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## WITHANOLIDES FROM *DATURA INNOXIA*

Bina S. Siddiqui,\* Shamsul Arfeen, Farhana Afshan, and Sabira Begum

International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi,

Karachi-75270 Pakistan

Tel: Off: (92-21) 9243199 Fax: (92-21) 9243191

E-mail: bina@khi.comsats.net.pk

**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  $11\alpha$ ,  $12\beta$ -dihydroxy ( $20R$ ,  $22R$ )-21,24-epoxy-1-oxowitha-2, 5, 25(27)-trien-22, 26-olide (**1**) and ( $20R$ ,  $22R$ )- $5\beta$ ,  $6\beta$ - $14\alpha$ ,  $15\alpha$ -21,24-triepoxy-1-oxowitha-2,25(27)-dien-22,26-olide (**2**) respectively by analysis of spectroscopic data.

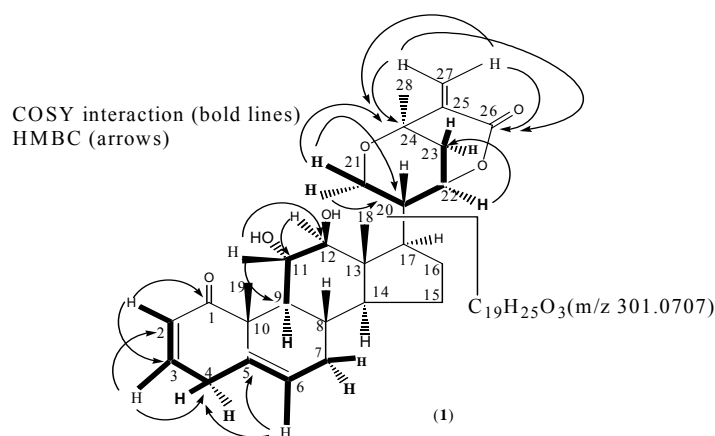
## INTRODUCTION

*Datura innoxia* belongs to the family Solanaceae which is known to contain various tropane alkaloids (hyoscyamine, hyoscyne and atropine)<sup>1,2</sup> and withanolides, a group of C-28 steroids having  $\delta$ -lactone in bicyclic side chain related to ergostane nucleus.<sup>3</sup> Several withanolides have displayed various types of biological activity, such as cytotoxic,<sup>4</sup> anticancer,<sup>5</sup> immunosuppressive,<sup>6</sup> anti-inflammatory<sup>7</sup> and hepatoprotective<sup>8</sup> 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  $11\alpha$ ,  $12\beta$ -dihydroxy- ( $20R$ ,  $22R$ )-21,24-epoxy-1-oxowitha-2, 5, 25 (27)-trien-22, 26-olide (**1**) and ( $20R$ ,  $22R$ )- $5\beta$ ,  $6\beta$ -  $14\alpha$ ,  $15\alpha$  - 21, 24-triepoxy-1-oxowitha-2, 25(27)-dien-22, 26-olide (**2**) respectively based on spectral studies.

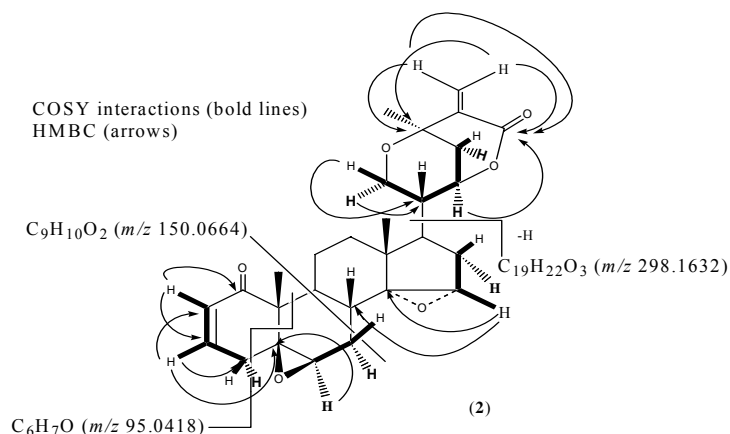
## RESULTS AND DISCUSSION

The molecular ion peak [ $M^+$ ] of witharifeen (**1**) at  $m/z$  468 in the EI-MS spectrum and  $m/z$  468.2492 in the HR-EI-MS spectrum corresponded to the molecular formula  $C_{28}H_{36}O_6$ . The UV ( $\lambda_{max}$  225 nm) and IR ( $\nu_{max}$  1610, 1680, 1720  $cm^{-1}$ ) spectra indicated  $\alpha,\beta$ -enone and  $\alpha,\beta$ -unsaturated  $\delta$ -lactone moieties generally encountered in a typical withanolide.<sup>9</sup> The IR spectrum further showed absorption for OH group at 3400  $cm^{-1}$ . In the upfield region of the  $^1H$ -NMR spectrum of **1** signals related to three methyl groups were observed as singlets at  $\delta$  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  $^1\text{H}$  NMR spectrum, two as doublet of double doublet of one proton each at  $\delta$  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  $\delta$  5.50 ( $J = 5.9, 2.5, 2.5, 2.5$  Hz, H-6), one as a br. singlet at  $\delta$  6.75 (H -27a) and remaining one as a very narrow doublet with fine splitting at  $\delta$  5.99 ( $J = 0.7$  Hz, d H-27b). Two multiplets (dddd) of one proton each resonated at  $\delta$  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  $\delta$  3.70 ( $J = 13.5, 3.0$  Hz, H-21b) and a doublet at  $\delta$  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  $\delta$  4.60. All the  $^1\text{H}$  and  $^{13}\text{C}$  NMR shifts could be conclusively assigned through COSY  $45^\circ$  as well as HMQC and HMBC experiments. The data recorded so far were in agreement with the published data of  $\Delta^5$ -withanolides with 21, 24-epoxy-1-oxowitha-2, 5, 25(27)-triene system.<sup>10-12</sup> 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  $\alpha,\beta$ -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  $\text{C}_{19}\text{H}_{25}\text{O}_3$  for the steroidal nucleus further supported the side chain comprising of  $\text{C}_9\text{H}_{11}\text{O}_3$ . These structural features left two oxygens to be justified and these were taken as two hydroxyl groups (IR  $\nu_{\text{max}}$  3400  $\text{cm}^{-1}$ ) at C-11 and C-12 as the  $^1\text{H}$ -NMR spectrum showed a double doublet at  $\delta$  4.16 ( $J = 11.5, 11.5$  Hz, H-11 $\beta$ ) and a doublet at  $\delta$  4.35 ( $J = 11.5$  Hz, H-12 $\alpha$ ). 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  $\delta$  76.0 (C-11) and 75.0 (C-12). Moreover C-12 also showed interaction with H-18 in the H y MBC plot. Stereochemistry of these hydroxyl groups was supported by NOESY interactions between H-11 $\beta$ , H-18 $\beta$  and H-19 $\beta$  and H-12 $\alpha$  and H-17 $\alpha$ . In light of these observations, the structure of **1** has been elucidated as 11 $\alpha$ , 12 $\beta$ -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).



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  $^1H$ -NMR spectrum which showed a doublet at  $\delta$  3.11 ( $J = 2.3$  Hz, H-6 $\alpha$ ) having a correlation with a carbon at  $\delta$  63.2 (C-6) in the HMQC spectrum and a signal as a doublet ( $J = 2.4$  Hz) at  $\delta$  3.41 (H-15 $\beta$ ) correlated with a carbon at  $\delta$  58.4 (C-15) in the HMQC spectrum and with carbons at  $\delta$  63.5 (C-14) and  $\delta$  34.7 (C-8) in the HMBC spectrum. The broad band  $^{13}C$ -NMR spectrum (Table 2) also had signals at  $\delta$  62.0 (C-5) and 63.5 (C-14). These values are comparable with the reported values in compounds with similar partial structures.<sup>13-16</sup> 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  $^1H$ -NMR spectrum of **2** signals related to four vinylic protons were observed, two as doublet of doublets at  $\delta$  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  $\delta$  6.76 (H-27a) and one as a narrow doublet at  $\delta$  5.98 ( $J = 0.8$  Hz, H-27b). The protons H-21a, H-21b and H-22 resonated at  $\delta$  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  $\delta$  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.<sup>3</sup> The latter observation provided further evidence in favor of 5 $\beta$ ,6 $\beta$  epoxide. Thus the structure of **2** has been elucidated as (20R, 22R)-5 $\beta$ , 6 $\beta$ -14 $\alpha$ ,15 $\alpha$ -21,24-triepoxy-1-oxowitha-2, 25(27)-dien-22, 26-olide (**2**).



## EXPERIMENTAL

**General** Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. UV and 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; EI, source at 250°C and 70 eV,  $m/z$  (rel.%).  $^1\text{H-NMR}$ , COSY, NOESY and  $J$ -resolved were recorded on Bruker spectrometer at 300 MHz; chemical shifts ( $\delta$ ) are in ppm relative to  $\text{SiMe}_4$  as internal standard, coupling constants ( $J$ ) in Hz and  $^{13}\text{C-NMR}$  on Bruker spectrometer, operating at HR 75 MHz. For TLC, preparative TLC and vacuum liquid chromatography (VLC) silica gel PF<sub>254</sub> (Merck) was used. Optical rotations: JASCO-DIP-360 digital polarimeter; CD spectra: JASCO-J-600 spectropolarimeter;  $\lambda$  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  $\text{Na}_2\text{CO}_3$  to separate the acidic and the neutral fractions. The EtOAc phase consisting of neutral fraction was washed with water, dried (anhyd  $\text{Na}_2\text{SO}_4$ ), 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  $\text{CHCl}_3$ -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  $\text{CHCl}_3$ -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  $\text{CHCl}_3$ -MeOH (9.5:0.5) was subjected to thick layer chromatography over silica gel with solvent system  $\text{CHCl}_3$  - 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 ( $\text{CHCl}_3$ -MeOH (9.6:0.4)) afforded compound (**2**) (12mg).

**Witharifeen (1)**: Colourless plates; mp 166-167°C (uncorrected);  $[\alpha]_{\text{D}}^{25}$  -109° ( $c$  0.09,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$ : 3400, 2920, 1720, 1680, 1610  $\text{cm}^{-1}$ ; UV(MeOH, nm, log  $\epsilon$ )  $\lambda_{\text{max}}$ : 225(4.200); CD (MeOH):  $[\theta]_{308.4}$  -34.74,  $[\theta]_{271.9}$  +8.60,  $[\theta]_{259.2}$  +5.66;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data: see Table 1; EIMS  $m/z$  (rel.int.,%) : 468 (100)  $[\text{M}^+]$ , 450 (20.0)  $[\text{M}^+-18]$ , 424 (20.3)  $[\text{M}^+-44]$ , 301(20), 285 (32.0), 241 (30), 167 (16.5), 94 (21.6), 83 (99.5). HREIMS  $m/z$ : 468.2492  $[\text{M}^+]$ (calcd for  $\text{C}_{28}\text{H}_{36}\text{O}_6$  468.2511), 301.0707 ( $\text{C}_{19}\text{H}_{25}\text{O}_3$ ), 167.1807 ( $\text{C}_9\text{H}_{11}\text{O}_3$ ).

**Daturalicin(2)**: Fine colorless needles; mp 140-141 °C (uncorrected);  $[\alpha]_D^{25}$  -26.7° (*c* 0.14, CHCl<sub>3</sub>). IR (CHCl<sub>3</sub>)  $\nu_{\max}$ : 2850, 1720, 1680 cm<sup>-1</sup>; UV(MeOH, nm, log  $\epsilon$ )  $\lambda_{\max}$ : 225(4.180); CD (MeOH):  $[\theta]_{307.1}$  -63.53,  $[\theta]_{267.7}$  +15.90,  $[\theta]_{254.0}$  +2.17; <sup>1</sup>H and <sup>13</sup>C NMR spectrum: see Table 2; EIMS *m/z* (rel.int.,%) : 466 (45) [M<sup>+</sup>], 451 (4), 395 (4), 329 (7), 298 (8), 195 (8), 151 (98), 94 (100). HREIMS *m/z* 466.2370 [M<sup>+</sup>](calcd for C<sub>28</sub>H<sub>34</sub>O<sub>6</sub> 466.2355), 298.1632 (C<sub>19</sub>H<sub>22</sub>O<sub>3</sub>; M<sup>+</sup>-side chain), 150.0664 (C<sub>9</sub>H<sub>10</sub>O<sub>2</sub>), 95.0418 (C<sub>6</sub>H<sub>7</sub>O).

Table 1: <sup>1</sup>H and <sup>13</sup>C NMR spectral data of **1** (CDCl<sub>3</sub>)

Position	$\delta_C$	$\delta_H$
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.  $^1\text{H}$ -,  $^{13}\text{C}$ -, NMR Spectral Data of **2** ( $\text{CDCl}_3$ )

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$
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

## REFERENCES

1. B. N. Sastri, 'The Wealth of India', Council of Scientific and Industrial Research, New Delhi, India. 1953, Vol. III, pp. 14-16.
2. R. L. Clarke, 'The Alkaloids', ed by R. H. Manske, Academic Press, New York, 1977, Vol. 16, pp. 84-180.

3. M. A. F. Jahromi, M. Manickam, M. Gupta, Y. Oshima, S. Hatakeyana, and A. B. Ray, *J. Chem. Res. (S)*, 1993, 234. (and the references cited therein).
4. S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch, R. M. Smith, J. Saenz-Renold, H. Schnoes, A. L. Burlingame, and D. H. Smith, *J. Org. Chem.*, 1969, **34**, 3858.
5. M. Suffness and J. Douros, *J. Nat. Prod.*, 1981, **45**, 1.
6. J. G. Luis, F. Echeverri, and A. G. Gonzalez, *Phytochemistry*, 1994, **36**, 769.
7. R. D. Budhiraja, S. Sudhir, and K. N. Garg, *Planta Medica*, 1984, **50**, 134.
8. S. Sudhir, R. D. Budhiraja, G. P. Miglani, B. Arora, L. C. Gupta, and K. N. Garg, *Planta Medica*, 1986, **52**, 61.
9. W. Frolow, A. B. Ray, M. Sahai, E. Glottlieb, and I Kirson, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1029.
10. N. Sultana, S. S. Ahmad, and S. I. Haider, *Phytochemistry*, 1987, **26**, 2641.
11. T. Mahmood, S. S. Ahmad, and S. Siddiqui, *Heterocycles*, 1988, **22**, 1013.
12. B. S. Siddiqui, S. Arfeen, and S. Begum, *Aust. J. Chem.* 1999, **52**, 905.
13. M. I. Choudhary, S. Abbas, S. A. Jamal, and Atta-ur-Rahman, *Heterocycles*, 1996, **42**, 555.
14. F. W. Wehrli and T. I. Nishida, in 'Progress in the Chemistry of Organic Natural Products', ed. by W. Herz, H. Grisebach, and G. W. Kirby, Wien-Springer-Verlag, New York, 1979, Vol. 36, pp. 104-109.
15. N. D. Laurence, D. S. Satyajit, and S. Vladimir, *Phytochemistry*, 1997, **44**, 509.
16. G. Mohini, M. Manickam, C. S. Subhash, A. S. Bagchi, and A. B. Ray, *Phytochemistry*, 1992, **31**, 2423.