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Bio-active cardenolides from the leaves of *Nerium oleander*

Sabira Begum [a], Bina S. Siddiqui [a,*], Razia Sultana [a], Atiya Zia [a], Amin Suria [b]

[a] *H. E. J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan*
[b] *Department of Pharmacology, Faculty of Health Sciences, The Aga Khan University, Karachi 74800, Pakistan*

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Abstract

A bioactivity directed isolation of the methanolic extract of the fresh, uncrushed leaves of *Nerium oleander* showing a central nervous system (CNS) depressant effect in mice has been undertaken. As a result, four CNS depressant cardenolides including a new cardenolide, neridiginoside and three known constituents, nerizoside, neritaloside and odoroside-H, have been isolated which exhibited CNS depressant activity in mice at a dose of 25 mg/kg. The structure of neridiginoside was elucidated as 3β-*O*-(D-diginosyl)-5β,14β-dihydroxy-card-20(22)-enolide, using spectroscopic methods including one-dimensional and two-dimensional NMR (COSY-45, NOESY, *J*-resolved, HMQC and HMBC). The known compounds have been indentified through spectral studies and comparison of data with those reported in the literature.

Keywords: *Nerium oleander*; Apocynaceae; Cardenolide; Neridiginoside; CNS activity

1. Introduction

Nerium oleander Linn (Syn. *N. odorum* Soland; *N. indicum* Mill), distributed in the Mediterranian region and sub-tropical Asia, is indigenous to the Indo–Pakistan subcontinent. The plant is commonly known as 'kaner' and its various parts are reputed as therapeutic agents in the treatment of swellings, leprosy, eye and skin diseases. The leaves possess cardiotonic, antibacterial, anticancer and antiplatelet aggregation activity, and depress the central nervous system (Nasir & Ali, 1982; Dymock, Warden & Hooper, 1891; Chopra, Nayar & Chopra, 1956; Manjunath, 1966; Nadkarni, 1976; Zia, Siddiqui, Begum & Suria, 1995; Farnaz, 1996). Investigations on different parts of the plant have revealed the presence of several glycosides, triterpenes and straight chain compounds (Siddiqui, Begum, Siddiqui & Lichter, 1995; Siddiqui, Hafeez & Siddiqui, 1986; Siddiqui, Begum, Hafeez & Siddiqui, 1987; Siddiqui, Siddiqui, Begum & Hafeez, 1990; Begum, Sultana & Siddiqui, 1997; Siddiqui, Sultana, Begum, Zia & Suria, 1997; Yamauchi, Yujiro & Yasuko, 1973). This paper deals with the activity directed isolation of the methanolic leaves extract, showing CNS depressant effect in mice, which furnished four bioactive cardenolides, including a new constituent, neridiginoside, and three known constituents, nerizoside (Siddiqui et al., 1997), neritaloside (Cabrera, Deluca, Seflides, Gros, Oberti, Crocket et al., 1993; Jager, Schlindler & Reichstein, 1959) and odoroside-H (Cabrera et al., 1993; Rittel, Hunger & Reichstein, 1953), all of them showing sedation in mice in a dose-dependant manner. The structural studies are based on ^{1}H and 2D NMR experiments (COSY-45, NOESY, *J*-resolved, HMQC and HMBC) and comparison of data of known compounds with those reported in the literature. The structure of neridiginoside was elucidated as 3β-*O*-(D-diginosyl)-5β,14β-dihydroxy-card-20(22)-enolide.

2. Results and discussion

Neridiginoside gave a positive test for cardenolides (Legal and Raymond test) (Fieser & Fieser, 1959). Its molecular formula, $C_{30}H_{46}O_8$, was obtained through a combined application of FAB +ve, EI, HRMS, ^{1}H NMR and ^{13}C NMR. Its UV spectrum showed a maximum at 219 nm, indicating the presence of an α,β-unsaturated γ-lactone (Yamaguchi, 1970), while

* Corresponding author. Tel.: +00-91-21-4968497-8; Fax: +00-92-4963373; E-Mail: hej@biruni.erum.com.pk.

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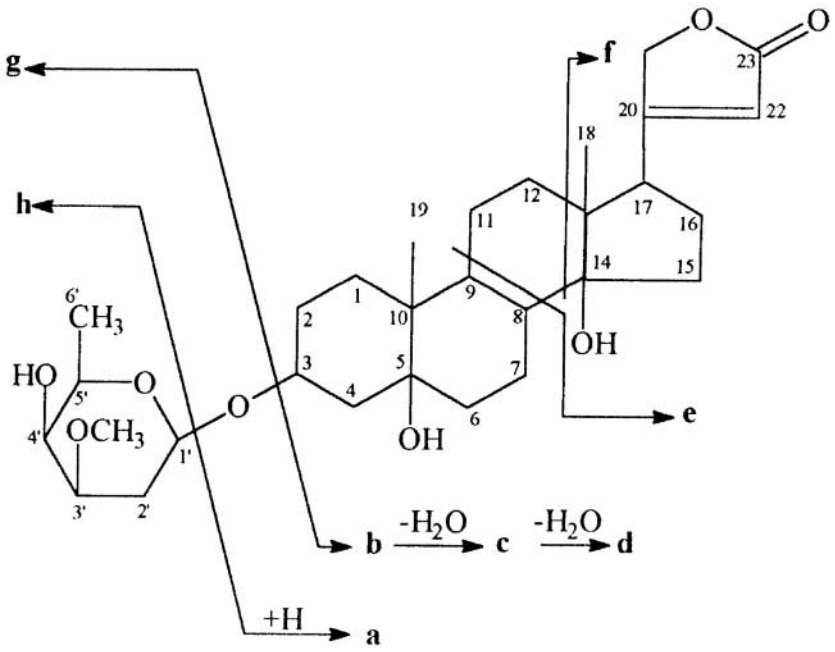

the IR spectrum showed characteristic bands at 3450 (OH), and 1780, 1740 (α,β-unsaturated γ-lactone) cm^{-1}. The ^1H NMR spectrum showed two double doublets of one-proton each at δ 4.98 and 4.80 for H-21a (J = 18.0, 1.5 Hz) and H-21b (J = 18.0, 1.5 Hz), and a one-proton triplet at δ 5.85 (J = 1.5 Hz) for H-22. Two singlets at δ 1.00 and 1.05 were attributed to H-18 and H-19, respectively, and a one-proton double doublet at δ 2.74 to H-17 (J = 9.5, 6.0 Hz). A broad singlet at δ 4.05 ($W_{1/2}$ = 7.0 Hz) was assigned to the equatorial carbinylic proton at C-3. Thus the eight double bond equivalents exhibited by the molecular formula were satisfied by the four rings of the steroidal skeleton, the α,β-unsaturated lactone ring and the ring of the sugar moiety, which was indicated by the NMR spectra (Table 1) as follows.

The ^1H NMR and ^{13}C NMR data demonstrated one molecule of sugar by an anomeric proton at δ 4.44 for H-1′ (*dd*, J = 9.5, 2.0 Hz) connected to the anomeric carbon at δ 97.8 in the HMQC spectrum. The chemical shift and coupling constants of H-1′ suggested β-linkage of the sugar. The connectivity of H-1′ with $H-2_{ax}'$ and $H-2_{eq}'$, both the $H-2_{ax}'$ and $H-2_{eq}'$ with H-1′ and H-3′, of H-3′ with $H-2_{ax}'$, $H-2_{eq}'$ and H-4′, of H-4′ with H-3′ and H-5′ and of H-5′ with H-4′ and H-6′ in the COSY-45 spectrum led us to assign all the protons of the sugar moiety. The relationship of various protons was also confirmed by proton decoupling experiments. Their multiplicities and coupling constants were attained from the normal ^1H NMR spectrum and 2D *J*-resolved. Thus, $H-2_{ax}'$ showed a doublet of double doublet at δ 1.68 (J = 12.5, 12.0, 9.5), H-2′ eq showed a doublet of double doublet at δ 1.94 (J = 12.5, 5.0, 2.0 Hz), H-3′ appeared as a one-proton doublet of double doublet at δ 3.33 (J = 12.0, 5.0, 3.5 Hz), H-4′ resonated as a one-proton broad triplet at δ 3.67 (J = 3.5 Hz) whereas H-5′ appeared as a doublet of quartet at δ 3.40 (J = 6.5, 3.5 Hz). H-6′ resonated as a three-proton doublet at δ 1.32 (J = 6.5 Hz) while OCH_3 protons resonated at δ 3.38 as a singlet. All these observations established that the sugar was β-D-diginose. The functionalities so far exemplified by the spectral data justified six oxygens out of eight of the molecular formula. The remaining two oxygens were accounted for by two hydroxyl groups located on quaternary carbinylic carbons since the ^{13}C NMR data showed two quaternary carbinylic carbons at δ 74.5 and 85.9. They were assigned to C-5 and C-14, respectively, through comparision with the data of compounds with similar substitutions reported in the literature (Reynolds, Maxwell, Telang, Bedaisie & Ramcharan, 1995). The ^{13}C NMR shifts of ring A and B carbons further suggested the β-orientation of the hydroxyl group at C-5, i.e., a *cis* A/B ring junction (Reynolds et al., 1995; Wehrli & Nishida, 1979). The positions of the hydroxyl groups were further confirmed by the significant mass fragments (*vide* structure). The carbon chemical shifts were conclusively assigned on the basis of ^{13}C NMR (broad band and DEPT) and HMQC spectra and comparison with the data of similar compounds (Reynolds et al., 1995; Wehrli & Nishida, 1979). Thus, this compound was assigned the structure as 3β-*O*-(D-diginosyl)-5β,14β-dihydroxy-card-20(22)-enolide. It may be noted that the aglycone has been reported earlier as periplogenin (Lehmann, 1897; Jacobs & Hoffmann, 1928). Neridiginoside, as well as nerizoside, odoroside-H and

Table 1
^{1}H NMR and ^{13}C NMR data of neridiginoside[a]

C	δ_C	H	δ_H	Multiplicity	J (Hz)
1	26.89	1a	1.82	*m*	–
		1b	1.42	*m*	–
2	27.01	2a	1.61	*m*	–
		2b	1.51	*m*	–
3	72.20	3	4.05	*br s*	$W_{1/2}=7.0$
4	35.03	4a	2.15	*dd*	10.0,4.5
		4b	1.60	*dd*	10.0,5.0
5	74.50	5	–	–	–
6	32.04	6a	1.95	*m*	–
		6b	1.70	*m*	–
7	17.60	7a	1.51	*m*	–
		7b	1.38	*m*	–
8	36.94	8	1.79	*m*	–
9	35.54	9	1.76	*m*	–
10	35.19	10	–	–	–
11	22.47	11a	2.10	*m*	–
		11b	1.10	*dddd*	14.0,3.0,3.0,3.0
12	40.38	12a	1.57	*ddd*	11.5,4.5,3.0
		12b	1.38	*ddd*	11.5,11.0,4.5
13	50.42	13	–	–	–
14	85.90	14	–	–	–
15	29.86	15a	1.62	*ddd*	14.5, 6.5, 3.0
		15b	1.48	*m*	–
16	27.38	16a	2.15	*m*	–
		16b	1.74	*m*	–
17	51.84	17	2.74	*dd*	9.5, 6.0
18	18.42	18	1.00	*s*	–
19	25.36	19	1.05	*s*	–
20	174.30	20	–	–	–
21	73.37	21a	4.98	*dd*	18.0, 1.5
		21b	4.80	*dd*	18.0, 1.5
22	117.90	22	5.85	*t*	1.5
23	174.20	23	–	–	–
1′	97.80	1′	4.44	*dd*	9.5, 2.0
2′	31.71	2′	1.94	*ddd*	12.5, 5.0,.2.0
			1.68	*ddd*	12.5, 12.0, 9.5
3′	78.03	3′	3.33	*ddd*	12.0, 5.0, 3.5
4′	67.19	4′	3.67	*br t*	3.5
5′	70.40	5′	3.40	*dq*	6.5, 3.5
6′	16.80	6′	1.32	*d*	6.5
$-OCH_3$	55.73	–	3.38	*s*	–

[a] The assignments are based on COSY-45, *J*-resolved, HMQC and HMBC spectra.

neritaloside, showed sedation in mice at 25 mg/kg dose.

3. Experimental

3.1. General

Mps were determined on a Gallenkamp melting-point apparatus and are uncorr. UV and IR spectra were recorded on Hitachi-u-3200 and Jasco A-302 spectrometers, respectively. The EI, FAB and HRMS were recorded on Finnigan MAT-112, MAT-312 and JMS HX-110 spectrometers, respectively. The ^{1}H NMR spectra was taken in $CDCl_3$ on a Bruker AM-500 FT–NMR spectrometer operating at 500 MHz, while the ^{13}C NMR (broad band and DEPT) spectra were obtained in $CDCl_3$ on the same instrument operating at 125 MHz. The spectra were referenced to the residual solvent signals. The chemical shifts are in ppm (δ) and coupling constants (J) are in Hz. The ^{13}C NMR (Table 1) spectral assignment was made partly through DEPT, HMQC and HMBC and partly through a comparison of the chemical shifts with the published data for similar compounds (Cabrera et al., 1993; Reynolds et al., 1995; Wehrli & Nishida, 1979). Assignments of protons were based on COSY-45, decoupling and NOESY experiments. The purity of compounds was monitored on TLC with silica gel PF_{254}: mobile phase: $CHCl_3$–MeOH (9.80:0.20). Silica gel E. Merck 9385 was used for flash CC (Model Eyela).The leaves of *Nerium oleander* were collected from the Karachi region. The plant was authenticated by Prof. Dr. Syed Irtifaq Ali of the Department of Botany, University of Karachi, and a voucher specimen (N.ol-1) was deposited in the Herbarium of the same department.

3.2. Biological activity

Mice of NMRI strain weighing between 18–22 g were used. They were maintained under standard colony conditions in our animal house. The volume of injection was 10 ml/kg body weight. The samples were dissolved in 5% tween 80 and given intraperitoneally. The animals were observed continuously for half an hour and then at every 30 min for 6 hours. The behavior was scored according to the modified procedure as described by Irwin (1962).

3.3. Extraction and isolation

Fresh and uncrushed leaves (40 kg) were extracted with MeOH at room temp. The concentrated syrupy residue obtained on removal of the solvent from the combined extracts under red. pres. showed CNS depressant effects in mice. It was shaken out with EtOAc and H_2O. The EtOAc layer was extracted with 4% aq. Na_2CO_3 sol to separate the acidic from the neutral fraction. The EtOAc layer containing the neutral fraction was washed, dried over anhydrous Na_2SO_4 and charcoaled. The charcoal bed was washed successively with EtOAc and MeOH–C_6H_6 (1:1). The solvent from the combined EtOAc filtrate and washings was evaporated and the fraction was marked as N-1. The residue from MeOH–C_6H_6 eluate was marked as N-2. Both these fractions were tested for their effects on the central nervous system (CNS). N-1 was found to be active and showed a CNS depressant activity at a dose of 50 mg/kg, whereas N-2 was inactive at this dose. N-1

was divided into petrol soluble (NPE) and insoluble (NPEI) fractions. NPE was inactive up to 50 mg/kg and was not pursued further in the present studies. The active petroleum ether insoluble fraction was dissolved in a minimum quantity of MeOH and kept cold overnight. A white crystalline residue settled down which was filtered and the filtrate was again kept for crystallization. Several crystalline crops thus obtained were combined and recrystallized from the same solvent. The colourless flowers of needles ultimately obtained were identified as ursolic acid through comparison of its spectral data with those reported in the literature (Yamaguchi, 1970; Seo, Tomita & Tori, 1975). The residue from the filtrate of ursolic acid was marked as B-1. Both ursolic acid and B-1 were tested for their activity on CNS. B-1 being the active fraction was again treated with petrol to give petrol soluble (B-3) and insoluble (B-2) fractions. These were again tested for their activity on CNS. B-2 showed CNS depressant activity with convulsions while B-3 exhibited depressant activity with hypnosis. It was subjected to further separation using VLC (petrol–EtOAc, followed by $CHCl_3$–MeOH, in order of increasing polarity). On combining the eluates on the basis of TLC, 14 fractions (Fr-1 to Fr-14) were ultimately obtained. The main fraction (Fr-2) possessing significant sedative hypnotic activity was subjected to further purification through VLC (petroleum ether–EtOAc, in increasing order of polarity) which afforded 20 fractions (NO-1 to NO-20) on combining the eluates on the basis of TLC. NO-15, NO-17 and NO-18 were pure constituents and were subjected for determination of their effect on CNS of mice. Their structures were determined as 3β-*O*-(D-2-*O*-methyl digitalosyl)-14β-hydroxy-5β-carda 16,20 (22)-dienolide (nerizoside) (Siddiqui et al., 1997), 3β-*O*-(D-digitalogyl)-14β-hydroxy, 16β-acetoxy-5β-carda-20(22)-enolide (neritaloside) (Cabrera et al., 1993; Jager et al., 1959) and 3β-*O*-(D-digitalosyl)-14β-hydroxy-5β-carda-20 (22)-enolide (odoroside-H) (Cabrera et al., 1993; Rittel et al., 1953), respectively. NO-14 on crystallization with MeOH afforded neridiginoside as fine needles and was characterized as 3β-*O*-(D-diginosyl)-5β,14β-dihydroxy-card-20(22)-enolide.

3.4. Neridiginoside

Fine needles (MeOH) (75 mg), mp. 195.8–196.5; $[\alpha]_D^{26}$ 38.31 (*c*, 0.522 $CHCl_3$) HR–MS *m*/*z*: 390.2410 ($C_{23}H_{34}O_5$, fragment *a*), 373.2318 ($C_{23}H_{33}O_4$, fragment *b*), 355.2252 ($C_{23}H_{31}O_3$, fragment *c*), 337.2108 ($C_{23}H_{29}O_2$, fragment *d*), 208.1161 ($C_{12}H_{16}O_3$, fragment *e*), 181.0882 ($C_{10}H_{13}O_3$, fragment *f*), 161.0850 ($C_7H_{13}O_4$, fragment *g*) and 145.0901 ($C_7H_{13}O_3$, fragment *h*). UV λ_{max}^{MeOH} nm: 219. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780 and 1740 cm^{-1}. ^1H NMR and ^{13}C NMR: Table 1.

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