H_{33}O_3$, ion **c**) in the EIMS, and indicated clearly that rings A−D of **1** were identical to those of azadirone.[1,3] The presence of a γ-hydroxybutenolide moiety was indicated by the ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2), in which two one-proton signals at δ 7.16 (br s) and 5.89 (m) were assigned to H-22 and H-23, respectively. The latter signal shifted to δ 6.87 (J = 0.9 Hz) upon acetylation. Furthermore, signals for the lactone including a hemiacetal carbon were observed at δ 96.0 and 97.4 (C-23), 145.6 and 145.8 (C-22), 169.5 and 169.1 (C-21), and 130.8 and 128.8 (C-20). The appearance of double signals for these carbons indicated **1** to be an epimeric mixture at C-23.[6] In light of these spectral data, the structure of **1** was assigned as 24,25,26,27-tetranorapoeupha-7α-acetoxy-23ξ-hydroxy-21,23-epoxy-1,-14,20(22)-trien-3,21-dione.

Isoazadironolide (**2**) showed UV, IR, MS, and ^1H and ^{13}C NMR spectral data similar to those of azadironolide (**1**). The presence of a 21-hydroxybut-20(22)-en 21,23-γ-lactone side chain in **2** differentiated this compound from **1**. This side chain was indicated by the presence of two one-proton signals at δ 5.80 (m, H-21, shifted to δ 6.78 upon acetylation) and δ 5.98 (br s, H-22) in the ^1H NMR spectrum, while the double signals in the ^{13}C NMR spectrum (Table 2) at δ 158.7 and 161.6 (C-20), 97.4 and 97.6 (C-21), 119.2 and 119.1 (C-22), and 169.7 and 169.4 (C-23) suggested the C-21 epimeric nature of **2**. Hence, the structure of **2** was elucidated as 24,25,26,27-tetranorapoeupha-7α-acetoxy-21,23-epoxy-21ξ-hydroxy-1,14,20(22)-trien-3,23-dione.

The stereochemistry of both **1** and **2** has been established by NOESY spectral analysis which showed the spatial proximity of H-1 to H-2; H-7 to H-6β and H-30; and H-18 to H-22 in each case. The latter interraction suggested that the butenolide side chain at C-17 is α oriented. In **1** an additional interaction of H-18 to H-22 was also observed.

Azadiradionolide (**3**) was assigned the molecular formula $C_{28}H_{34}O_6$ by HRMS. Its UV and IR spectra exhibited absorption bands for an α,β-unsaturated carbonyl and a lactone, a trisubstituted double bond, and geminal methyl groups. A tetracyclic triterpenoidal nucleus was indicated by the presence of five quaternary methyls in the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **3**. Furthermore, the signals at δ 7.12 and 5.88 (each ^1H, d, J = 10.2, Hz; H-1 and H-2) in the ^1H NMR spectrum and at δ 156.7 (C-1), 126.0 (C−2), and 203.6 (C-3), in the ^{13}C NMR spectrum, indicated the presence of a ring A 1-en-3-one system[3,7] which was supported by a diagnostic mass fragment at *m*/*z* 137.0971 ($C_9H_{13}O$, fragment **a**). Three one-proton double doublets were observed at δ 2.20 (J = 12.1, 2.8 Hz; H-5), 5.31 (J = 3.1, 3.1 Hz; H-7), and 2.46 (J = 11.0, 6.1 Hz; H-9) while two one-proton signals resonated as singlets at δ 5.98 (H-15) and 3.32 (H-17). The acetoxy methyl protons resonated at δ 1.93 as a singlet. These spectral data revealed that rings A−D of **3** are identical with those of azadiradione.[3,7] The presence of an α,β-unsaturated γ-lactone attached to C-17 in **3** was indicated by the ^1H and ^{13}C NMR signals resonating at δ 7.54 (dd, J = 1.9, 0.9 Hz; H-22), 4.88 (dd, J = 18.6, 0.9 Hz; H-23a), and 4.96 (dd, J = 18.6, 1.9 Hz; H-23b), while the ^{13}C NMR spectrum exhibited signals at δ 128.7 (C-20), 174.6 (C-21), 150.1 (C-22), and

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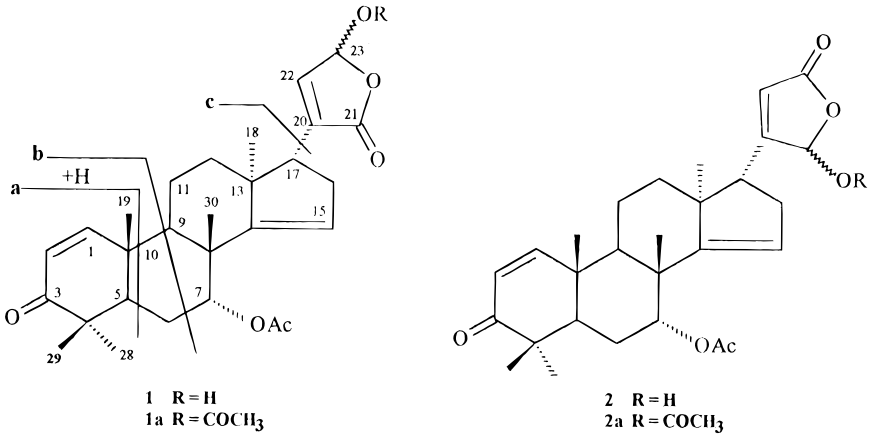
Chart 1



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1a R = $COCH_3$

2 R = H
2a R = $COCH_3$

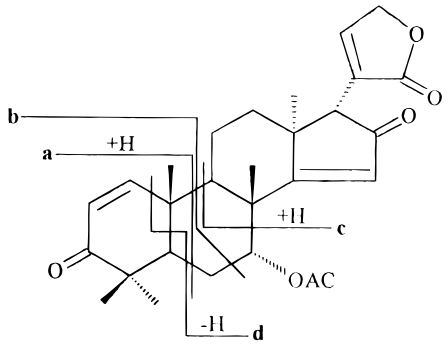

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Table 1. ^{1}H NMR Data and ^{1}H−^{1}H COSY NMR Correlations of Triterpenoids **1**−**3**

proton (s)	**1**	COSY	**2**	**3**	COSY
1	7.12 (d, 10.2)	5.87 (H-2)	7.12 (d, 10.2)	7.12 (d, 10.2)	5.88 (H-2)
2	5.87 (d, 10.2)		5.85 (d, 10.2)	5.88 (d, 10.2)	–
5	2.09 (dd, 13.0, 2.7)	1.87 (H-6α) 1.79 (H-6*β*)	2.09 (dd, 13.0, 2.7)	2.20 (dd, 12.1, 2.8)	1.91 (H-6α) 1.97 (H-6*β*)
6	1.87 (α, m) 1.79 (*β*, m)		1.74−1.97 (α, m) 1.74−1.97 (*β*, m)	1.91 (α, m) 1.97 (*β*, m)	
7	5.26 (t, 3.1)	1.87 (H-6α) 1.79 (H-6*β*)	5.26 (t, 3.2)	5.31 (t, 3.1)	1.91 (H-6α) 1.97 (H-6*β*)
9	2.41 (dd, 11.7, 4.6)	1.74 (H-11α) 1.97 (H-11*β*)	2.40 (dd, 11.7, 4.6)	2.46 (dd, 11.0, 6.1)	2.00 (H-11α) 2.05 (H-11*β*)
11	1.74 (α, m) 1.97 (*β*,m)		1.74−1.97 (α, m) 1.74−1.97 (*β*, m)	2.00 (α, m) 2.05 (*β*, m)	
12	1.55 (α, m) 1.85 (*β*, m)		1.50−1.74 (α, m) 1.74−1.97 (*β*, m)	1.75 (α, m) 1.90 (*β*, m)	
15	5.38 (dd, 3.1, 2.7)		5.39 (m)	5.98 (s)	
16	2.51 (α, m) 2.31 (*β*, m)		2.51 (m) 2.31 (m)	– –	
17	2.15 (m)		2.15 (m)	3.32 (brs)	
18	0.98 (s)		1.00 (s)	1.05 (s)	
19	1.16 (s)		1.16 (s)	1.24 (s)	
21	–		5.80 (m)	–	
22	7.16 (brs)		5.98 (brs)	7.54 (dd, 1.9, 0.9)	4.88 (H-23a) 4.96 (H-23b) 3.32 H-17)
23	5.89 (m)		–	4.88 (a, dd, 18.6, 0.9) 4.96 (b, dd, 18.6, 1.9)	
28	1.07 (s)		1.05 (s)	1.08 (s)	
29	1.07 (s)		1.07 (s)	1.08 (s)	
30	1.23 (s)		1.23 (s)	1.34 (s)	
OCO*Me*	1.94 (s)		1.93 (s)	1.93 (s)	

71.1 (C-23). All the assignments noted above were confirmed by ^{1}H−^{13}C COSY (Table 2) and COSY-45 (Table 1) spectra.

The stereochemistry of **3** was established by employing a NOESY experiment, which showed spatial connectivities of H-1 to H-2; H-7 to H-30; H-15 to H-17; H-18 to H-22; and H-22 to H-23. In light of these spectral data, the structure of **3** has been deduced as 24,25,26,27-tetranor-apoeupha-7α-acetoxy-21,23-epoxy-1,14,20(22)-trien-3,16,21-trione.

Table 2. ^{13}C NMR Chemical Shifts (δc/ppm) of Terpenoids (**1**−**3**) and $^{1}H-^{13}C$ COSY NMR Data for Compound **3**

position	**1**	**2**	**3**	$^{1}H-^{13}C$ COSY (δ_H)
1	158.1	158.1	156.7	7.12
2	125.5	125.1	126.0	5.88
3	204.0	203.8	203.6	−
4	45.3	45.3	44.1	−
5	46.2	46.6	46.3	2.20
6	23.8	23.9	23.5	1.91, 1.97
7	71.6	72.0	74.1	5.31
8	42.7	42.8	44.9	−
9	38.6	38.6	38.1	2.46
10	40.0	41.1	40.0	−
11	16.3	16.3	15.9	2.00, 2.05
12	33.9	34.0	30.6	1.75, 1.90
13	48.9	46.9	47.9	−
14	156.9	157.6	193.2	−
15	119.9	119.9	123.5	5.98
16	34.6	34.9	203.8	−
	34.7	35.0		
17	51.9	54.4	59.5	3.32
	52.0	53.1		
18	19.9	20.0	26.2	1.05
19	19.0	19.0	19.0	1.24
20	130.8	158.7	128.7	−
	128.8	161.6		
21	169.5	97.4	174.6	−
	169.1	97.6		
22	145.6	119.2	150.1	7.54
	145.8	119.1		
23	97.4	169.7	71.1	4.88, 4.96
	96.0	169.4		
28	21.2	21.3	27.0	1.08
29	27.1	27.1	21.3	1.08
30	29.7	29.7	26.6	1.34
*OCO*Me	170.0	170.0	169.4	−
OCO*Me*	21.2	21.1	20.9	1.93

Experimental Section

General Experimental Procedures. Optical rotations were measured on JASCO DIP-360 digital polarimeter. UV (in MeOH) and IR (in $CHCl_3$) spectra were measured on Hitachi-3200 and JASCO-A302 spectrophotometers, respectively. The ^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker Aspect AM-400 (**1** and **2**) and AM-300 (**3**) NMR spectrometers operating at 400 and 300 MHz, respectively, while ^{13}C NMR spectra (BB and DEPT) were recorded in $CDCl_3$ on a Bruker Aspect AM-400 spectrometer operating at 100 MHz (**1** and **2**) and a Bruker Aspect AM-300 spectrometer operating at 75 MHz (**3**). The chemical shifts are recorded in ppm (δ) and coupling constants (J) are in Hz. The ^{13}C NMR spectral assignments of **1**−**3** have been partly made through BB, DEPT, and $^{1}H-^{13}C$ COSY spectra and partly through comparison of chemical shifts with those of azadirone[1,3] and azadiradione.[3,7] Silica gel GF-254 was used for vacuum-liquid chromatography[8,9] and flash column chromatography was performed on Eyela EF-10 (Silica gel, E. Merck 9385). HPLC was performed on a Shimadzu apparatus LC-6A with a UV SPD-6A spectrometer (detection at 230 nm) and a techsphere C18 column (300 mm × 10 mm i.d.) (HPLC Technology Ltd., St. West Macclesfield, Cheshire, U.K.): mobile phase, 70% aqueous MeOH; flow rate, 3 mL/min.

Plant Material. The fruit coats were obtained from the fresh uncrushed, ripe neem fruits (*A. indica* A. Juss.) identified by Prof. Dr. S. I. Ali, Department of Botany, University of Karachi, and voucher specimen NM-1 has been deposited in the herbarium, Department of Botany, University of Karachi. The fruits were collected from the Karachi region in July 1988.

Extraction and Isolation. The plant material (20 kg) was extracted repeatedly with EtOH (three times) at room temperature. The thickish brown residue obtained from the combined extracts on removal of the solvent in vacuo was partitioned between EtOAc and H_2O. The EtOAc phase was treated with 4% Na_2CO_3 to separate the acidic from the neutral fractions. The EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under a vacuum. The residue was successively extracted with petroleum ether and Et_2O. The residue from the Et_2O-soluble portion was partitioned between 90% aqueous MeOH−petroleum ether (1:1). After the usual workup, the aqueous MeOH phase afforded 200 g of a gummy residue, 180 g of which was subjected to vacuum-liquid chromatography (VLC; petroleum ether and petroleum ether−EtOAc mixtures of increasing polarity). The petroleum ether−EtOAc (2:8 and 1:9), EtOAc, and EtOAc−MeOH (9:1, 8:2, 7:3, and 6:4) eluates were combined on the basis of TLC. Removal of the solvent afforded residue E, which was subjected to VLC ($CHCl_3$−MeOH mixtures of increasing polarity). The $CHCl_3$−MeOH (9.9:0.1) eluates furnished various fractions, of which fractions 9 and 10 (2.5 g) and 5 and 6 (5.2 g) were combined on the basis of TLC. Combined fractions 9 and 10 (2 g) were subjected to flash column chromatography (silica gel, $CHCl_3$ and $CHCl_3$−MeOH mixtures of increasing polarity). The $CHCl_3$ eluate furnished a fraction (93.9 mg) with one major and two minor spots on TLC, which was subjected to preparative TLC ($CHCl_3$−MeOH; 9.9:0.1), affording a major component (55 mg) showing a single spot on TLC. The ^{1}H and ^{13}C NMR spectra indicated that it was still a mixture of two constituents, which after a number of trials, could ultimately be separated into compounds **1** and **2** using precoated cards of silica gel (Riedel-de Haen 37360 DC cards SIF, silica gel GF-254, thickness 0.2 mm; $CHCl_3$−MeOH−AcOH, 9.9:0.1:0.01) in a ratio of 1:1 as amorphous powders. A portion (2.6 g) of combined fractions 5 and 6 was subjected to flash column chromatography (petroleum ether and petroleum ether−EtOAc mixtures of increasing polarity). The petroleum ether−EtOAc (7.5:2.5) eluate (58 mg) showed a broad single spot by TLC. However, its ^{1}H NMR spectrum indicated a major along with a minor component, which was purified by reversed-phase HPLC (70% aqueous MeOH) to afford **3** as an amorphous powder (35 mg).

Azadironolide (1): $[\alpha]^{28}_D$ +8.9° (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} 227 nm; IR ($CHCl_3$) ν_{max} 3450 (OH), 1760 (α,β-unsaturated γ-lactone), 1725 (ester carbonyl), 1660 (conjugated cyclohexenone), 1600, 820 (trisubstituted double bond), 1375 (geminal methyl) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive) *m/z* 469 $[MH]^+$, HREIMS *m/z* 468.2468 [M^+, $C_{28}H_{36}O_6$ requires 468.2511] (45), 408.2365 [M^+ − HOAc, $C_{26}H_{32}O_4$] (54), 407.2287 ($C_{26}H_{31}O_4$) (36), 369.2443 [$C_{24}H_{33}O_3$, fragment **c**] (49), 310.2259 ($C_{22}H_{30}O$) (74), 309.2150 ($C_{22}H_{29}O$) (80), 215.1379 ($C_{15}H_{19}O$) (37), 201.1314 ($C_{14}H_{17}O$) (46), 187.1285 ($C_{10}H_{19}O_3$) (55), 175.1146 ($C_{12}H_{15}O$) (42), 150.1059 [$C_{10}H_{14}O$, fragment **b**] (89), 149.1004 ($C_{10}H_{13}O$) (61), 137.0970 [$C_9H_{13}O$, fragment **a**] (100), 121.0651 (C_8H_9O) (58).

Acetylation of azadironolide (1): a solution of **1** (15 mg) in pyridine (1 mL) and Ac_2O (2 mL) was kept overnight at room temperature. Workup as usual afforded an acetate (**1a**), 8.5 mg, as an amorphous powder. UV (MeOH) λmax 225 nm; IR ($CHCl_3$) ν_{max} 1760 (α,β-unsaturated γ-lactone), 1730 (ester carbonyls), 1665 (conjugated cyclohexene). ^{1}H NMR ($CDCl_3$) δ 6.87 (d, J = 0.9 Hz), 1.93 (s, OAc), 2.01 (s, OAc); HRMS *m/z* 510.2620 [M^+, $C_{30}H_{38}O_7$, requires 510.2617], EIMS *m/z* 510 [M^+] (17), 450 (56), 390 (25), 369 (37), 150 (78), 137 (100).

Isoazadironolide (2): $[\alpha]^{28}_D$ +25.0° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} 228 nm; IR ($CHCl_3$) ν_{max} 3650 (OH), 1755 (α,β-unsaturated γ-lactone), 1730 (ester carbonyl), 1665 (conjugated cyclohexenone), 1620, 820 (trisubstituted double bond), 1375 (geminal methyl cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive) *m/z* 469 $[MH]^+$; HREIMS *m/z* 468.2469 [M^+, $C_{28}H_{36}O_6$ calcd for 468.2511] (25), 408.2365 (51), 369.2443 ($C_{24}H_{33}O_3$) (49), 310.2259 ($C_{22}H_{30}O$) (74), 215.1379 ($C_{15}H_{19}O$) (35), 201.1314 ($C_{14}H_{17}O$) (44), 187.1285 ($C_{10}H_{19}O$) (55), 175.1146 ($C_{12}H_{15}O$) (40), 150.1059 ($C_{10}H_{14}O$) (90), 149.1004 ($C_{10}H_{13}O$) (60, 137.0969 ($C_9H_{13}O$) (100), 121.0649 (C_8H_9O) (56).

Acetylation of isoazadironolide (2): isoazadironolide (15 mg) was acetylated in the same manner as **1** to yield **2a** (7.8 mg; amorphous powder). UV (MeOH) λ_{max} 227 nm; IR ($CHCl_3$) ν_{max} 1765 (α,β-unsaturated γ-lactone), 1725 (ester carbonyls),

1660 (conjugated cyclohexene). ^{1}H NMR ($CDCl_3$) δ 6.78 (d, J = 4.1 Hz), 1.94 (s, OAc), 2.04 (s, OAc); HRMS m/z 510.2619 [M^+, $C_{30}H_{38}O_7$, requires 510.2617], EIMS m/z 510 [M^+] (21), 450 (61), 390 (23), 369 (40), 150 (90), 137 (100).

Azadiradionolide (3): $[\alpha]^{28}_D$ +87.9° (c 0.25, $CHCl_3$); UV (MeOH) λ_{max} 230 nm; IR ($CHCl_3$) ν_{max} 1680, 1760 (α,β-unsaturated carbonyl and lactone), 1625, 820 (trisubstituted double bond), 1375 (geminal methyl) cm^{-1}; ^{1}H and ^{13}C NMR, Tables 1 and 2; FDMS m/z 466; HREIMS m/z 466.2388 [M^+, $C_{28}H_{34}O_6$ calcd for 466.2355] (44), 406.2159 [M^+ − HOAc, $C_{26}H_{30}O_4$] (96), 391.1921 ($C_{25}H_{27}O_4$) (32), 356.1624 [$C_{21}H_{24}O_5$, fragment **d**] (5), 286.1548 ($C_{18}H_{22}O_3$) (18), 285.1505 ($C_{18}H_{21}O_3$) (20), 283.1348 ($C_{18}H_{19}O_3$) (24), 245.1168 [$C_{15}H_{17}O_3$, fragment **c**] (60), 150.1053 [$C_{10}H_{14}O$, fragment **b**] (32), 137.0971 [$C_9H_{13}O$, fragment **a**] (24), 121.0679 ($C_{10}H_{14}O$) (100).

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