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REVISED STRUCTURES OF DATURALACTONE AND 12-OXOWITHANOLIDE

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Key Word Index—*Datura quercifolia*; Solanaceae; withanolides; revised structures; daturalactone; 12-oxowithanolide.

Abstract—Recently Dhar *et al.* reported the isolation of two new steroidal lactones from the leaves of *Datura quercifolia* HBK and formulated them as 5α,12α,17α-trihydroxy-1-oxo-6α, 7α-epoxy-22 *S*-witha-2,24-dienolide and 5α,17α-dihydroxy-1, 12-dioxo-6α,7α-epoxy-22 *S*-witha-2,24-dienolide on the basis of UV, IR, NMR and MS studies. Further detailed chemical and spectral studies have led to revised structures, namely 5α, 12α-dihydroxy-1-oxo-6α,7α: 24α, 25α-diepoxy-20 *S*, 22 *R*-with-2-enolide and 5α-hydroxy-1, 12-dioxo-6α, 7α: 24α, 25α-diepoxy-20 *S*, 22 *R*-with-2-enolide, respectively, for the above two compounds.

INTRODUCTION

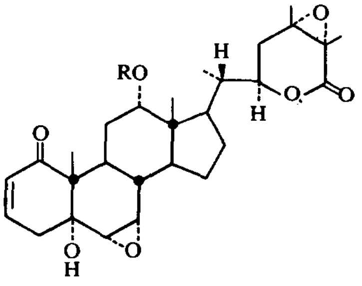
In previous papers [1, 2] we dealt with the structures of two steroidal lactones of the withanolide series, isolated from *Datura quercifolia* HBK. Further physico-chemical investigations have now led us to revise the structures of these two compounds.

RESULTS AND DISCUSSION

Daturalactone (**1a**) exhibited λ_{max} at 223 nm and two IR bands in the carbonyl region at 1695 (α,β-unsaturated ketone) and 1729 cm^{-1}. This latter band can be attributed to a saturated δ-lactone. This was also suggested by the catalytic hydrogenation under different conditions and with different catalysts of the compounds which gave a dihydro-derivative **2**, IR 1709 and 1725 cm^{-1}. Absence of an α,β-unsaturated δ-lactone moiety was also suggested by the negative Cotton effect in CD at 232 nm; in Δ^{24}-22*S*-withenolides the CD-absorption has been observed at around 260 nm ($\Delta\varepsilon \approx -3$) [3]. Attention is also called to the signals of the 27- and 28-methyl protons in the ^{1}H NMR spectrum (cf. Table 1) which appeared as singlets at δ 1.50 and 1.58, a position upfield from where the corresponding methyls in α,β-unsaturated δ-lactones resonate. The upfield position of these methyl signals cannot be accounted for by an *S*-configuration at C-22 because such compounds also give peaks at around δ 2.0 [4]. Since these signals appeared as singlets and downfield from the corresponding signals of saturated δ-lactones, this suggested that the C-24 and C-25 positions were tetrasubstituted and therefore contained an oxygen function, most probably an epoxide.

The positions and multiplicity of two vinylic hydrogen signals at δ 5.81 (*dq*, 10; 3; 1) and 6.6 (*dq*, 10; 4.5; 3) were attributed to the C-2 and C-3 protons, respectively, in a steroidal Δ^2-en-1-one system. In the hydrogenated product (**2**), these signals in the olefinic region disappeared. Such a position of the enone-chromophore was also in accordance with the negative Cotton effect in the CD spectrum at 337 nm which suggested furthermore the 5α-configuration [5].

The two signals at δ 3.06 (*d*, 4) and 3.35 (*dd*, 4; 1) were attributed to the C-6 and C-7 protons in a 5α-OH-6α,7α-epoxy steroid because their position and multiplicity agreed exactly with those displayed by withanolides having a similar substitution pattern [6]. This assignment was further supported by the HI reduction which yielded the trienone **3**, λ_{max} 358 nm. The ^{1}H NMR of **3** showed a downfield shift of the signals of the vinylic C-6 and C-7 protons which resonated at 5.85 (*d*, 10) and 6.11 (*dd*, 10; 2.5); the C-4 proton signal also appeared as a vinylic signal at 5.95 (*d*, 5.5) due to elimination of the 5α-OH and one C-4 proton. The downfield position of



1a R = H
1b R = Ac
2 R = H; 2,3-H$_2$

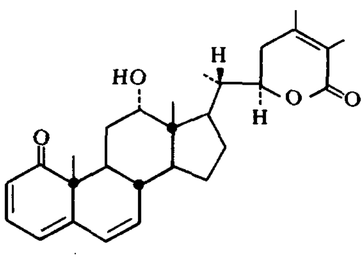


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Table 1. ^{1}H NMR signals of daturalactone (**1a**)

Compound	2-H	3-H	6-H	7-H	12-H	22-H
1a	5.81*dq*	6.60*dq*	3.06*d*	3.35*d*	4.00*m*	4.55*m*
	(10, 3, 1)*	(10, 4.5, 3)	(4)	(4.1)		
1b	5.81*dq*	6.60*dq*	3.06*d*	3.35*d*	5.08*m*	4.55*m*
	(10, 3, 1)	(10, 4.5, 3)	(4)	(4.1)		
2			3.08*d*	3.32*d*	4.03*m*	4.60*m*
			(4)	(4.1)		
3	5.92*d*	6.99*dd*	5.85*d*	6.11*dd*	4.09*m*	4.38*dt*
	(9.5)	(9.5, 5.5)	(10)	(10, 2.5)		(12.5, 3, 3)
4			3.05*d*	3.28*d*	4.02*m*	4.40*dt*
			(4)	(4.1)		(12.5, 3)
5	5.85*d*	6.60*dq*	3.08*d*	3.42*d*		4.55*m*
	(10)	(10, 4.5, 3)	(4)	(4.1)		
5†	5.66*d*	6.61*dd*	2.98*d*	3.14*d*		4.41*m*
	(10)	(10, 5, 2.5)	(4.5)	(4.5)		

* *J*(Hz) in parentheses.
† Recorded in DMSO.

the C-27 and C-28 methyl proton signals (1.90, *s* and 1.95 *s*) in **3** suggested the appearance of a double bond between C-24 and C-25, i.e. the presence of the usual withenolide lactone system [7]. This assumption was also supported [3] by a positive CD curve at 253 nm. It was also interesting to note that in the reductive cleavage of **1a** with Zn–Cu couple besides double bond hydrogenation in ring A only one epoxide (that at C-24, C-25) was cleaved with the formation of a double bond. The ^{1}H NMR spectrum of this compound **4** showed the absence of signals due to vinylic C-2, C-3 protons and the characteristic downfield position of the C-27 and C-28 methyl proton signals at δ 1.91 (*s*) and 1.96 (*s*) (cf. HI reduction [7]). Both the HI reduction and the reductive cleavage substantiate further the proof for the presence of an epoxide at C-24, C-25.

Daturalactone formed a monoacetate (**1b**) in which the single proton peak at δ 4.00 was deshielded to 5.08 indicating the geminal position of the —OH to this proton. The assignment of the position of the —OH was based on the relationship of **1** with its 12-dehydroderivative (**5**) isolated from the same plant and obtained from **1a** also by Jones' oxidation.

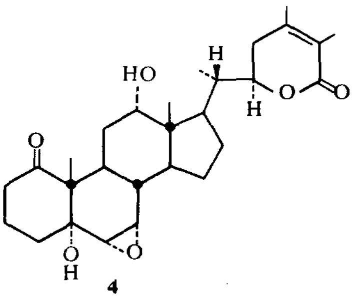
Compound **5** showed λ_{max} at 225 nm and the bands in the carbonyl region in the IR at 1683 (α,β-unsaturated ketone), 1730 (α,β-epoxy-δ-lactone) and 1700 cm^{-1} (six-membered ring ketone). ^{1}H NMR signals of **5** closely resembled the corresponding signals in the spectrum of daturalactone (**1a**) with the exception of the signals related to the C-18 protons and the absence of one proton signal at δ 4.00. In **5** the C-18 protons resonated at δ 1.1; this suggested the presence of a carbonyl group, because this is the only position which can strongly deshield the C-18 protons. This assignment was also supported by a positive Cotton effect at 286 nm [8].

The relationship between **1a** and **5** fixed the —OH group of **1a** at the C-12 position. From the half-band width of the C-12 proton signal (W_H = 5.7 Hz; X-part of ABX pattern) it followed that the C-12 proton had an equatorial position because in this position it bisects the 11-methylene protons almost equally, resulting in weak splitting (2.5 Hz) [9, 10]. The configuration of the 24/25-epoxide has been assigned α because the ^{1}H NMR spectrum of **5** in DMSO completely corresponded to that of the oxidation product of Nic 7, whose 24α,25α-epoxide structure has been determined by X-ray crystallography [11]. The IR spectrum of the former compound was also completely superimposable on the IR of the latter compound.

The MS of **1a** and **5** were in accordance with the proposed structures; the base peak at *m/e* 125 which appeared in both spectra and which is believed to be characteristic for the Δ^{24}-enelactone moiety (cleavage of C(20)–C(22) bond) can be assigned to the ion **6** formed by double fission of ring B [12].

EXPERIMENTAL

Mps. are uncorr.; UV spectra were recorded in MeOH; IR spectra were recorded of KBr pellets; ^{1}H NMR spectra were



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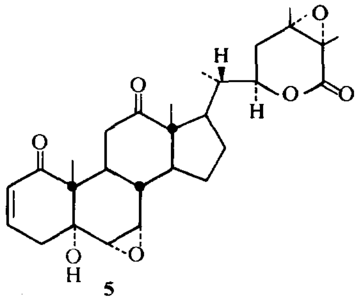

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and its derivatives (recorded in $CDCl_3$)

18-CH_3	19-CH_3	21-CH_3	27,28-$(CH_3)_2$	Others
0.88*s*	1.18*s*	1.02*d* (5)	1.50*s*, 1.58*s*	
0.80*s*	1.21*s*	1.02*d* (5)	1.50*s*, 1.58*s*	2.01*s* acetate-CH_3
0.74*s*	1.21*s*	1.02*d* (5)	1.50*s*, 1.59*s*	3.22, $(OH)_2$
0.83*s*	1.25*s*	1.08*d* (6)	1.90*s*, 1.95*s*	5.95*d*, 4-H (5.5)
0.76*s*	1.20*s*	1.10*d* (6)	1.91*s*, 1.96*s*	3.24, $(OH)_2$
1.10*s*	1.26*s*	0.90*d* (7)	1.52*s*, 1.58*s*	
1.054*s*	1.16*s*	0.77*d* (7)	1.44*s*, 1.46*s*	

recorded at 100 MHz using TMS as internal standard. TLC was carried out on chromatoplates of Si gel G (Merck).

Isolation procedure. Crushed fresh leaves of *Datura quercifolia* HBK (2 kg) were extracted 2× with 3 l. portions of cold C_6H_6 for 3 hr; the combined extracts were concd to 400 ml and kept at 0° for 24 hr. A pale green solid (1.3 g) deposited which was chromatographed on a Si gel column (−100/+200 mesh, 42 × 3.2 cm). Elution with C_6H_6–EtOAc (20:1, 20:2, 20:20) afforded **1a** (650 mg, from 20:20 eluent mixture) and crude **5** (90 mg, from 20:2 mixture).

Daturalactone (**1a**), (20S, 22R)-6α,7α; 24,25-diepoxy-5,12α-*dihydroxy-1-oxo-5α-with-2-enolide.* Mg 260–61° (petrol–C_6H_6). UV λ_{max} nm: 223; IR ν_{max} cm^{-1}; 3600–3500 (CH), 1729 (δ-lactone) and 1695 (C=O): CD (acetonitrile); $\Delta\varepsilon_{337} = +1.69$, $\Delta\varepsilon_{234} = -2.88$, $\Delta\varepsilon_{194} - +163$. (Found: C, 68.87; H, 7.74; M^+ 486. $C_{28}H_{38}O_7$ requires: C, 69.13; H, 7.82%, M^+ 486).

Acetylation of **1a** *to* **1b**. **1a** (50 mg) was acetylated with Ac_2O in Py overnight at room temp. to give **1b** (48 mg), mp 248° (petrol–EtOAc), IR ν_{max} cm^{-1}: 3610–3220 (OH), 1740 (C=O), 1727 (δ-lactone) and 1695 (C=O). (Found: C, 67.98; H, 7.53, M^+ 528. $C_{30}H_{40}O_8$ requires: C, 68.19; H, 7.58%; M^+ 528).

Hydrogenation of **1a** *to* **2**. **1a** (100 mg) was hydrogenated (1 mol H_2) over 5% Pd–C in EtOH at room temp. and atmos. pres. to give **2** (86 mg), mp 235–236° (EtOAc); IR ν_{max} cm^{-1}: 3500 (OH), 1726 (δ-lactone) and 1709 (C=O); CD: $\Delta\varepsilon_{296} = +0.72$, $\Delta\varepsilon_{232} = -2.83$, and $\Delta\varepsilon_{190} = +5.8$. (Found: C, 68.62; H, 8.0; M^+ 488. $C_{28}H_{40}O_7$ requires: C, 68.85; H, 8.2%; M^+ 488).

Reaction of **1a** *with* HI. To an ice-cold soln of **1a** (150 mg) in Me_2CO (60 ml) HI (57%, 2 ml) was added dropwise. The mixture was set aside for 1 hr at room temp. It was then diluted with H_2O and extracted with $CHCl_3$. The extract was washed with aq. sodium thiosulphate and H_2O and dried. The residue, which showed two compounds, was separated by PLC on Si gel G (0.5 mm thick; C_6H_6–EtOAc, 2:1). Extraction of the upper yellow band afforded **3** as a yellow solid (40 mg), mp 241–43° (EtOAc), UV λ_{max} nm: 358; IR ν_{max} cm^{-1}: 3500 (OH), 1685 (br, C=O) and 1645; CD: $\Delta\varepsilon_{404} = +1.8$; $\Delta\varepsilon_{350} = -4.3$, $\Delta\varepsilon_{253} = +3.6$, $\Delta\varepsilon_{226} = -3.4$, and $\Delta\varepsilon_{199} = +8$. Values were not reproducible because the sample decomposed during measurement. (Found: C, 74.30; H, 7.94; M^+ 436. $C_{28}H_{36}O_5$ requires: C, 74.33; H, 7.96%; M^+ 436).

Reaction of **1a** *with* Zn–Cu *couple.* **1a** (150 mg), Zn–Cu couple (9 g; prepared according to lit. [13], and dry EtOH (15 ml) were refluxed for 60 hr. The mixture was filtered and the filtrate vacuum-dried. The residue on chromatography on Si gel gave **4** (35 mg) as the major product, mp 250–52° (EtOAc), CD: $\Delta\varepsilon_{313} = +0.38$, $\Delta\varepsilon_{304} = +0.70$, $\Delta\varepsilon_{254} = +3.96$, $\Delta\varepsilon_{220} = +6.50$ (sh), $\Delta\varepsilon_{197} = +16.4$. (Found: C, 71.32, H, 8.10: M^+ 472. $C_{28}H_{40}O_6$ requires: C, 71.18; H, 8.47%; M^+ 472).

(20S, 22R)-6α,7α; 24,25-*Diepoxy-5-hydroxy-1,12-dioxo-5α-with-2-enolide* (**5**). The crude **5** was purified by repeated crystallization from petrol–$CHCl_3$: mp 303–5°, UV λ_{max} nm: 225, IR ν_{max} cm^{-1}: 3526, 3510 (OH), 1730 (α,β-epoxy-δ-lactone), 1700 (C=O) and 1683 (C=O); CD: $\Delta\varepsilon_{346} = -2.03$, $\Delta\varepsilon_{338} = -2.09$, $\Delta\varepsilon_{288} = +1.00$, $\Delta\varepsilon_{233} = -3.14$, $\Delta\varepsilon_{189} = +26.6$. (Found: C, 69.1; H, 7.51; M^+ 484. $C_{28}H_{36}O_7$ requires: C, 69.42; H, 7.43%; M^+ 484).

Oxidation of **1a** *to* **5**. To an ice-cold soln of **1a** (100 mg) in dry Me_2CO (200 ml) was added Jones' reagent dropwise until a brown colour appeared. The mixture was stirred until the reaction was complete (monitored by TLC). It was then poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 3% aq. $NaHCO_3$ and H_2O. The extract was dried, evapd and crystallized from petrol–$CHCl_3$ to give a crystalline solid which resembled the natural compound (**5**) in all physicochemical properties.

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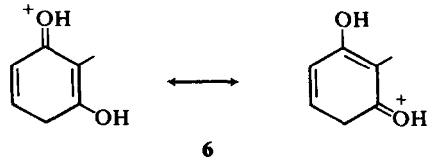

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