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WITHANOLIDES FROM *DATURA INNOXIA*

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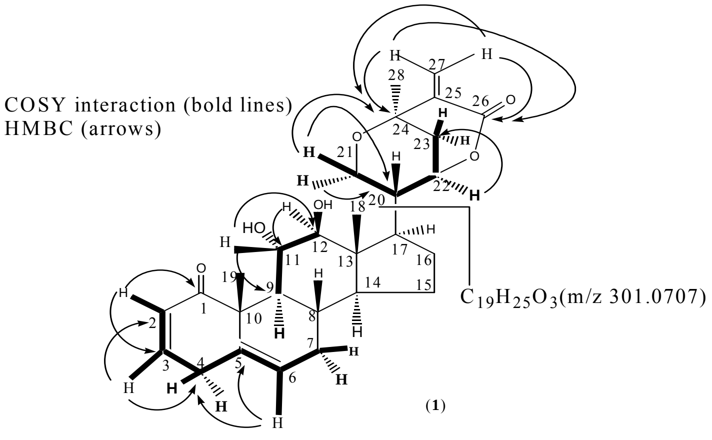
Abstract-The methanolic extract of the aerial parts of *Datura innoxia* afforded two new withanolides namely witharifeen (**1**) and daturalicin (**2**). Their structures were established as 11*α,* 12*β*-dihydroxy (20*R*, 22*R*)-21,24-epoxy-1-oxowitha-2, 5, 25(27)-trien-22, 26-olide (**1**) and (20*R*, 22*R*)-5*β*, 6*β*-14*α*,15*α*-21,24-triepoxy-1-oxowitha-2,25(27)-dien–22,26-olide (**2**) respectively by analysis of spectroscopic data.

INTRODUTION

Datura innoxia belongs to the family Solanaceae which is known to contain various tropane alkaloids (hyoscyamine, hyoscine and atropine)[1,2] and withanolides, a group of C-28 steroids having *δ*-lactone in bicyclic side chain related to ergostane nucleus.[3] Several withanolides have displayed various types of biological activity, such as cytotoxic,[4] anticancer,[5] immunosuppressive,[6] anti-inflammatory [7] and hepatoprotective[8] properties. In this Note the isolation and structure elucidation of two new withanolides witharifeen (**1**) and daturalicin (**2**) are reported. Their structures were elucidated as 11*α,* 12*β*-dihydroxy-(20*R*, 22*R*)-21,24-epoxy-1-oxowitha-2, 5, 25 (27)-trien-22, 26-olide (**1**) and (20*R*, 22*R*)-5*β*, 6*β*- 14*α*, 15*α* - 21, 24-triepoxy-1-oxowitha-2, 25(27)-dien-22, 26-olide (**2**) respectively based on spectral studies.

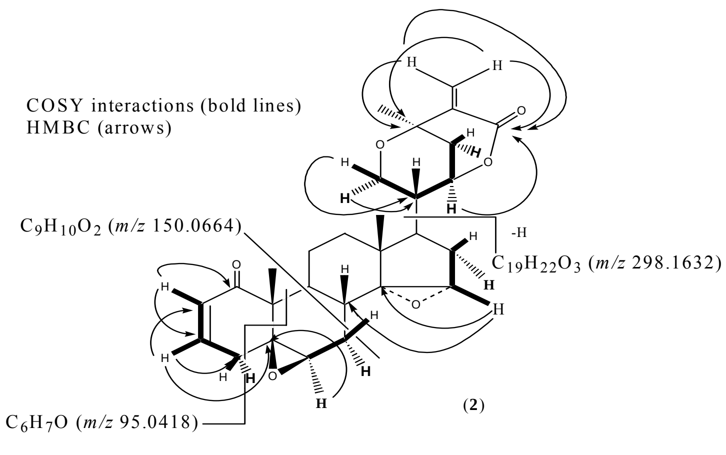
RESULTS AND DISCUSSION

The molecular ion peak [M^+] of witharifeen (**1**) at *m/z* 468 in the EI-MS septrum and *m/z* 468.2492 in the HR-EI-MS spectrum corresponded to the molecular formula $C_{28}H_{36}O_6$. The UV (λ_{max} 225 nm) and IR (ν_{max} 1610, 1680, 1720 cm^{-1}) spectra indicated α,β-enone and α,β-unsaturated δ-lactone moieties generally encountered in a typical withanolide. [9] The IR spectrum further showed absorption for OH group at 3400 cm^{-1}. In the upfield region of the ^{1}H-NMR spectrum of **1** signals related to three methyl groups were observed as singlets at δ 0.71 (H−18), 1.31 (H−19) and 1.42 (H-28). Five vinylic protons were observed in the low field region of the ^{1}H NMR spectrum, two as doublet of double doublet of one proton each at δ 5.87 (J = 10.0, 2.5,1.2 Hz; H-2) and 6.74 (J = 10.0, 5.0, 2.5 Hz; H-3), one as a doublet of quartet at δ 5.50 (J = 5.9, 2.5, 2.5, 2.5 Hz, H-6), one as a br. singlet at δ 6.75 (H -27a) and remaining one as a very narrow doublet with fine splitting at δ 5.99 (J = 0.7 Hz, d H-27b). Two mutiplets (dddd) of one proton each resonated at δ 2.80 (J = 21.0, 2.5, 2.5, 1.2; Hz, H-4b) and 3.25 (J = 21.0, 5.0, 2.5, 2.5 Hz, H -4a). Two ethereal protons resonating as a double doublet at δ 3.70 (J = 13.5, 3.0 Hz, H-21b) and a doublet at δ 3.95 (J = 13.5 Hz, H-21a) indicated the presence of a primary - tertiary ether linkage. H-22 appeared as a broad singlet at δ 4.60. All the ^{1}H and ^{13}C NMR shifts could be conclusively assigned through COSY 45^{o} as well as HMQC and HMBC experiments. The data recorded so far were in agreement with the published data of Δ^5 -withanolides with 21, 24-epoxy-1-oxowitha-2, 5, 25(27)-triene system.[10-12] The molecular formula of **1** displayed eleven double bond equivalents four of which were accounted for by the rings of the steroidal skeleton, two by the *α,β*-unsaturated carbonyl system in ring A, one by the isolated C=C double bond at C-5 one by the ether ring between C-21 and C-24 and three by the lactone moiety between C-22 and C-26 with an exocyclic double bond at C-25 (C-27). A MS fragment at *m/z* 301.0707 corresponding to $C_{19}H_{25}O_3$ for the steroidal nucleus further supported the side chain comprising of $C_9H_{11}O_3$. These structural features left two oxygens to be justified and these were taken as two hydroxyl groups (IR ν_{max} 3400 cm^{-1}) at C-11 and C-12 as the ^{1}H-NMR spectrum showed a double doublet at δ 4.16 (J = 11.5, 11.5 Hz, H-11β) and a doublet at δ 4.35 (J = 11.5 Hz, H-12α). These two protons showed interaction with each other in the COSY plot and each of them had a cross peak in the HMQC with the respective carbons at δ 76.0 (C-11) and 75.0 (C-12). Moreover C-12 also showed interaction with H-18 in the HyMBC plot. Stereochemistry of these hydroxyl groups was supported by NOESY interactions between H-11*β*, H-18*β* and H-19*β* and H-12*α* and H-17*α*. In light of these observations, the structure of **1** has been elucidated as 11*α*, 12*β*-dihydroxy-(20*R*, 22*R*)-21, 24-epoxy-1-oxowitha-2, 5, 25 (27)-trien-22, 26-olide, which got substantial evidence from the MS spectral data (*vide* EXPERIMENTAL).



(1)

The molecular ion peak (M^+) of daturalicin (**2**) at *m/z* 466 in the EI-MS spectrum and at *m/z* 466.2370 in the HR-EI-MS spectrum corresponded to the molecular formula $C_{28}H_{34}O_6$. The NMR spectrum of **2** showed that it also belongs to the withanolide class of steroids possessing two epoxy rings one between C-5 and C-6 and the other between C-14 and C-15 apart from the C-21-C-24 epoxy linkage. Further, the ion at *m/z* 298.1632 ($C_{19}H_{22}O_3$; *vide* structure) in the HR-EI-MS spectrum revealed that the side chain of **2** is identical with that of **1**. The epoxides between C-5/C-6 and C-14/C-15 were evident from the ^{1}H-NMR spectrum which showed a doublet at δ 3.11 (J = 2.3 Hz, H-6α) having a correlation with a carbon at δ 63.2 (C-6) in the HMQC spectrum and a signal as a doublet (J = 2.4 Hz) at δ 3.41 (H-15β) correlated with a carbon at δ 58.4 (C-15) in the HMQC spectrum and with carbons at δ 63.5(C-14) and δ 34.7(C-8) in the HMBC spectrum. The broad band ^{13}C-NMR spectrum (Table 2) also had signals at δ 62.0 (C-5) and 63.5 (C-14). These values are comparable with the reported values in compounds with similar partial structures.[13-16] The MS spectral fragments at *m/z* 298.1632 ($C_{19}H_{22}O_3$), 150.0664 ($C_9H_{10}O_2$) and 95.0418 (C_6H_7O) provided supportive evidences (*vide* structure). In the low field region of the ^{1}H-NMR spectrum of **2** signals related to four vinylic protons were observed, two as doublet of double doublets at δ 6.00 (J = 10.0, 2.5, 1.2 Hz, H-2), and 6.85(J = 10.0, 5.0, 2.5 Hz, H-3), one as a broad singlet at δ 6.76 (H-27a) and one as a narrow doublet at δ 5.98 (J = 0.8 Hz, H-27b). The protons H-21a, H-21b and H-22 resonated at δ 3.87 (*d*, J = 13.2 Hz), 3.72 (*dd*, J = 13.2, 2.7 Hz) and 4.62 (br *s*) respectively in analogy with that in compound (**1**). Three methyl singlets appeared at δ 0.99 (H-18), 1.32 (H-19) and 1.41 (H-28). The observed cotton effect at 254 nm (positive) and 345 nm (positive) suggested 22R configuration and *cis* A/B ring junction respectively.[3] The latter observation provided further evidence in favor of 5β,6β epoxide. Thus the structure of **2** has been elucidated as (20*R*, 22*R*)-5β, 6β-14α,15α-21,24-triepoxy-1-oxowitha-2, 25(27)-dien–22, 26-olide (**2**).



(2)

EXPERIMENTAL

General Melting points were determind on a Gallenkamp melting point apparatus and are uncorrected. UVand IR Spectra were recorded on Hitachi U-3200 and JASCO-A-302 spectrophotometers respectively. EI was recorded on Finnigan Mat 311A mass spectrometer; source at 250°C and 70 eV; *m/z* (rel.%). EIMS was recorded on JEOL JMS-HX-110 mass spectrometer; El, source at 250°C and 70 eV, *m/z* (rel.%). ^{1}H-NMR, COSY, NOESY and *J*-resolved were recorded on Bruker spectrometer at 300 MHz; chemical shifts (δ) are in ppm relative to $SiMe_4$ as internal standard, coupling constants (J) in Hz and ^{13}C-NMR on Bruker spectrometer, operating at HR 75 MHz. For TLC, preparative TLC and vacuum liquid chromatography (VLC) silica gel PF_{254} (Merck) was used. Optical rotations: JASCO-DIP-360 digital polarimeter; CD spectra: JASCO-J-600 spectropolarimeter; λ in nm (mdeg).

Extraction and Isolation

The fresh undried aerial parts of *D. innoxia* (22 kg) were extracted with methanol (90 L) at rt (five times), 48 h each time. The combined methanolic extract was freed of the solvent in vacuo to give a thick syrup (450 g). This was partitioned between ethyl acetate (EtOAc) and water. The EtOAc phase was treated with 4% aqueous Na_2CO_3 to separate the acidic and the neutral fractions. The EtOAc phase consisting of neutral fraction was washed with water, dried (anhyd Na_2SO_4), and freed of the solvent. The residue (202 g) thereby obtained was divided into petroleum ether soluble (150 g) and petroleum ether insoluble (50 g) fractions. A part of the petroleum ether insoluble fraction (17.5 g) was subjected to vacuum liquid chromatography (VLC, silica gel, PF-254; petroleum ether, EtOAC and then $CHCl_3$-MeOH in increasing order of polarity). The fractions were combined on the basis of TLC and total 27 fractions were ultimately obtained. Fraction No.7 which eluted with $CHCl_3$-MeOH (9.9:0.1) was active under UV light (254 nm) and showed a single spot on TLC. On recrystallization from methanol this afforded **1** (50 mg). Fraction No.13 which eluted with $CHCl_3$-MeOH (9.5:0.5) was subjected to thick layer chromatography over silica gel with solvent system $CHCl_3$ - MeOH (9.7:0.3). It separated into seven bands of which band No.5 was the major band. This band on further separation over TLC plate ($CHCl_3$-MeOH (9.6:0.4)) afforded compound (**2**) (12mg).

Witharifeen (**1**): Colourless plates; mp 166-167°C (uncorrected); $[\alpha]_D^{25}$ -109° (*c* 0.09, $CHCl_3$);.IR ($CHCl_3$) ν_{max}: 3400, 2920, 1720, 1680, 1610 cm^{-1}; UV(MeOH, nm, log ε) λ_{max}: 225(4.200); CD (MeOH): $[\theta]_{308.4}$–34.74, $[\theta]_{271.9}$+8.60, $[\theta]_{259.2}$ +5.66; ^{1}H and ^{13}C NMR spectral data: see Table 1; EIMS *m/z* (rel.int.,%) : 468 (100) [M$^+$] , 450 (20.0) [M$^+$-18], 424 (20.3) [M$^+$-44], 301(20), 285 (32.0), 241 (30), 167 (16.5), 94 (21.6), 83 (99.5). HREIMS *m/z*: 468.2492 [M$^+$](calcd for $C_{28}H_{36}O_6$ 468.2511), 301.0707 ($C_{19}H_{25}O_3$), 167.1807 ($C_9H_{11}O_3$).

Daturalicin(**2**): Fine colorless needles; mp 140-141 °C (uncorrected); $[\alpha]_D^{25}$ -26.7° (*c* 0.14, $CHCl_3$). IR ($CHCl_3$) ν_{max} : 2850, 1720, 1680 cm^{-1}; UV(MeOH, nm, log ε) λ_{max}: 225(4.180); CD (MeOH): $[\theta]_{307.1}$ –63.53, $[\theta]_{267.7}$ +15.90, $[\theta]_{254.0}$ +2.17 ;^{1}H and ^{13}C NMR spectrum: see *Table 2*; EIMS *m/z* (rel.int.,%) : 466 (45) [M^+] , 451 (4), 395 (4), 329 (7), 298 (8), 195 (8), 151 (98), 94 (100). HREIMS *m/z* 466.2370 [M^+](calcd for $C_{28}H_{34}O_6$ 466.2355), 298.1632 ($C_{19}H_{22}O_3$; M^+-side chain), 150.0664 ($C_9H_{10}O_2$), 95.0418 (C_6H_7O).

Table 1: ^{1}H and ^{13}C NMR spectral data of **1** ($CDCl_3$)

Position	δ_C	δ_H
1	204.6	
2	127.0	5.87 (ddd, 10.0, 2.5, 1.2)
3	145.4	6.74 (ddd, 10.0, 5.0, 2.5)
4a	33.5	3.25 (dddd, 21.0, 5.0, 2.5, 2.5)
4b		2.80 (dddd, 21.0, 2.5, 2.5, 1.2)
5	136.0	-
6	124.6	5.50 (dq, 5.9, 2.5)
7a	30.3	2.07 (m)
7b		1.90 (m)
8	34.7	1.60 (m)
9	36.9	2.10 (m)
10	50.0	-
11	75.0	4.16 (dd, 11.5, 11.5)
12	76.0	4.35 (d, 11.5)
13	46.4	-
14	55.6	N.A
15	23.5	1.73 (m)
16	25.8	1.77 (m)
17	47.9	1.68 (m)
18	13.0	0.71 (s)
19	18.8	1.31 (s)
20	40.1	1.74 (m)
21a	60.6	3.95 (d, 13.5)
21b		3.70 (dd, 13.5, 3.0)
22	74.5	4.60 (br s)
23a	33.0	1.92 (dd, 14.0, 3.0)
23b	-	2.15 (dd, 14.0, 2.0)
24	70.1	-
25	139.0	-
26	165.3	-
27a	130.0	6.75 (br s)
27b		5.99 (d, 0.7)
28	25.5	1.42

Chemical shifts are in (ppm) and J values (in Hz) are presented in parenthesis
N.A: Not assigned due to overlapped signals.

Table 2. ^{1}H-, ^{13}C-, NMR Spectral Data of **2** (CDCl$_3$)

Position	δ_C	δ_H
1	203	-
2	129.2	6.00 (ddd, 10.0, 2.5, 1.2)
3	144.0	6.85 (ddd, 10.0, 5.0, 2.5)
4a	33.1	2.43 (m)
4b	-	2.31 (m)
5	62.0	
6	63.2	3.11 (br d, 2.3)
7	31.0	1.24, 2.05 (m)
8	34.7	1.10 (m)
9	36.9	1.30 (m)
10	51.5	
11	23.5	1.51 (m)
12a	26.5	1.84 (m)
12b	-	1.26 (m)
13	47.2	-
14	63.5	-
15	58.4	3.41 (d, 2.4)
16a	38.7	2.12(m)
16b	-	1.10 (m)
17	48.3	1.28 (m)
18	11.5	0.99 (s)
19	14.7	1.32 (s)
20	40.0	1.85 (m)
21a	60.6	3.87 (d, 13.2)
21b		3.72 (dd, 13.2, 2.7)
22	75.6	4.62 (br s)
23a	33.4	1.84 (dd, 14.0, 3.0)
23b		2.14 (dd, 14.0, 2.0)
24	69.4	-
25	139.0	-
26	166.0	-
27a	129.0	6.76 (br s)
27b		5.98 (d, 0.8)
28	26.5	1.41 (s)

Chemical shifts are in (ppm) and J values (in Hz) are presented in parenthesis

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