Phytochemistry 71 (2010) 2205–2209

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Chlorinated and diepoxy withanolides from *Withania somnifera* and their cytotoxic effects against human lung cancer cell line

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ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 16 August 2010
Available online 31 October 2010

Keywords:
Withania somnifera
Withanolides
Withaferin A
Cytotoxicity
Lung cancer cell line (NCI-H460)

ABSTRACT

Phytochemical studies on the aerial parts of *Withania somnifera* L. Dunal. (Solanaceae) led to the isolation of a chlorinated steroidal lactone (27-acetoxy-4β,6α-dihydroxy-5β-chloro-1-oxowitha-2,24-dienolide), a diepoxy withanolide (5β,6β,14α,15α-diepoxy-4β,27-dihydroxy-1-oxowitha-2,24-dienolide), and withaferin A. Their structures were elucidated by using spectroscopic techniques. All three compounds exhibited a growth inhibition and cytotoxic activity against human lung cancer cell line (NCI-H460), with withaferin A being the most potent (GI_{50} = 0.18 μg/mL and LC_{50} = 0.45 μg/mL) among three compounds tested.

1. Introduction

Withanolides are a group of steroidal lactones, isolated from various plants of the family Solanaceae, including *Withania somnifera*, distributed in the South Asian sub-continent (Said, 1970). In Pakistan, *W. somnifera* is used traditionally for the treatment of gout, and lumbago. It is also used as anti-bronchial and anti-asthmatic by local people (Said, 1970). In Ayurveda, *Withania* has been claimed to posses potent aphrodisiac, sedative, rejuvenative, and life prolonging properties (Nadkarni, 1976; Williamson, 2002). Other biological activities of extracts of various parts of *W. somnifera* include cholinesterase inhibition of whole plant (Choudhary et al., 2004, 2005), anti-inflammatory *via* COX-2 enzyme inhibition of leaves extract (Jayaprakasam and Nair, 2003), antibacterial activity of extracts of leaves and roots (Owais et al., 2005), and sex hormones deficiency regulation in diabetic rats from roots extract (Kiasalari et al., 2009). Previous chemical investigations on *Withania* have resulted in the isolation, and identification of several withanolides with anti-glycation (Maurya et al., 2008), and anti-pyretic activities (Ali et al., 1997).

Plants have been reputed as basic source of many anti-cancer constituents such as vinblastine, and vincristine (*Catharanthus roseus*), etoposide, and teniposide (*Podophyllum* species), paclitaxel (*Taxus brevifolia*) (Shoeb, 2006), vinorelbine, and docetaxel (semi-synthetic derivative from *Taxus baccata*) (Schmidt and Bastians, 2007), irinotecan (Dholwani et al., 2008), and topotecan (semi-synthetic derivatives from *Camptotheca acuminata*) (Srivastava et al., 2005). Among various cancers, lung cancer is the leading cause of mortality worldwide, contributing to about one million deaths each year (Sun et al., 2007). The standard chemotherapeutic regimens include vinblastine and cisplatin (VP) in combination with mitomycin (MVP), cyclophosphamide, doxorubicin with methotrexate, procarbazine (CAMP) (Bonomi et al., 1989). The other chemotherapeutic combinations include vincristine (CAV) and cisplatin with etoposode (PE) (Fukuoka et al., 1991) or paclitaxel (Schiller et al., 2002). Furthermore, aqueous mistletoe extract, containing vinorelbine, along with cisplatin reported to improve the quality of life in non-small cell lung cancer patients (Piao et al., 2004).

It is well established that various compounds of *Withania* species, such as withaferin A from the leaves (Jayaprakasam et al., 2003), and ashwagandhanolides from the roots of *W. somnifera* (Subbaraju et al., 2006) are known to posses anti-cancer properties. They have been reported to inhibit cell growth of various human cancer cell lines including lung cancer (NCI-H460).

Other species, i.e. *Withania aduensis*, and *Withania riebeckii*, used in Yemini traditional medicines, also exhibited cytotoxicity against lung cancer cell lines (A-427 and LCLC-103H) (Mothana et al., 2007). The mechanism by which withanolides demonstrate antiproliferative, antimetastatic, antiangiogenic, anti-invasive, and proapoptotic activities has been associated with the suppression of NF-κB and NF-κB-regulated gene products (Ichikawa

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doi:10.1016/j.phytochem.2010.08.019

et al., 2006). Furthermore, withanolides, isolated from the stems, leaves, and roots of *Tubocapsicum anomalum*, arrested human lung cancer cells (A549, H358, and H226) at G_1 phase of the cell cycle (Chang et al., 2007).

Recently, compounds other than withanolides, i.e. L-asparaginase isolated from fruits of *W. somnifera* also exhibited a growth inhibitory effect against lymphoblastic leukemia (Oza et al., 2010).

As a part of our on going research on various *Withania* species, several bioactive withanolides have been isolated from *W. somnifera* (Choudhary et al., 2005). In this context, three withanolides (**1**, **2**, and **3**) were isolated and characterized from the methanolic extract of the aerial parts of *W. somnifera*. Considering, the reported cytotoxic potential of withanolides, the present investigation was undertaken to assess the cytotoxicity of aforementioned compounds against human lung cancer cell line (NCI-H460).

2. Results and discussion

The structure of withanolide **1** was deduced largely on the basis of NMR and mass spectrometry (Fig. 1). The FAB-MS displayed a *pseudo*-molecular ion peak at *m*/*z* 549 $[M+H]^+$. The molecular formula $C_{30}H_{41}O_7Cl$ was deduced from HRFAB-MS $[M+H]^+$ (*m*/*z* 549.2655). The presence of chlorine in the compound was deduced by an isotopic peak at *m*/*z* 551 (18%). In the EI-MS, a fragment ion at *m*/*z* 513 indicated the loss of a chlorine in the form of hydrochloric acid. The IR spectrum ($CHCl_3$) displayed bands at 1737, 1710, 1682, and 3478 cm^{-1}, indicating the presence of carbonyl and hydroxyl functionalities.

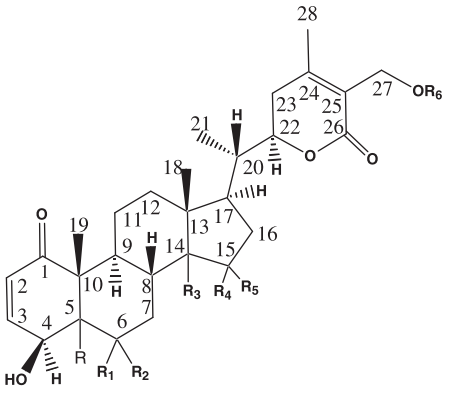
The broad-band decoupled, and DEPT ^{13}C NMR (pyridine, Table 1) spectra displayed 30 carbon signals, including a carbonyl carbon (δ_C 201.5), two olefinic carbons (δ_C 126.5 and 147.0), and an α,β-unsaturated δ-lactone moiety (δ_C 165.2, 122.0, and 157.7). The ^{13}C NMR spectrum showed a downfield signal (δ_C 79.6) due to the electron withdrawing effect of the chlorine at the quaternary C-5. The two oxygenated methine carbon signals at δ_C 64.8, and 65.9 were assigned to C-4, and C-6, respectively. H-4 (δ_H 5.34) showed COSY interactions with H-3 (δ_H 6.82). The allylic methine H-4, geminal to the OH group, resonated at δ_H 5.34 indicated its *pseudo axial* orientation in ring A. The chlorine group at C-5 was proposed to have a β-orientation (Ceccherelli et al., 1991), because of the presence of a strong NOESY interaction between H-4 (δ_H 5.34), and H-9 (δ_H 1.55), while the absence of its NOESY interactions with the C-19 methyl protons (δ_H 1.58) further supported the α-orientation of H-4. In addition to this, the H-9 methine (δ_H 1.55) showed NOESY interaction with H-14 (δ_H 1.25) which supported the proposed stereochemistry at C-9, and C-14, respectively. The C-19 β-methyl protons (δ_H 1.58) showed NOESY interactions with the C-6 methine proton (δ_H 4.69), indicating the β-orientation of H-6 (Fig. 2). The stereochemistry assigned at C-4, and C-6 was also supported by comparison of the spectroscopic data with those reported in the literature (Nittala et al., 1981, compounds **1a**, and **5**). The downfield chemical shifts of the AB doublets for the C-27 methylene protons (δ_H 5.21 and 5.13, $J_{27a,b}$ = 11.6 Hz) supported the presence of an acetyl group at C-27. A multiplet at δ_H 4.42 was assigned to the characteristic methine C-22 proton of the lactone moiety. The stereochemistry at C-20, and C-22 were extensively studied previously (Hsieh et al., 2007, compound **1**).

The two olefinic signals at δ_H 6.20, and 6.82 were due to the C-2, and C-3 vinylic protons, respectively. The three tertiary methyls appeared as singlets at δ_H 0.53, 1.58, and 2.00 (C-18, C-19, and C-28), respectively, while a 3H doublet at δ_H 0.93 ($J_{21,20}$ = 6.5 Hz) was assigned to the C-21 secondary methyl protons. The acetyl methyl carbon was resonated at δ_H 2.02. The presence of hydroxyl substituents at C-4, and C-6 were deduced from the long-range HMBC interactions of H-4 with C-2 (δ_C 126.5), C-3 (δ_C 147.0), and C-5 (δ_C 79.6), and a weak interaction with C-1 (δ_C 201.5). The H-6 (δ_H 4.69) showed HMBC interactions with C-5 (δ_C 79.6), and C-10 (δ_C 58.1), while the H-9 (δ_H 1.55) showed HMBC interactions with C-8 (δ_C 35.3), C-10 (δ_C 58.1), and a weak interaction with C-5 (δ_C 79.6) (Fig. 3). The HMBC interactions of the C-19 methyl protons with the chloro substituted C-5 (δ_C 79.6), and C-9 (δ_C 46.4)

Table 1
^1H and ^{13}C NMR data of compound **1** (C_5D_5N) and **2** ($CDCl_3$).

Carbon No.	**1**		**2**	
	δ ^{13}C (ppm)	δ ^1H [J(Hz)]	δ ^{13}C (ppm)	δ ^1H [J(Hz)]
1	201.5	–	202.3	–
2	126.5	6.20 d (10.2)	132.2	6.18 d (10.2)
3	147.0	6.82 dd (10.1, 3.4)	141.8	6.91 dd (9.9, 4.0)
4	64.8	5.34 br s	69.9	3.67 d (6.0)
5	79.6	–	63.8	–
6	65.9	4.69 dd (12.7, 4.42)	62.6	3.18 br s
7	30.1	2.29, 1.88 m	31.1	1.35, 1.65 m
8	35.3	1.51 m	44.1	1.00 m
9	46.4	1.55m	29.5	2.45m
10	58.1	–	57.5	
11	23.9	1.42 m	27.2	1.38 m, 1.61
12	39.2	1.74 m	24.2	1.89 m, 1.73
13	43.1		47.6	–
14	51.7	1.25 m	60.6	–
15	27.0	1.50 m	56.0	3.31 br s
16	39.7	1.88 m	39.3	1.09 m, 1.92 m
17	54.9	1.25 m	51.9	1.12 m
18	11.7	0.53 s	11.6	0.66 s
19	10.4	1.58 s	17.0	1.37 s
20	39.0	1.88 m	38.7	1.82 m
21	13.3	0.93 d (6.5)	13.3	0.96 d (6.6)
22	78.2	4.42 m	78.7	4.40 m
23	30.1	2.03 m, 2.26	29.3	1.99 m, 2.45 m
24	157.7	–	152.7	–
25	122.0	–	125.6	–
26	165.2	–	167.0	–
27	58.5	5.21 d (11.6), 5.13 d (11.6)	56.8	4.27 d (12.3), 4.24 d (12.8)
28	20.2	2.00 s	20.0	2.01 s
29	170.6			
30	20.6	2.11 s		

Coupling constants (*J* in Hz) in parenthesis.
S, singlet; d, doublet; m, multiplet; dd, double doublet and br s, broad singlet.



1. R = β Cl , R_1= α OH, R_2 = β H, R_3 = α H, R_4 = R_5 = H, R_6 = -$C^{29}OC^{30}H_3$
2. R = R_1 = O, R_2 = α H, R_3 = R_4 = O, R_5 = β H, R_6 = H
3. R = R_1 = O, R_2 = α H, R_3 = α H, R_4 = R_5 = H, R_6 = H

Fig. 1. Structures of compounds **1**, **2**, and **3**.