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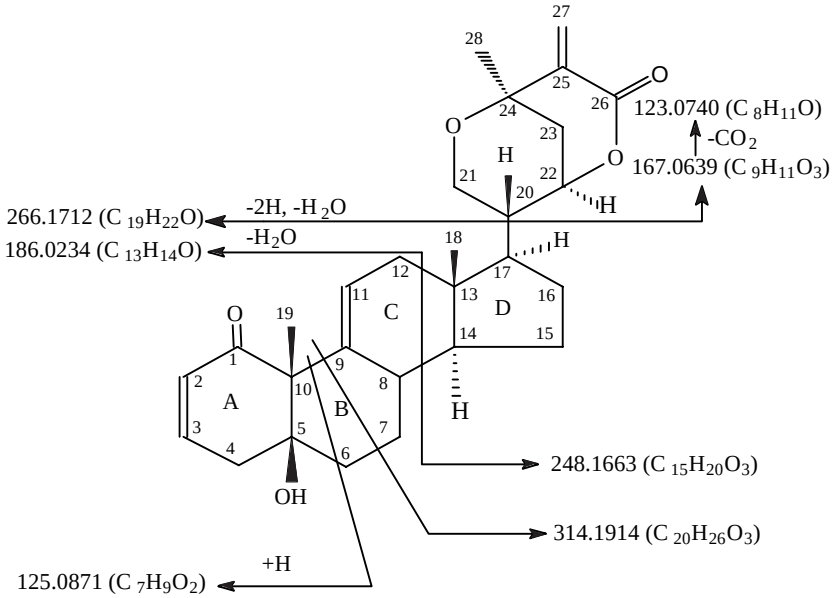
TWO NEW WITHANOLIDES FROM THE AERIAL PARTS OF *DATURA INNOXIA*

Bina S. Siddiqui*, Imran Ali Hashmi, and Sabira Begum

H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi–75270, Pakistan

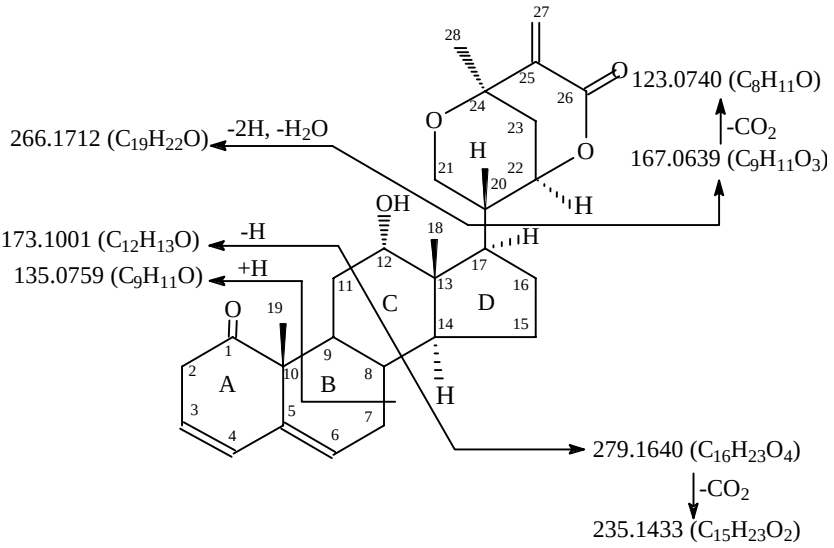
Abstract- Two new withanolides named as withametelinol-A (**1**) and withametelinol-B (**2**) have been isolated from the aerial parts of *Datura innoxia*. Their structures have been elucidated as (20*R*, 22*R*)-21,24-epoxy-5β-hydroxy-1-oxowitha-2,9(11),25(27)-trienolide and (20*R*, 22*R*)-21,24-epoxy-12α-hydroxy-1-oxowitha-3,5,25(27)-trienolide respectively through spectroscopic studies.

Datura innoxia syn. *D. metel* belongs to the family Solanaceae[1] and is reputed for the presence of withanolides.[2] Withanolids are C_{28}–steroidal lactones, built on ergostane–framework and reported to have cytotoxic,[3] anticancer,[4] hepatoprotective[5] and anti-inflammatory[6] properties. Present paper deals with the isolation and structure elucidation of two new withanolides withametelinol-A (**1**) and withametelinol-B (**2**) along with a known withanolide characterized as daturilin (**3**).[7] The new constituents have been characterized on the basis of spectral studies, including 1- and 2-D NMR, whereas the known compound has been identified through comparison of its spectral data with those reported in literature.



Significant MS fragments of 1

The HREIMS of **1** showed the molecular ion peak at *m/z* 452.2506 corresponding to the molecular formula $C_{28}H_{36}O_5$, [calcd 452.2562] along with following MS fragments 434.2602 [M^+-H_2O, $C_{28}H_{34}O_4$, base peak], 314.1914 [$C_{20}H_{26}O_3$], 266.1712 [$C_{19}H_{22}O$], 248.1663 [$C_{15}H_{20}O_3$], 186.0234 [$C_{13}H_{14}O$], 167.0639 [$C_9H_{11}O_3$] and 123.0740 [$C_8H_{11}O$]. In IR spectrum transmissions in the hydroxy region (ν_{max}, 3600 cm^{-1}) were observed in addition to the bands for olefin (ν_{max}, 1620 cm^{-1}), enone (ν_{max}, 1680 cm^{-1}) and α,β-unsaturated δ-lactone (ν_{max}, 1720 cm^{-1}) moieties. It showed UV maxima at 224 nm. The data including ^1H and ^{13}C NMR spectra (Table 1) indicated that **1** belongs to withanolide series with a bicyclic side chain.[8] The ^1H NMR displayed resonances for three methyl groups located at tertiary carbon atoms at δ 0.69 (s, H-18), 1.34 (s, H-19) and 1.43 (s, H-28) and five vinylic protons. Out of these, one proton with doublet of double doublet resonating at δ 5.85 (*J* = 10.0, 3.1 and 1.2 Hz) was assigned to H-2 and the other proton at δ 6.76 (*J*=10.0, 5.0 and 2.5 Hz) to H-3. A double doublet at δ 5.62 (*J*=5.4 and 2.1 Hz) was accounted for H-11. The remaining two vinylic protons appearing at δ 6.74 and 6.00 (H-27a and H-27b, respectively) as broad singlets, indicated the exo double bond in the side chain. The ^1H NMR further showed signals for an oxymethylene group at δ 3.90 (dd, *J*=13.3 and 4.0 Hz, H-21a) and 3.70 (dd, *J*=13.3 and 3.0 Hz, H-21b) and an oxymethine proton at δ 4.64 (br s, H-22). Of the five oxygen atoms present in **1**, three are associated with an enone and δ-lactone functions, one forms the ether linkage of the bicyclic steroidal side chain and the remaining oxygen atom is present as a hydroxyl group (IR, 3600 cm^{-1}) located at a non-protonated carbon atom (br band, δ 78.30). The index of unsaturation was accounted for as follows: four by the steroidal nucleus, two by the enone, one by the isolated C=C double bond, three by the δ-lactone moiety with an exocyclic double bond at C-25(27) and one by the ether ring between C-21 and C-24. The hydroxyl group was placed at C-5 on the basis of an important interaction between C-5 and H-3 in the HMBC spectrum along with other correlations.



Significant MS fragments of 2

The stereochemistry of C-5 was determined as 5β by comparison of ^{13}C value with the data of similar compounds (δ 72-77 for 5α-hydroxy)[10, 14-16] and (δ 78-79 for 5β-hydroxy). Its position at C-5 was also supported by the significant mass fragment at *m/z* 125.0871 corresponding to $C_7H_9O_2$[9] and comparison of ^{13}C-NMR data with those published for similar structures.[10,17,18]

Table 1. ^{1}H- and ^{13}C-NMR spectral data of compound (**1**)

C/H No.	δ_C [a)]	δ_H [b)], m, *J* (Hz)	COSY correlations	HMBC correlations
1	204.29	-	-	H-11
2	127.95	5.85, ddd (10.0, 3.1, 1.2)	H-3	H-4b
3	145.14	6.76, ddd (10.0, 5.0, 2.5)	H-2, H-4a/b	H-4a/b
4a	33.52	3.27, ddd (21.0, 2.5, 1.2)	H-3, H-4b	-
4b		2.73, ddd (21.0, 5.0, 3.1)	H-3, H-4a	-
5	78.30	-	-	H-3
6	30.76	1.95	-	-
7	22.50	1.20	-	-
8	42.87	1.79	-	-
9	135.97	-	-	H-19
10	50.53	-	-	H-3, H-4b
11	124.60	5.62, dd (5.4, 2.1)	H-12a/b	-
12a	39.71	1.75, m	H-11	-
12b		1.55, m		
13	42.64	-	-	H-18
14	56.00	1.20	-	-
15	23.67	2.21	-	-
16	26.53	1.70	-	-
17	47.63	1.75	-	H-21a/b
18	12.77	0.69, s	-	-
19	18.46	1.34, s	-	-
20	39.88	1.90, m	H-21a/b, H-22	-
21a	60.53	3.90, dd (13.3, 4.0)	H-20, H-21b	H-22
21b		3.70, dd (13.3, 3.0)	H-20, H-21a	-
22	75.63	4.64, br s	H-20, H-23b	H-21a/b
23	33.27	2.04, dd (14.0, 1.3)	H-23b	H-28
		1.89, dd (14.0, 4.1)	H-22, H-23a	-
24	69.35	-	-	H-27a/b, H-21a/b, H-22, H-28
25	139.02	-	-	H-27b, H-23a
26	165.33	-	-	H-27a/b, H-22
27a	129.95	6.74, s	H-27b	-
27b		6.00, s	H-27a	-
28	24.10	1.43, s	-	-

a) run at 100 MHz in $CDCl_3$ (Assignments were made by means of HMQC.)

b) run at 300 MHz in $CDCl_3$ (*J* in Hz)

Table 2. ^{1}H- and ^{13}C-NMR spectral data of compound (**2**)

C/H No.	δ_C [a]	δ_H [b] m, *J* (Hz)	COSY correlations	HMBC correlations
1	207.00	-	-	-
2a	39.45	2.74, ddd (20.0, 5.5, 2.6)	H-2b, H-3, H-4	
2b	-	3.22, dd (20.0, 6.3)	H-2a, H-3	
3	129.53	5.58, ddd (9.5, 6.3, 5.5)	H-2a/b, H-4	
4	130.63	6.04, dd (9.5, 2.6)	H-2a, H-3	H-3,H-6
5	120.50	-	-	-
6	n.o.*	5.62, dd (5.7, 2.6)	-	-
7	29.27		-	-
8	29.61	1.18 m	-	-
9	39.93	2.16 m	-	
10	49.74	-	-	H-4, H-6
11	31.80	1.75, m	-	H-8
		2.00, m		
12	73.01	3.85	-	H-18
13	48.80	-	-	-
14	53.43		-	-
15	25.30	1.36 m	-	-
16	25.68	1.72	-	-
17	48.69	1.40	-	H-18
18	13.96	0.71, s	-	-
19	22.59	1.32, s	-	-
20	40.01	1.75, m	-	-
21	60.51	3.81, dd (13.6, 2.9)		H-17
		3.98, dd (13.6, 3.3)		-
22	77.30	4.64, br s		
23a	33.07	2.11, dd (14.0, 1.3)	H-22, H-23b	H-28
23b		1.89, dd (14.0, 4.0)	H-22, H-23a	-
24	69.90	-	-	H-27a/b, H-28
25	138.31	-	-	H-27b
26	165.55	-	-	H-27a/b
27a	129.54	6.01, s	H-27b	-
27b		6.75, s	H-27a	-
28	23.28	1.43, s	-	-

[a] run at 125 MHz in $CDCl_3$ (Assignment were made by means of HMQC)
[b] run at 300 MHz in $CDCl_3$ (*J* in Hz)

The isolated double bond was placed at C-9(11) on the basis of HMBC spectrum showing interaction between C-9 and H-19. The stereochemistry at C-20, C-22 and C-24 depicted in the structure was derived through comparable proton and ^{13}C-NMR shifts with those of compounds having same stereochemistry.[2, 8, 17] In light of these observations, the structure of **1** has been elucidated as (20R, 22R)-21,24-epoxy-5β-hydroxy-1-oxowitha-2,9(11),25(27)-trienolide.

Compound (**2**) showed a molecular ion peak at *m/z* 452.2589, in the HREIMS corresponding to the molecular formula $C_{28}H_{36}O_5$ [calcd 452.2562] along with other MS fragments at 434.2471 [M^+-H_2O, $C_{28}H_{34}O_4$], 266.1712 [M^+-side chain-H_2O-2H, $C_{19}H_{22}O$], 173.1001 [$C_{12}H_{13}O$], 167.0639 [$C_9H_{11}O_3$], 135.0759 [$C_9H_{11}O$]. The twin maxima in UV spectrum**,** at 223 and 234 nm, indicated the presence of an α,β-unsaturated δ-lactone and a heteroannular diene chromophore.[11] Its IR spectrum displayed transmissions for a hydroxyl group (ν_{max}, 3400 cm^{-1}), an α,β-unsaturated δ-lactone (ν_{max}, 1720 cm^{-1}) and a carbonyl group (ν_{max}, 1712 cm^{-1}). It did not show any absorption for the enone carbonyl. The ^{1}H-NMR spectrum of **2** showed striking resemblance with **1**. The only observable difference was in the chemical shifts and splitting of three olefinic protons of rings A and B and the chemical shifts of 19-methyl of **2** (Table 2). An additional oxymethine proton signal was also observed at δ 3.85 (t, 3.5 Hz, H-12β). The ^{1}H-NMR spectral signals for three vinylic protons at δ 6.04 (dd, *J*=9.5 and 2.6 Hz, H-4), 5.62 (dd, *J*=5.4, 2.6 and 2.1 Hz, H-6) and 5.56 (ddd, *J*=9.5, 8.2 and 2.1 Hz, H-3) and UV maximum (234 nm) suggested a 3, 5-dien-1-one system.[12] The hydroxyl group was placed at C-12 on the basis of comparison of spectral data with those of similar compounds.[13]

EXPERIMENTAL

General Experimental Procedures. UV spectra were measured for methanolic solution on a Hitachi UV–3200 spectrophotometer. IR spectra were recorded for chloroform solutions on a JASCO A-302 spectrophotometer. ^{1}H-, ^{13}C-NMR (broad band and DEPT), COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker Aspect AM 300 spectrometer operating at 300 (^{1}H) and 75 MHz (^{13}C) for solutions in $CDCl_3$. MS spectra were run on a JEOL JMS–HX110 (HREIMS, 70 eV) and a Varian Mat 311 A (EIMS, 70 eV) instrument. Optical rotations were obtained by using a JASCO DIP–360 polarimeter and are given in units of deg cm^{-2} g^{-1}. All TLC was performed on Merck Kieselgel 60 GF_{254}. Vacuum–liquid chromatography (VLC) was performed on Merck silica gel 60 GF_{254}. Petrol used was of the boiling range 60–80^o.

Plant material. Aerial parts of *Datura innoxia* (22 kg) were collected in the month of April from Karachi, Pakistan and identified by Dr. Surayya Khanum, Department of Botany, University of Karachi. A voucher specimen [No. KUH–GH 668553] has been deposited in the herbarium of the same department.

Extraction and Isolation The fresh undried aerial parts (22 kg) of *D. innoxia* were repeatedly extracted with methanol (40 L) at rt (five times) for 72 h and combined methanolic extract was evaporated under vacuum. The thick syrupy residue (200 g) was partitioned between ethyl acetate (EtOAc) and water.

The EtOAc phase after usual workup and removal of solvent under reduced pressure was divided into petrol ether soluble (22 g) and petrol ether insoluble (5 g) fractions. The petrol ether insoluble fraction was subjected to column chromatography (silica gel, petrol ether, petrol ether-$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH, MeOH in order of increasing polarity). The column fractions eluted with petrol ether-$CHCl_3$ (1:1) yielded a pure constituent characterized as **1** (26 mg). The column fractions which eluted with $CHCl_3$–MeOH (9.5:0.5) gave a crystalline residue on removal of the solvent. It showed two spots on TLC which were purified through thick layer chromatography (silica gel, $CHCl_3$–MeOH, 9.5:0.5, eluted for three times) to give **2** (29 mg) and **3** (15 mg.).

Withametelinol A (**1**), colorless fine needles (26 mg) from cold MeOH; mp 212-214 ^oC; $[\alpha]^{28}_D$ + 32.4^o (*c*, 0.074 in MeOH); UV λ_{max} (MeOH): 224 nm; IR ν_{max} ($CHCl_3$) cm^{-1}: 3600 (OH), 1720 (α,β–unsaturated δ–lactone), 1680 (enone); HREIMS *m/z* (rel. int.), 452.2506 [M^+, $C_{28}H_{36}O_5$, calcd 452.2562] (7) 434.2602 [M^+-H_2O, $C_{28}H_{34}O_4$] (100) 314.1914 [$C_{20}H_{26}O_3$] (22), 266.1712 [$C_{19}H_{22}O$] (27),248.1663 [$C_{15}H_{20}O_3$] (18),186.0234 [$C_{13}H_{14}O$](17), 167.0639 [$C_9H_{11}O_3$] (20), 125.0871 [$C_7H_9O_2$] (18), 123.0740 [$C_8H_{11}O$] (23); ^{1}H- and ^{13}C-NMR (Table 1).

Withametelinol B (**2**), colorless fine needles; mp 197-198 ^oC; $[\alpha]^{28}_D$ + 0.7^o (c, 0.28 in MeOH); UV λ_{max} (MeOH): 223 and 234 nm; IR ν_{max}($CHCl_3$) cm^{-1}: 3400 (OH), 1720 (α,β–unsaturated δ–lactone), 1712 (C=O); HREIMS *m/z* (rel. int.): 452.2589 [M^+, $C_{28}H_{36}O_5$, calcd 452.2562] (32), 434.2471 [M^+,-H_2O, $C_{28}H_{34}O_4$] (89), 279.1640 [$C_{16}H_{23}O_4$] (24), 266.1712 [M^+, -side chain -H_2O -2H, $C_{19}H_{22}O$] (28), 235.1433 [$C_{15}H_{23}O_2$] (20), 173.1001 [$C_{12}H_{13}O$] (28), 167.0639 [$C_9H_{11}O_3$] (20), 135.0759 [$C_9H_{11}O$] (27), 123.0740 [$C_8H_{11}O$] (16); ^{1}H- and ^{13}C-NMR (Table 2).

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