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A NOVEL WITHANOLIDE FROM *DATURA METEL*

SALIMUZZAMAN SIDDIQUI, NAHEED SULTANA, S. SALMAN AHMAD and S. IMTIAZ HAIDER

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 32, Pakistan

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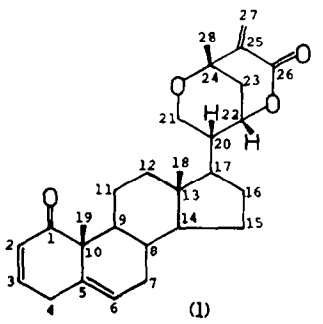
Abstract—A novel withanolide, daturilin, has been isolated from the alcohol soluble extract of the fresh leaves of *D. metel* and its structure established as 1-oxo-21,24*S*-epoxy-(20*S*,22*S*)-witha-2,5,25-trienolide through spectral studies including 2D NMR.

INTRODUCTION

In an earlier communication the isolation and structure elucidation of a new tropane alkaloid datumetine [1] has been reported from the alcoholic extract of the leaves of *D. metel*. L. Extended studies in the neutral constituents of the same plant material have led to the isolation and structure elucidation of a novel withanolide, daturilin (**1**). A few withanolides have been reported earlier from other species of *Datura* eg. *D. quercifolia*, [2–6], *D. ferox* [7], *D. stramonium* [8] and its variety *violaceae* [9], but this is the first instance of the isolation of a withanolide from *D. metel*. Further, although the C-21, C-24 epoxide ring has been reported in the triterpenes of tirucallane skeleton [10, 11], there is no precedence for an epoxide ring in a withanolide skeleton.

RESULTS AND DISCUSSION

From the acid-insoluble fraction of the alcoholic extract of *D. metel* leaves, a colourless crystalline constituent daturilin (**1**) mp 206° has been obtained through solvent fractionation and preparative thin layer chromatography. The molecular ion $[M]^+$ at *m/z* 436 and high resolution MS corresponded to the molecular formula $C_{28}H_{36}O_4$ and the prominent peaks at *m/z* 269 and 268 were indicative of M-side chain fragment with and without transfer of hydrogen. The intensity and position of the UV band at 218 nm (ε 16318) indicated two α,β-unsaturated carbonyl chromophores without extended conjugation. The IR spectrum exhibited bands at 1680 and 1720 cm^{-1} for α,β-unsaturated ketone and α,β-unsaturated δ-lactone groupings respectively [12], both characteristic of withanolides. IR and MS spectra did not show the presence of any hydroxyl group in **1**, which was also confirmed through negative results of attempted acetylation. In the low field region of the ^1H NMR spectrum of **1**, signals related to five vinylic proton were observed. Two one-proton doublets of double doublets at δ5.78 (J = 10.11, 3.24, 1.23 Hz) and δ6.69 (J = 10.11, 5.02, 2.49 Hz) have been assigned to H-2 and H-3 respectively. H-6 appeared as a double triplet at δ5.60 (J = 6.06, $W_{1/2}$ = 1.71 Hz) and the remaining two vinyl protons resonating at δ5.99 and δ6.56 as narrow doublets (J = 0.78 Hz) were indicative of H-27a and H-27b of the exo double bond in the side



(1)

chain. Two double doublets, of one proton each, with a large geminal coupling constant (observed by J resolve technique) resonating at δ2.87 (J = 21.30, 4.92 Hz) and at δ3.34 (J = 21.3, 2.49 Hz) have been attributed to H-4a and H-4b. The protons of the methyl groups located at non-protonated carbons appeared as singlets at δ0.77 (H-18), 1.24 (H-19) and 1.41 (H-28). These data were suggestive of a 2,5-diene-1-one structure for **1** and were in agreement with the published data of Δ^5-withanolides [13–15]. Two ethereal protons, one as a sharp doublet at δ3.90 (J = 13.23 Hz, H-21β) and other as a double doublet at δ3.66 (J = 13.23, 2.64 Hz, H-21α), indicated the presence of a primary–tertiary ether linkage. The signal for H-22 appeared at δ4.63 as a broad singlet, showing coupling with H-20 and H-23 as observed by COSY-45, H-23a and H-23b resonated as double doublets at δ2.14 (J = 14.13, 1.65 Hz) and at δ1.95 (J = 14.07, 3.87 Hz) respectively. Both the double doublets became sharp doublets (J = 14.16 Hz) on homo-decoupling of the H-22 at δ4.6. All these assignments have been confirmed through homo-decoupling, J. resolve, COSY-45 and NOESY experiments.

The stereochemistry of various centres has been established through a n.O.e difference measurements. Irradiation of the multiplet of H-20 (δ1.82) resulted in 7.23, 11.18 and 5.92% increase in the intensities of H-18 (δ0.77), H-22 (δ4.6) and H-28 (δ1.4) respectively; similarly irradiation of H-18 enhanced the signal of H-20 (7.23%). On the other hand, 9.86 and 7.23% enhancements of H-20 and H-28 signals were observed on irradiation of H-22. Furthermore, 7.89 and 5.92% increase in the intensities of H-22 and H-20 signals was induced by the irradiation of H-28. These observations revealed that H-20, H-22, H-28 and H-18 are located in the same plane, and led to the conclusion that they are β-oriented since the angular methyls in the ergostane series have a β-disposition. In

Table 2. n.O.e. difference measurements of daturilin (1)

No.	Irradiated at (δ)	n.O.e%
1.	0.77 (H-18)	2.63 (H-2) 2.63 (H-19) 7.23 (H-20) 2.30 (H.21β)
2.	1.41 (H-28)	2.63 (H-2) 5.92 (H-20) 7.89 (H-22) 11.84 (H-27b)
3.	1.82 (H-20)	3.28 (H-2) 7.23 (H-18) 4.1 (H-21α) 2.63 (H-21β) 11.18 (H-28) 5.92 (H-28)
4.	3.66 (H-21α)	3.28 (H-2) 1.97 (H-20) 15.78 (H-21β)
5.	3.90 (H-21β)	3.94 (H-2) 1.31 (H-18) 16.44 (H-21α)
6.	4.63 (H-22)	3.28 (H-2) 9.86 (H-20) 7.23 (H-20)
7.	5.99 (H-27a)	1.131 (H-3) 32.23 (H-27b) 4.6 (H-28)
8.	6.56 (H-27b)	3.28 (H-2) 25.0 (H-27a)

Table 1. ^{13}C NMR assignments of daturilin (1)

Carbons	Chemical shift* (δ)	Multiplicity (DEPT)	Carbons	Chemical shift* (δ)	Multiplicity (DEPT)
1	204.15	*s*	15	24.124	*t*
2	128.00	*d*	16	26.556	*t*
3	145.11	*d*	17	47.699	*d*
4	33.524†	*t*	18	12.751	*q*
5	136.06	*s*	19	18.976	*q*
6	124.60	*d*	20	42.939	*d*
7	33.334†	*t*	21	60.574	*t*
8	33.297	*d*	22	75.629	*d*
9	39.963	*d*	23	30.795	*t*
10	50.544	*s*	24	69.355	*s*
11	23.678	*t*	25	139.20	*s*
12	39.749	*t*	26	165.26	*s*
13	42.662	*s*	27	129.74	*t*
14	56.051	*d*	28	25.627	*q*

*All values are in ppm (δ) with respect to TMS.
†Assignments may be interchanged.

addition to the above observations, it has been noted that irradiation of H-18 shows 2.38% n.O.e. at δ3.99 (H-21β), which suggested that H-21α and H-21β are proximate to H-18. Further, H-18, H-20, H-21α, H-21β, H-27b and H-28 caused n.O.e. of variable percentages on H-2 (Table 1) while H-3 was effected only by H-27a. All these n.O.e. interactions served to establish the '*S*' configuration at C-20, C-22 and C-24.

The ^{13}C NMR assignments of **1** (Table 2) have been based on comparison with the related peaks of cholesterol [16] and a steroidal lactone (20*R*, 22*R*)-14α, 20α-dihydroxy-1-oxo-witha-2,5,16,24-tetraenolide [17] and confirmed by spin echo and polarization transfer (DEPT) experiments. The peaks at δ165.26 and δ204.15 were characteristic for the carbonyl carbon of α,β-unsaturated δ-lactone and α,β-unsaturated ketone moieties respectively, whereas the methylene carbon of the exo-double bond was observed at δ129.74. The chemical shift of C-17 (δ47.699) of **1** is somewhat upfield as compared to cholesterol due to the γ-gauche effect induced by the oxygen atom of ether group [18]. In the light of these data the structure 1-oxo-21,24*S*-epoxy- (20*S*, 22*S*)-witha-2,5,25-trienolide has been assigned to daturilin (**1**).

EXPERIMENTAL

General. Mp is uncorr. DEPT experiments were carried out with $\theta = 45°$, 90° and 135°.

Plant material. The leaves of *D. metel* (5 kg) collected from Karachi, Pakistan in March 1985 were identified by Dr. S. I. Ali, Department of Botany, University of Karachi, and a voucher specimen has been deposited in the herbarium of that department.

Isolation. The fresh, undried leaves of *D. metel* were cut into small pieces and repeatedly extracted with EtOH at room temp. The combined extracts were concd under red. pres. to a viscous liquid which was kept overnight at about 20°. The gummy mass that settled was separated and repeatedly triturated with 10% HOAc, until free from alkaloids. The HOAc insoluble fraction was treated with petrol to remove the fatty matter and divided into Et_2O soluble and insoluble fractions; the latter was extracted with MeOH, rejecting a negligible quantity of resinous material. The dark green MeOH soluble fraction was heavily charcoaled and the charcoal bed repeatedly eluated with MeOH and MeOH–C_6H_5 (1:1). The charcoal filtrate and MeOH eluates were combined and freed of the solvent under red. pres. The residue (8.2 gm) was again treated with petrol and Et_2O to remove the residual fatty matter, extracted with $CHCl_3$ and subjected to prep. TLC. (silica gel GF 254 with fluorescent indicator, C_6H_6–Me_2CO; 9:1). The UV active band with R_f 0.82 yielded daturilin (**1**) on usual work-up as a colourless crystallizate which on recrystallization from petrol–$CHCl_3$ (9:1) formed needles (372 mg) mp 206–7° $[\alpha]_D^{23}$ −100.60 ($CHCl_3$; *c* 0.4866). UV λ_{max}^{MeOH} nm: 218; IR ν_{max}^{KBr} cm^{-1}: 1650, 1680, 1720 and 1125; HRMS *m/z* (rel. int.%): 436.2626 (M^+ calcd. for $C_{28}H_{36}O_4$ 436.2613) (50.83), 421.2346 $(M-Me)^+$ (3.55), 354.2200 $(C_{23}H_{30}O)^+$ (6.17), 269.1910 (M − side chain) (2.91), 268.1824 $(C_{19}H_{24}O)^+$ (6.55), 253.1594 $(C_{18}H_{21}O)^+$ (7.0), 227.1416 $(C_{16}H_{19}O)^+$ (758), 225.1281 $(C_{16}H_{17}O)^+$ (10.52), 187.1119 $(C_{13}H_{15}O)^+$ (7.13), 185.0971 $(C_{13}H_{13}O)^+$ (13.3), 173.0969 $(C_{12}H_{13}O)^+$ (2.26), 171.0812 $(C_{12}H_{11}O)^+$ (23.37). ^{1}H NMR (300 MHz) (C_3D_6O), δ: 0.77 (3H, *s*, H-18), 1.24 (3H, *s*, H-19), 1.41 (3H, *s*, H-28), 1.82 (1H, *m*, H-20), 1.95 (1H, *dd*, $J_{23a,23b}$ = 14.07, $J_{23b,22}$ = 3.87 Hz, H-23b), 2.14 (1H, *dd*, $J_{23b,23a}$ = 14.13, $J_{23a,22}$ = 1.65 Hz, H-23a), 2.87 (1H, *dd*, $J_{4a,4b}$ = 21.30, $J_{4a,3}$ = 4.92 Hz, H-4a), 3.34 (1H, *dd*, $J_{4b,4a}$ = 21.30, $J_{4b,3}$ = 2.94 Hz, H-4b), 3.66 (1H, *dd*, $J_{21\alpha,21\beta}$ = 13.23, $J_{21\alpha,20}$ = 2.64 Hz, H-21α), 3.90 (1H, *d*, $J_{21\alpha,21\beta}$ = 13.23 Hz, H-21β), 4.63 (1H, *br s*, $W_{1/2}$ = 3.1 Hz, H-22), 5.60 (1H, *dt*, $J_{7,6}$ = 6.0 H-6), 5.78 (1H, *ddd*, $J_{2,3}$ = 10.1, $J_{4a,2}$ = 3.24, $J_{4b,2}$ = 1.23 Hz, H-2), 5.99 (1H, *d*, $J_{27a,27b}$ = 0.78 Hz, H-27a), 6.56 (1H, *d*, $J_{27a,27b}$ = 0.78 Hz, H-27b) and 6.69 (1H, *ddd*, $J_{2,3}$ = 10.11, $J_{3,4a}$ = 5.02, $J_{3,4b}$ = 2.49 Hz, H-3).

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