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NEW MELIACIN ANALOGUES FROM EPOXYAZADIRADIONE

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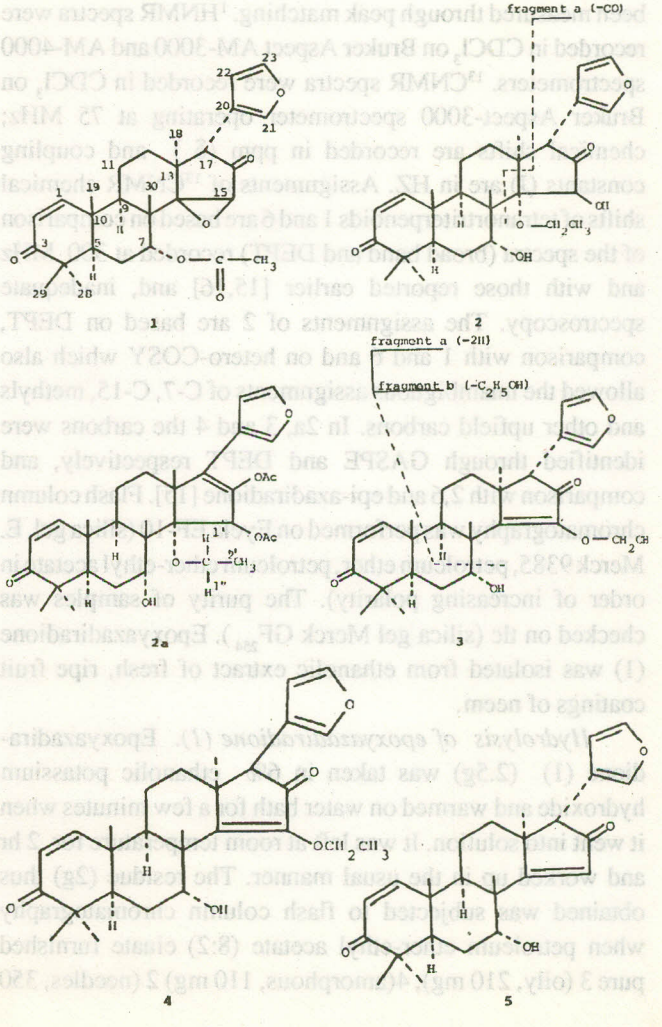
Hydrolysis of epoxyazadiradione (1) with ethanolic potash has led to several new analogues with highly oxygenated ring D, possessing potential pharmacological significance. These include three major products 7-deacetoxy, 14-ethoxy, 7,15 β -dihydroxy, 14,15-dihydroazadiradione (2), 7-deacetoxy, 7-hydroxy, 15- ethoxyazadiradione (3) and 7-deacetoxy, 7-hydroxy, 15-ethoxy-epi- azadiradione (4) and two minor compounds, 7-deacetoxy, 7,16- dihydroxy, 15-ethoxy, Δ^{16}- azadirone (7) and 7-deacetoxy, 7,15β, 16-trihydroxy, 14-ethoxy, 14, 15-dihydro, Δ^{16}-azadirone (8). Their structures have been elucidated through chemical and spectral studies. The present studies which provide a simple route for introducing new moieties e.g. enol ether, di-enolate ether and enol in the meliacin nucleus bear a potential utilization in the synthesis of natural products. Of particular mention, is the substitution of an alkyloxy group at the olefinic carbon of azadiradione, via epoxyazadiradione. The triterpenoids azadiradione (6), hydrolysis products 2 and 3, and epoxyazadiradione (1) were tested for their activity against house flies (*Musca domestica*. L). 6,2 and 3 showed LD_{50} 0.85,8 and 7.8 μ g/fly respectively, whereas 1 was ineffective on these concentrations.

Key words : New meliacin derivatives; 7-deacetoxy, 14-ethoxy, 7,15β-dihydroxy, 14,15-dihydroazadiradione; 7-deacetoxy, 7- hydroxy, 15-ethoxyazadiradione; azadiradione; 7-deacetoxy, 7- hydroxy, 15-ethoxy-epi-azadiradione; 7-deacetoxy, 7,16-dihydroxy, 15-ethoxy- Δ^{16}-azadirone; 7-deacetoxy, 7,15β, 16-trihydroxy, 14- ethoxy, 14,15-dihydro, Δ^{16}-azadirone; pesticidal activity.

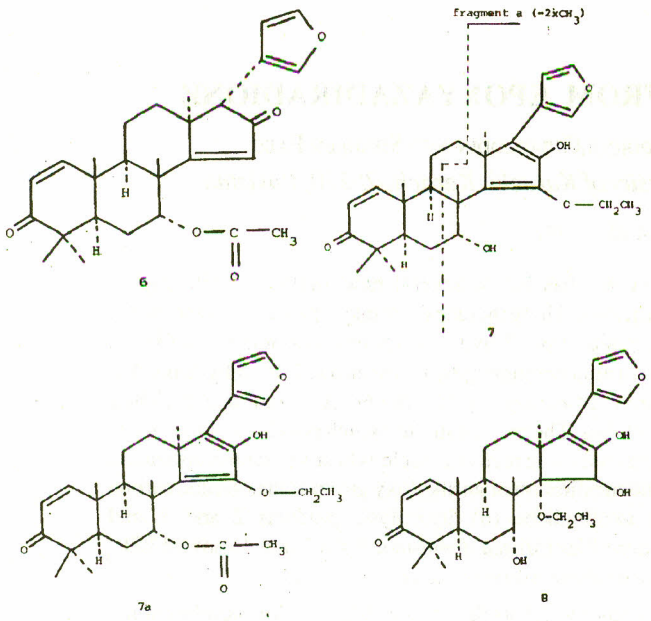
Introduction

In view of the therapeutic [1,2] and pesticidal properties [3-7] attributed to various parts of *Azadirachta indica* (Neem), comprehensive investigations on its different parts have been undertaken by various groups of workers [8-11]. Azadirachtin a tetranortriterpenoid of the plant isolated in 1968 [12] has been found to be a very active phagorepellent and systemic growth disruptor [13,14] and as such has been a focus of considerable research efforts [3-5]. The fact that other main triterpenoids, namely azadiradione (6) and epoxyazadiradione (1) of the fresh fruits isolatable using simple procedures have not been tested for their biological activity in respect of pest control prompted us to carry out chemical studies on epoxyazadiradione, followed by biological testings of (1), (6) and new analogues 2 and 3 against house flies (*Musca domestica* L.). These studies present a possible utilization of the constituents of *A. indica* and their analogues other than azadirachtin in pest control, and provide a simple method of introducing new functionalities for instance, the enol ether (3), the dienolate ether (7) and the enol (8) moiety to be utilized in the synthesis of natural products. Since transformation of azadiradione (6) to epoxyazadiradione (1) is already known [8] 3,7 and 8 can be regraded as derivatives of azadiradione and thus give a simple and interesting route for introducing an alkyloxy substituent at the olefinic carbon.



Experimental

Melting point was recorded in glass capillary tube and is uncorrected. IR (in $CHCl_3$) and UV (in MeOH) spectra were



measured on JASCO-A-302 and Hitachi U-3200 spectrophotometers respectively; mass spectra were recorded on Finnigan MAT-112 spectrometer and exact masses have been measured through peak matching. ^{1}HNMR spectra were recorded in $CDCl_3$ on Bruker Aspect AM-3000 and AM-4000 spectrometers. ^{13}CNMR spectra were recorded in $CDCl_3$ on Bruker Aspect-3000 spectrometer operating at 75 MHz; chemical shifts are recorded in ppm (δ) and coupling constants (J) are in HZ. Assignments of ^{13}CNMR chemical shifts of tetranortriterpenoids 1 and 6 are based on comparison of the spectra (broad band and DEPT) recorded at 300 MHz and with those reported earlier [15,16] and, inadequate spectroscopy. The assignments of 2 are based on DEPT, comparison with 1 and 6 and on hetero-COSY which also allowed the unambiguous assignments of C-7, C-15, methyls and other upfield carbons. In 2a, 3 and 4 the carbons were identified through GASPE and DEPT respectively, and comparison with 2,6 and epi-azadiradione [15]. Flash column chromatography was performed on Eyela EF-10 (silica gel, E. Merck 9385, petroleum ether, petroleum ether-ethyl acetate in order of increasing polarity). The purity of samples was checked on tlc (silica gel Merck GF_{254}). Epoxyazadiradione (1) was isolated from ethanolic extract of fresh, ripe fruit coatings of neem.

Hydrolysis of epoxyazadiradione (1). Epoxyazadiradione (1) (2.5g) was taken in 6% ethanolic potassium hydroxide and warmed on water bath for a few minutes when it went into solution. It was left at room temperature for 2 hr and worked up in the usual manner. The residue (2g) thus obtained was subjected to flash column chromatography when petroleum ether-ethyl acetate (8:2) eluate furnished pure 3 (oily, 210 mg), 4(amorphous, 110 mg) 2 (needles, 350 mg, mp 220-223°) and an impure fraction A in the order of increasing polarity. This fraction was further purified through thick layer chromatography ($CHCl_3$-MeOH 9.5:0.5) affording 7 (amorphous, 12.5mg) and 8 (amorphous, 10 mg).

7-Deacetoxy, 14-ethoxy, 7,15β-dihydroxy, 14,15-dihydroazadiradione (2). UVλ_{max} (MeOH)nm: 217, 208; IR υ_{max} ($CHCl_3$) cm^{-1}: 3450 (OH), 2800-2950 (C-H), 1752 (α-hydroxy cyclopentanone), 1710 (cyclohexenone), 1664 (C=C), 1370 (CH_3) and 1520, 875 (β substituted furan ring). EIMS m/z (%): 470 (M$^+$, $C_{28}H_{38}O_6$) (7), 442.2781 ($C_{27}H_{38}O_5$) (32), 424.2543 ($C_{27}H_{36}O_4$) (8), 406.2138 ($C_{26}H_{30}O_4$) (7), 396.2318 ($C_{26}H_{36}O_3$) (14), 378 (10),360 (24), 345 (14), 327 (21), 299 (19), 264 (24), 167.0744 ($C_9H_{11}O_3$, fragment a) (100), 149 (50), 137 (50), 121 (73), 109 (51), 95 (60), 81 (62) and 69 (89). ^{1}HNMR Table 1; ^{13}CNMR Table 3.

*Acetylation of 2 to 2*a. To a solution of 2 (15 mg) in pyridine (0.7 ml) acetic anhydride (1.5 ml) was added and the reaction mixture kept for two days at room temperature. On usual work up the diacetate (2a) was obtained as an amorphous powder (12.5 mg) showing a single spot on tlc; UV λ_{max} nm: 228, 216, 204. IR υ_{max} ($CHCl_3$) cm^{-1}: 3450, 1740, 1662 and 1365; EIMS m/z (%): 512 [M$^+$] (1.5), 494.2709 ($C_{30}H_{38}O_6$) (14), 479.2430 ($C_{29}H_{35}O_6$) (14), 452.2558 ($C_{28}H_{36}O_5$) (9), 406 (15), 391 (8), 363 (8), 255 (4), 231 (5), 209 (19), 167 (53), 149 (25), 95 (20) and 83 (100). ^{1}HNMR Table 1; ^{13}CNMR Table 3.

7-Deacetoxy, 7 α-hydroxy, 15-ethoxy azadiradione (3). UV λ_{max} (MeOH) nm: 261, 206; IR υ_{max} ($CHC1_3$) cm^{-1}: 3400-3550 (OH), 2880 (C-H), 1720 (cyclopentenone), 1705 (cyclohexenone), 1660 (C=C), 1370 (CH3) and 1525,875 (β-substituted furan ring). EIMS m/z (%): 452 (M$^+$, $C_{28}H_{36}O_5$) (6), 406.2143 ($C_{26}H_{30}O_4$) (100), 389.2112 ($C_{26}H_{29}O_3$) (9), 270.1251 ($C_{17}H_{18}O_3$, fragment a) (14), 226.0998 ($C_{15}H_{14}O_2$, fragment b) (31), 149 (14), 137 (24), 135 (18) 109 (30) and 81 (25). ^{1}HNMR Table 1; ^{13} CNMR Table 3.

7-Deacetoxy, 7 α-hydroxy, 15-ethoxy, epi- azadiradione (4). UV λ_{max} (MeOH) nm: 266, 220; IR υ_{max} ($CHCl_3$) cm^{-1}: 3375-3500, 2950 1700- 1720, 1665 and 1375. EIMS m/z (%): 452 (M$^+$, $C_{28}H_{36}O_5$) (48), 437 (47), 406 (100), 255 (20), 227 (18), 149 (38), 137 (37) 135 (26), 109 (43), 95 (40) and 83 (90). ^{1}HNMR Table 2; ^{13}CNMR Table 3.

7-Deacetoxy, 7α, 16-dihydroxy, 15- ethoxy, Δ^{16} azadirone (7). UV λ_{max} (MeOH) nm: 218,204; IR υ_{max} ($CHCl_3$) cm^{-1}: 3550, 2900, 1710, 1665, 1375, 1325, 1130 and 990; EIMS m/z (%): 452 (M$^+$, $C_{28}H_{36}O_5$) (2), 422.2078 ($C_{26}H_{30}O_5$) (86), 407.1860 ($C_{25}H_{27}O_5$) (23), 394.2134 ($C_{25}H_{30}O_4$) (17), 287 (13), 231.0655 ($C_{13}H_{11}O_4$; f ragment a) (30), 149 (25), 137 (18), 119 (15), 95 (95) and 74 (100). ^{1}HNMR Table 2.

Acetylation of 7 to 7a. To a solution of 7 (10 mg) in pyridine (0.5 ml) acetic anhydride (1.5 ml) was added and the

TABLE 1. ^{1}H NMR SPECTRAL DATA (δ_H PPM AND J/Hz) OF TRITERPENOIDS.

Assignment	1	6	2	2a	3
H-1	7.155 d $J_{1,2}$ 10.20	7.112 d $J_{1,2}$ 10.24	7.205 d $J_{1,2}$ 10.17	7.271 d $J_{1,2}$ 10.17	7.0.75 d $J_{1,2}$ 10.24
H-2	5.877 d $J_{2,1}$ 10.20	5.875 d $J_{2,1}$ 10.24	5.848 d $J_{2,1}$ 10.17	5.845 d $J_{2,1}$ 10.17	5.807 d $J_{2,1}$ 10.24
H-5	2.178 dd $J_{5,6\beta}$ 12.00 $J_{5,6\alpha}$ 3.79	2.203 dd $J_{5,6\beta}$ 12.72 $J_{5,6\alpha}$ 2.92	2.469 dd $J_{5,6\beta}$ 1341 $J_{5,6\alpha}$ 3.24	2.438 dd $J_{5,6\beta}$ 1341 $J_{5,6\alpha}$ 3.15	2.386 dd $J_{5,6\beta}$ 1293 $J_{5,6\alpha}$ 2.73
H–6α	1.834 m	1.881 ddd J_{gem} 14.98 $J_{6\alpha,5}$ 2.92 $J_{6\alpha,7}$ 2.40	1.822 m	1.801 m	1.769-1.865 m
H-6β	1.872 ddd J_{gem} 14.88 $J_{6\beta,5}$ 12.00 $J_{6\beta,7}$ 2.79	1.925 ddd J_{gem} 14.98 $J_{6\beta,5}$ 12.72 $J_{6\beta,7}$ 3.32	2.091 dddd J_{gem} 14.64 $J_{6\beta,5}$ 13.41 $J_{6\beta,7}$ 3.10 $J_{6\beta.OH}$ 1.53	2.152 m	1.769-1.865 m
H-7	4.718 t $J_{7,6\beta}$ 2.79 $J_{7,6\alpha}$ 2.79	5.309 dd $J_{7,6\beta}$ 3.32 $J_{7.6\alpha}$ 2.40	3.938 ddd $J_{7,6\beta}$ 3.10 $J_{7.6\alpha}$ 3.10 $J_{7,OH}$ 3.10	3.947 ddd $J_{7,6\beta}$ 3.09 $J_{7,6\alpha}$ 3.09 $J_{7,OH}$ 3.09	4.599 t $J_{7,6\alpha}$=$J_{7,6\beta}$ 3.01
H-9	2.625 dd $J_{9,11\beta}$ 12.24 $J_{9,11\alpha}$ 3.79	2.488 dd $J_{9,11\beta}$ 9.60 $J_{9,11\alpha}$ 4.50	2.595 dd $J_{9,11\beta}$ 11.17 $J_{9,11\alpha}$ 2.88	2.657 dd $J_{9,11\beta}$ 12.87 $J_{9,11\alpha}$ 2.28	2.495 dd $J_{9,11\beta}$ 9.66 $J_{9,11\alpha}$ 5.55
H-11α	1.903 m	1.852 dddd J_{gem} 15.00 $J_{11\alpha,9}$ 4.50 $J_{11\alpha,12\alpha}$ 3.20 $J_{11\alpha,12\beta}$ 2.90	1.856 m	1.802 m	1.769-1.865 m
H–11β	1.991 dddd J_{gem} 14.89 $J_{11\beta,9}$ 12.24 $J_{11\beta,12\alpha}$ 9.90 $J_{11\beta,12\beta}$ 4.00	2.083 dddd J_{gem} 15.00 $J_{11\beta,9}$ 9.60 $J_{11\beta,12\alpha}$ 8.00 $J_{11\beta,12\beta}$ 4.00	1.927 dddd J_{gem} 17.70 $J_{11\beta,9}$ 11.17 $J_{11\beta,12\alpha}$ 10.05 $J_{11\beta,12\beta}$ 2.58	2.100 m	2.008 m
H-12α	1.799 ddd J_{gem} 17.60 $J_{12\alpha,11\beta}$ 9.90 $J_{12\alpha,11\alpha}$ 5.60	1.843 ddd J_{gem} 15.00 $J_{12\alpha,11\beta}$ 8.00 $J_{12\alpha,11\alpha}$ 3.20	2.232 ddd J_{gem} 13.11 $J_{12\alpha,11\beta}$ 10.05 $J_{12\alpha,11\alpha}$ 2.37	2.372 ddd J_{gem} 12.12 $J_{12\alpha,11\beta}$ 8.70 $J_{12\alpha,11\alpha}$ 2.09	1.929 ddd J_{gem} 15.66 $J_{12\alpha,11\beta}$ 12.96 $J_{12\alpha,11\alpha}$ 2.76
H–12β	2.108 ddd J_{gem} 17.60 $J_{12\beta,11\alpha}$ 5.60 $J_{12\beta,11\beta}$ 4.00	2.054 ddd J_{gem} 15.00 $J_{12\beta,11\beta}$ 4.00 $J_{12\beta,11\alpha}$ 2.90	1.661 ddd J_{gem} 13.11 $J_{12\beta,11\beta}$ 2.58 $J_{12\beta,11\alpha}$ 2.58	1.710 m	1.996 ddd J_{gem} 15.66 $J_{12\beta,11\beta}$ 3.99 $J_{12\beta,11\alpha}$ 2.82

Table 1 (contd.)

(Table 1 contd.)

H–15	3.399 s	5.861 s	3.470 s	4.191 s	–
H-17	3.886 br s	3.410 br s	4.101 br s	-	3.353 br s
H-18	1.066 s	1.029 s	1.153 s	1.154 s	0.965 s
H-19	1.207 s	1.240 s	1.104 s	1.096 s	1.059 s
H-21	7.560 ddd	7.461 ddd	7.325 ddd	7.858 dd	7.429 ddd
	$J_{21,23}$ 1.70	$J_{21,23}$ 1.84	$J_{21,23}$ 1.65	$J_{21,23}$ 1.56	$J_{21,23}$ 1.71
	$J_{21,22}$ 0.88	$J_{21,22}$ 0.80	$J_{21,22}$ 0.80	$J_{21,22}$ 0.87	$J_{21,22}$ 0.75
	$J_{21,17}$ 0.88	$J_{21,17}$ 0.80	$J_{21,17}$ 0.80		$J_{21,17}$ 0.75
H-22	6.230 ddd	6.259 dd	6.173 dd	6.527 dd	6.229 dd
	$J_{22,23}$ 1.70	$J_{22,23}$ 1.84	$J_{22,23}$ 1.65	$J_{22,23}$ 1.89	$J_{22,23}$ 1.71
	$J_{22,21}$ 0.88	$J_{22,21}$ 0.80	$J_{22,21}$ 0.80	$J_{22,21}$ 0.87	$J_{22,21}$ 0.75
H-23	7.387 t	7.411 t	7.384 t	7.403 dd	7.383 t
	$J_{23,22}$ 1.70	$J_{23,22}$ 1.84	$J_{23,22}$ 1.65	$J_{23,22}$ 1.89	$J_{23,22}$ 1.71
	$J_{23,21}$ 1.70	$J_{23,21}$ 1.84	$J_{23,21}$ 1.65	$J_{23,21}$ 1.56	$J_{23,21}$ 1.71
H-28	1.038 s	1.079 s	1.254 s	1.096 s	1.183 s
H-29	1.074 s	1.085 s	1.107 s	1.098 s	1.140 s
H-30	1.218 s	1.328 s	1.289 s	1.246 s	1.268 s
H-1'	-	-	4.161 qd	3.811 qd	4.445 qd
			J_{gem} 9.42	J_{gem} 9.09	J_{gem} 9.75
			$J_{1'2'}$ 7.08	$J_{1'2'}$ 7.02	$J_{1'2'}$ 7.08
H-1"	-	-	3.730 qd	3.621 qd	4.104 qd
			J_{gem} 9.42	J_{gem} 9.09	J_{gem} 9.75
			$J_{1",2'}$ 7.08	$J_{1",2'}$ 7.02	$J_{1",2'}$ 7.08
H-2'	-	-	1.266 t	1.261 t	1.291 t
			$J_{2',1'}$=	$J_{2',1'}$=	$J_{2',1'}$=
			$J_{2',1"}$ 7.08	$J_{2',1"}$ 7.02	$J_{2',1"}$ 7.08
7-OH	-	-	4.377 dd	4.242 dd	2.129 m
			$J_{OH,7}$ 3.10	$J_{OH,7}$ 3.09	
			$J_{OH,6\beta}$ 1.53	$J_{OH,6\beta}$ 1.80	
15–OH	–	–	1.862 br s	–	–
OAc	2.009 s	1.933 s	–	1.685 s	
				2.262 s	–

TABLE 2. ^{1}H NMR SPECTRAL DATA (δ_H PPM AND J/HZ) OF TRITERPENOIDS.

Assignment	4	7	7a	8
H-1	7.088 d	7.095 d	7.092 d	7.232 d
	$J_{1,2}$ 10.22	$J_{1,2}$ 10.24	$J_{1,2}$ 10.24	$J_{1,2}$ 10.16
H-2	5.817 d	5.837 d	5.861 d	5.854 d
	$J_{2,1}$ 10.22	$J_{2,1}$ 10.24	$J_{2,1}$ 10.24	$J_{1,2}$ 10.16
H-5	2.390 dd	2.412 dd	2.132 dd	2.412 dd
	$J_{5,6\beta}$ 12.85	$J_{5,6\beta}$ 13.12	$J_{5,6\beta}$ 13.28	$J_{5,6\beta}$ 13.64
	$J_{5,6\alpha}$ 2.75	$J_{5,6\alpha}$ 2.64	$J_{5,6\alpha}$ 2.50	$J_{5,6\alpha}$ 3.08

(contd.)

(Table 2 contd.)

H-6α	1.749 m	1.819 ddd J_{gem} 17.24 $J_{6\alpha,5}$ 2.64 $J_{6\alpha,7}$ 5.52	1.896-2.083 m	1.504-1.679 m
H-6β	1.931 m	1.498-1.683 m	1.465-1.749 m	1.504-1.679 m
H-7	4.617 t $J_{7,6\beta}$=$J_{7,6\beta}$ 2.66	4.614 ddd $J_{7,6\beta}$ 2.52 $J_{7,6\alpha}$ 2.52 $J_{7,OH}$ 2.52	5.635 t $J_{7,6\beta}$ 2.52 $J_{7,6\alpha}$ 2.52	3.922 ddd $J_{7,6\beta}$ 3.04 $J_{7,6\alpha}$ 3.04 $J_{7,OH}$ 3.04
H-9	2.430 dd $J_{9,11\beta}$ 9.24 $J_{9,11\alpha}$ 4.88	2.441 dd $J_{9,11\beta}$ 11.68 $J_{9,11\alpha}$ 6.64	2.375 dd $J_{9,11\beta}$ 11.20 $J_{9,11\alpha}$ 5.48	2.784 m
H-11α	1.662-1.703 m	1.498-1.683 m	1.465-1.749 m	1.504-1.679 m
H-11β	1.662–1.703 m	1.909-2.003 m	1.896-2.083 m	1.866-2.061 m
H-12α	1.484 m	1.498-1.683 m	1.465-1.749 m	1.504-1.679 m
H-12β	1.484 m	1.909-2.003 m	1.896-2.083 m	1.866-2.061 m
H-15	-	-	-	3.725 s
H-17	3.238 br s	-	-	-
H-18	1.388 s	1.170 s	1.162 s	1.167 s
H-19	1.153 s	1.083 s	1.264 s	1.247 s
H-21	7.196 ddd $J_{21,23}$ 1.90 $J_{21,22}$ 0.78 $J_{21,17}$ 0.49	7.279 dd $J_{21,23}$ 1.72 $J_{21,22}$ 0.88	7.291 dd $J_{21,23}$ 1.72 $J_{21,22}$ 0.88	7.283 dd $J_{21,23}$ 1.71 $J_{21,22}$ 0.90
H-22	6.094 dd $J_{22,23}$ 1.90 $J_{22,21}$ 0.78	6.273 dd $J_{22,23}$ 1.72 $J_{22,21}$ 0.88	6.261 dd $J_{22,23}$ 1.72 $J_{22,21}$ 0.88	6.283 dd $J_{22,23}$ 1.71 $J_{22,21}$ 0.90
H-23	7.329 dt $J_{23,22}$ 1.90 $J_{23,21}$ 1.90 $J_{23,17}$ 0.41	7.336 t $J_{23,22}$ 1.72 $J_{23,21}$ 1.72	7.337 t $J_{23,22}$ 1.72 $J_{23,21}$ 1.72	7.367 t $J_{23,22}$ 1.71 $J_{23,21}$ 1.71
H-28	1.149 s	1.261 s	1.070 s	1.083 s
H-29	1.077 s	1.147 s	1.076 s	1.105 s
H-30	1.240 s	1.326 s	1.360 s	1.273 s
H-1'	4.483 qd J_{gem} 9.67 $J_{1',2'}$ 7.08	4.538 qd J_{gem} 9.72 $J_{1',2'}$ 7.08	4.367 qd J_{gem} 9.64 $J_{1',2'}$ 7.08	4.042 qd J_{gem} 9.48 $J_{1',2'}$ 7.08
H-1"	4.134 qd J_{gem} 9.67 $J_{1",2'}$ 7.08	4.125 qd J_{gem} 9.72 $J_{1",2'}$ 7.08	4.160 qd J_{gem} 9.64 $J_{1",2'}$ 7.08	3.753 qd J_{gem} 9.48 $J_{1",2'}$ 7.08
H-2'	1.314 t $J_{2',1'}$=$J_{2',1"}$ 7.08	1.343 t $J_{2',1'}$=$J_{2',1"}$ 7.08	1.288 t $J_{2',1'}$=$J_{2',1"}$ 7.08	1.270 t $J_{2',1'}$=$J_{2',1"}$ 7.08
7-OH	2.21 m	2.177 br s	-	3.089 s
16-OH	-	3.135 br s	3.114 s	3.089 s
OAc	-	-	1.955 s	-

TABLE 3. ^{13}C NMR CHEMICAL SHIFTS (δ_C PPM) OF TRITERPENOIDS.

Carbons	1	6	2	2a	3	4
1.	157.53	157.37	158.79	159.21	157.38	157.58
2.	125.79	125.78	125.78	125.60	125.63	125.70
3.	204.16	203.79	205.83	204.99	204.81	204.92
4.	44.23	43.95	44.60	44.60	43.41	44.13
5.	46.70	46.02	45.70	46.21	44.45	44.58
6.	24.23	23.34	28.70	28.71	25.17	25.44
7.	73.66	73.81	71.05	71.18	70.62	70.63
8.	43.16	44.45	49.41[a]	49.41[b]	44.02[c]	42.43[d]
9.	39.72	38.11	39.99	39.67	37.17	37.10
10.	39.68	39.89	40.40	40.40	40.13	40.26
11.	16.06	15.68	16.95	15.93	15.87	15.92
12.	29.11	30.20	35.10	35.16	30.88	26.70
13.	42.50	47.84	46.84[a]	45.72[b]	46.69[c]	47.09d
14.	72.58	192.19	85.83	86.55	165.42	168.45
15.	57.24	123.15	82.05	84.62	146.84	146.90
16.	208.41	204.81	213.24	143.20	200.91	203.03
17.	50.96	60.61	57.85	121.50	59.09	57.72
18.	27.05	26.88	27.45	27.43	26.82	32.30
19.	20.00	18.88	20.35	19.73	18.65	18.81
20.	116.64	118.36	118.99	118.00	118.23	121.52
21.	141.59	141.52	141.08	141.43	141.45	140.31
22.	110.96	111.05	111.25	109.48	111.07	109.83
23.	142.46	142.62	142.68	143.09	142.61	143.15
28.	25.00	26.33	26.50	28.29	26.68	26.94
29.	21.25	21.15	21.06	21.02	21.27	21.38
30.	19.00	26.14	19.96	21.02	23.28	23.11
O-C-CH$_3$	169.75	169.44	-	169.95	-	-
				169.81		
O-C-CH$_3$	21.00	20.81	-	20.77	-	-
				20.71		
OCH$_2$CH$_3$			68.17	67.14	65.60	65.80
OCH$_2$-CH$_3$			14.91	15.31	15.77	16.03

[a, b, c, d] values may be interchanged.

reaction mixture kept for three days at room temperature. On usual workup the acetylated product 7a (6.3 mg) was obtained as an amorphous powder. UVλ_{max} (MeOH) nm: 225 203; IR υ_{max} ($CHCl_3$) cm^{-1}: 3500, 2850-2900, 1720, 1705, 1660, 1600 and 1360; EIMS m/z (%): 494 (M^+) (19), 451 (4), 434 (21), 422 (9), 405 (2), 231 (6), 165 (12), 149 (38), 109 (37), 95 (100), 83 (69) and 81 (50). ^{1}HNMR Table 2.

7-Deacetoxy,14-ethoxy,7α,15β,16-trihydroxy,14,15-dihydro-Δ^{16}-azadirone (8). UVλ_{max} (MeOH) nm: 214, 204; IR υ_{max} ($CHCl_3$) cm^{-1}: 3500, 2950, 1710, 1665, 1375, 1050 and 870. EIMS m/z (%): 470 (2), 424 (2), 423 (68), 408 (19), 346 (76), 327 (22), 299 (19), 271 (8), 231 (30), 183 (57), 175 (12), 163 (16), 149 (42), 137 (30), 121 (15), 109 (22), 95 (100), 69 (38) and 67 (15). ^{1}HNMR Table 2.

Results and Discussion

On alkaline hydrolysis, epoxyazadiradione (1) yielded a reaction mixture, which afforded several unusual constituents through flash column chromatography, three of them (2,3 and 4) as major and two (7 and 8) as minor constituents. The spectral data indicated that in each of them A,C and furan rings are intact and the changes occurred in rings B and D only. In 2 and 4 the stereochemistry of the skeleton was also affected by the alkaline treatment. Thus in 2 there is a cis C/D junction which is normally trans in naturally occurring tirucallane, euphane and lanostane series of triterpenoids [17]. On the other hand, the rare β -orientation of the furan ring at C-17 was noted in 4. Lack of the acetyl signal in the ^{1}H and ^{13}CNMR spectra (Table 1 and 3) of 2 and appearance of H-7 doublet of double doublet at δ 3.938 ($J_{7,6\alpha} = J_{7,6\beta} = J_{7,OH} = 3.0$) showed that the acetyl group has been hydrolysed. The multiplicity of this proton indicated that it is coupled with 6 α and β protons as well as with 7-OH. Two protons exchangeable with D_2O, resonating at δ 4.377 (dd, J_{OH}, 7 = 3.10, $J_{OH,6\beta} = 1.53$) and 1.862 (brs) showed two hydroxyl protons one of which (4.377) could be assigned to the hydroxyl group at C- 7 on the basis of its W- coupling with H-6B ($J_{OH,6\beta} = 1.53$). Shaking with D_2O resulted in the conversion of H-6β (dddd, $J_{gem} = 14.64$, $J_{6\beta,5} = 13.41$, $J_{6\beta,7} = 3.10$, $J_{6\beta,OH} = 1.53$) at δ2.091 to a doublet of double doublet ($J_{gem} = 14.64$, $J_{6\beta,5} = 13.41$, $J_{6\beta,7} = 3.10$) and H-7 signal to a triplet ($J_{7,6\alpha} = J_{7,6\beta} = 3.10$). The second hydroxyl function (δ 1.862) was placed at C-15 since H-15 appeared as a one-proton singlet at δ 3.470, which was confirmed by acetylation (acetic anhydride/pyridine; room temperature; 48 h) of 2 to 15-0-acetyl derivative (2a) in the ^{1}HNMR spectrum (Table 1) of which the singlet at δ 1.862 disappeared, while H-15 and 15- acetoxy methyl protons appeared at δ 4.191 and 1.685 respectively. Fruthermore, as expected, the hydroxyl group at C-7 remained intact in 2a, being axial in nature [8] and instead an acetoxy function was introduced at C-16 through enol tautomer of 2. This was evident from the downfield chemical shift of acetoxy protons (δ 2.262), absence of its geminal proton and H-17 resonance. The latter was noted in 2 at δ 4.101.

The ^{1} HNMR spectrum of 2 further showed two sets of quartets of doublets (each integrating for 1H) at δ 4.161 (J_{gem} = 9.42 $J_{1',2'}$ = 7.08, H-1'), 3.730 (J_{gem} = 9.42, $J_{1'',2'}$ = 7.08, H-1'') and a triplet (3H) at δ 1.266 ($J_{2',1'} = J_{2',1''} = 7.08$, H-2') which demonstrated that an ethoxy moiety has been incorporated at C-14.

The stereochemistry of various centres particularly of C-14 and C-15 in 2 was confirmed through NOED experiment. Thus irradiation of H-18 (δ 1.153) enhanced the signals of H-9, H- 15, H-1', H-1'', H-17, 7-OH, H-21 and H-22, whereas, irradiation of ethoxy methyl protons (δ 1.266, H-2') enhanced the signals of H-9, H-15, ethoxymethylene protons (H-1' and H- 1''), 7-OH, H-21 and H-22. Similarly signals of H- 6β, H-11β, H-7 and H-17 were enhanced on irradiating H-30

(δ 1.289). The interaction of H-18 with H-15, H- 21, H-22 and that of ethoxy methyl protons with H-9 and H-15 showed that they all lie in the same plane i.e. α and the C/D ring junction is cis.

The ^{1}HNMR spectral data (Table 1) of 3 were very similar to those of 7 α- deacetyl, 7-hydroxyazadiradione (5) [18]. However, the signal of H-15 was missing and instead two sets of one-proton quartets of doublets were observed at δ4.445 (J_{gem} = 9.75, $J_{1',2'}$ = 7.08) and 4.104 (J_{gem} = 9.75, $J_{1'',2'}$ 7.08) besides a three-proton triplet at δ1.291 ($J_{2',1'} = J_{2',1''}$ = 7.08) which demonstrated an ethoxy function at C-15, and that the opening of the epoxy ring has been followed by dehydration which was corroborated by the downfield resonance of the ethoxy protons as compared to those of 2. The upfield shift of C-14 (δ165.42) and downfield shift of C- 15 (δ 146.84) due to the C-15 ethoxy group as compared to those of azadiradione (6) [15] and (5) [18] further supported the assigned structure of 3. The formation of 3 from 1 can be envisaged through S_N2 attack of ethoxide ion at C- 15, the less highly substituted epoxide carbon which is considered as a normal pathway, followed by dehydration [19]. The higher yield of 2, on the other hand, is in analogy with the earlier observations that the presence of an electron withdrawing substituent inhibits reaction at the carbon atom to which it is attached [19]. Thus, it can be considered that a modified SN_2 mechanism operates in case of 2.

The spectral data of 4 were similar to those of 3, however, the resonance of H-18 (δ 1.388) and C-18 (δ32.30) as against (δ0.965) and (δ 26.82) in 3 showed that in 4 the furan ring is β- oriented as in the case of epi-azadiradione [15]. This stereochemistry was also supported by the 2D NOE interactions of H-30 with H-22 and H-17 with H-18.

7 Represents the enol tautomer of 3 as evident from its ^{1}HNMR spectrum (Table 2) in which the signal of H-17 disappeared while signals for two hydroxyl groups (δ 3.135 and 2.177), exchangeable with D_2O were observed. The rest of the chemical shifts for A,B,C and furan rings of 7 are similar to those of 3. Acetylation (acetic anhydride/pyridine, room temp; 72 h) of 7 afforded 7a, the ^{1}HNMR spectrum (Table 2) of which exhibited that it is the unexpected 7-acetyl derivative as the characteristic signal of H-7 at δ 4.614 (ddd, $J_{7,6\beta} = J_{7,6\alpha} = J_{7,OH}$ 2.54) of 7 shifted to δ5.635 (t, $J_{7,6\beta} = J_{7,6\alpha}$ 2.52). Further, one of the D_2O exchangeable protons remained unaffected (δ 3.135), while the other at δ 2.177 disappeared.

The spectral data of the product 8 indicated that this is the enol tautomer of 2. The isolation of the enol tautomer in this series may be explained as due to their stabilization through conjugation by furan ring in 8, and by both furan ring and the double bond at C-14 in 7.

The assignments of various protons in above discussed compounds are based on ^{1}H-^{1}H homodecoupling experiments, ^{1}HNMR recorded after shaking with D_2O and 2D experiments (COSY-45 and NOESY). The carbon chemical shifts in ^{13}CNMR spectra (Table 3) have been exactly assigned on the basis of ^{1}H-^{13}C-hetero-COSY experiments.

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