Phytochemistry Letters 17 (2016) 107–113

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Cytotoxic and anti-inflammatory salicin glycosides from leaves of *Salix acmophylla*

Zafar Ali Shah[a], Attiya Hameed[a], Aqeel Ahmed[b], Shabana U. Simjee[a,b], Almas Jabeen[b], Abid Ullah[c], Farzana Shaheen[a,*]

[a] H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[b] Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] Department of Zoology, University of Peshawar, Peshawar 25120, Pakistan

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 29 June 2016
Accepted 6 July 2016
Available online xxx

Keywords:
Salix acmophylla
Salicin glycosides
NMR spectroscopy
Intracellular reactive oxygen species
Cytotoxicity

ABSTRACT

The species of genus *Salix*, commonly known as Willow, are well known worldwide as rich source of medicinally important salicin derivatives and phenolic glycosides. The current study focuses on *Salix acmophylla* Bioss with the aim of identifying new bioactive constituents of this plant. Two new salicin glycosides, acmophyllin A (**1**), acmophyllin B (**2**) and five reported phenolic glycosides **3–7**, were identified from *S. acmophylla* Bioss. NMR and mass spectroscopic techniques were employed to elucidate the structure of secondary metabolites of *S. acmophylla*. The new salicin glycosides were evaluated against three different cancer cell lines i.e., PSN-1 (pancreatic cancer cells), MCF-7 (breast cancer cells) and NCI-H460 (lung cancer cells). The acmophyllin A (**1**) exhibited cytotoxicity in a dose dependent manner against all three cancer cells (IC_{50} ~35–40 μM). Acmophyllin B (**2**) exhibited mild activity against PSN-1 cells and MCF-7 cancer cells. In addition, compounds **5** and **6** showed potent inhibition of oxidative burst in zymosan activated neutrophils by chemiluminescence technique, while no other compound were found to inhibit the production of reactive oxygen species (ROS).

1. Introduction

The genus *Salix* (Willow) comprises of over 450 species which mainly occur in temperate and cold regions of Northern Hemisphere. Some species of *Salix* are well known herbs for their analgesic and anti-inflammatory activities. They have been used in rheumatic diseases, headache, fever and infections (Vainio and Morgan, 1997; Poblocka-Olech et al., 2010; Gawlik-Dziki et al., 2014; El-Shazly et al., 2012). *Salix* species are reported to contain many secondary metabolites such as terpenoids, flavonoids, phenolic acids, lignans, and salicin (natural aspirin) (El-Sayed et al., 2015; El-Shazly et al., 2012). Salicin derivatives have been found effective in treatment of pain, fever, and inflammation (Kim et al., 2015).

The plant *S. acmophylla* has wide occurrence in the Central Asia, eastern Turkey, Iran, Pakistan and Afghanistan (Iqbal et al., 2004). The methanolic extract of this plant was reported to have strong phytotoxicity. The current investigation on ethyl acetate extract of this plant afforded two new and five known phenolic glycosides from the *S. acmophylla*. The cytotoxicity of new constituents of this plant was evaluated against three different cancer cell lines. The isolated constituents of plants were also evaluated for their anti-inflammatory potential.

2. Results and discussion

The new salicin derivatives acmophyllin A (**1**) and acmophyllin B (**2**) (Fig. 1) were obtained from the purification of crude extract of *S. acmophylla* Boiss as white powder. The molecular formula of **1** was determined from the quasi-molecular ion $[M+Na]^+$ at *m*/*z* 533.1 in the ESI–MS. The molecular formula $C_{27}H_{26}O_{10}Na$ was deduced from the observed molecular ion peak $[M+Na]^+$ in the HRESI–MS at *m*/*z* 533.1409 (Calc. 533.1423 for $C_{27}H_{26}O_{10}Na$) (Supporting information, Fig. S2). This indicated fifteen degrees of unsaturation in compound **1**. Eleven degrees of unsaturation were accounted for eleven double bonds, while, remaining four degrees of unsaturation were due to four rings of compound **1**. and The IR spectrum of **1** showed absorption bands at 3413 cm^{-1} (OH),

* Corresponding author.
E-mail address: afnan.iccs@gmail.com (F. Shaheen).

http://dx.doi.org/10.1016/j.phytol.2016.07.013
1874-3900/

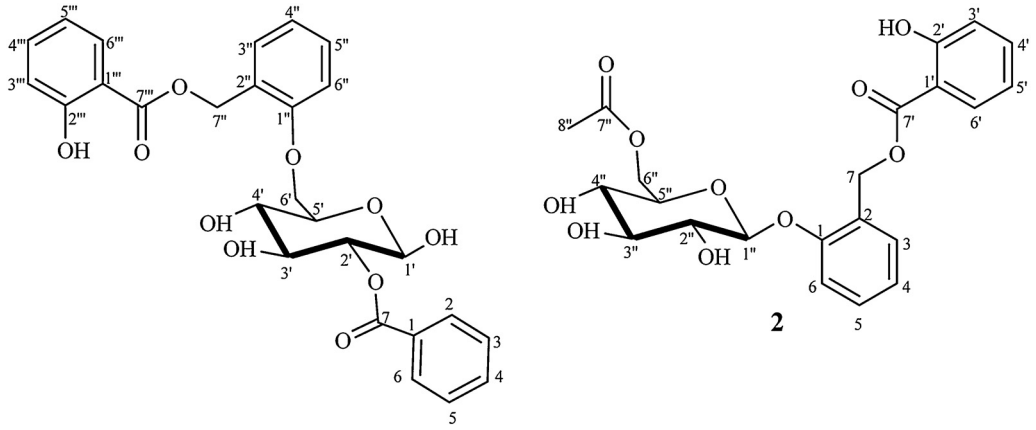

Fig. 1. The Structure of New Compounds **1** and **2**.

2923 cm^{-1} (C—H due to asymmetric stretching), 1398 cm^{-1}, 1616 cm^{-1} (C=C of benzene ring). The UV spectrum exhibited absorption bands at λ max (ε) at 307, 278, 237, 212 nm.

Analysis of ^{1}H NMR spectrum of **1** revealed the signals at δ_H 7.98 (2H, d, J = 7.6 Hz, H-2, 6), 7.31 (2H, m, H-3, 5), and 7.43 (1H, m, H-4) which implied the presence of benzoyl group, while, the signals at δ_H 7.30 (1H, m, H-6″), 7.03 (1H, m, H-3″) and 7.31 (2H, H-4″, 5″) indicated salicylic alcoholic moiety. The remaining signals at δ_H 6.88 (1H, d, J = 8.4 Hz, H-3‴), 7.41 (1H, m, H-4‴), 6.73 (1H, m, H-5‴) and 7.55 (1H, d, J = 8.0 Hz, H-6‴) were assigned to *ortho*-hydroxylbenzoyl group. The ^{13}C NMR spectral analysis of **1** revealed the presence of 27 carbons, including two methylene, eighteen methine and seven quaternary carbons (Table 1).

The structure of new compound **1** was fully supported by 2D-NMR spectral data such as HSQC, HMBC and COSY spectrum. In the HMBC spectrum (Fig. 2), the H-7″methylene protons H_a δ_H 5.20, d, (J = 12.4); H_b = δ_H 5.15, d, (J = 12.4) exhibited correlation with quaternary carbons C-7‴ (δ_C 170.8), C-1″ (δ_C 156.7), C-2″ (δ_C 125.9), which supported the linkage of salicylic alcoholic moiety with *ortho*-hydroxybenzoyl group *via* ester connection. Similarly, the correlation of aromatic proton H-6″ (δ_H 7.30) with C— 6′ (δ_C 62.4) methylene carbon of sugar unit, C-2″ (δ_C 125.9) and C-1″ (δ_C 156.7) supported the linkage of C-6′ position of glucose moiety with salicylic alcoholic group. H-2′ (δ_H 5.28) of sugar unit showed correlation with C-7 carbonyl carbon (δ_C 167.2) of benzoyl group.

In the COSY 45° spectrum (Fig. 2), correlations of H-2 and H-6 (δ_H 7.98) were observed with H-3 and H-5 (δ_H 7.31), respectively. While H-4 (δ_H 7.43) was found to be correlated with both H-3 and H-5 (δ_H 7.31). The overlapped signals of H-4″ (δ_H 7.31), H-5″ (δ_H 7.31), and H-6″ (δ_H 7.30) were correlated with H-3″ (δ_H 7.03) in the salicylic alcoholic group. The ^{1}H-^{1}H COSY correlation of H-3‴- H-4‴, H-4‴- H-5‴ and H-5‴- H-6‴ supported the *ortho*-hydroxyl benzoyl group in **1**. The ^{1}H NMR, ^{13}C NMR chemical shift, and COSY, HMBC connections for **1** are presented in Table 1.

Table 1
^{1}H- and ^{13}C NMR Spectral Data of Acmophyllin A (**1**) in CD_3OD.

C. No.	^{1}H-δ (J Hz)	^{13}C (δ)	Multiplicity	HMBC Correlations	COSY Correlations
1	–	131.5	C		
2	7.98, d, ($J_{2,3}$ = 7.6)	130.9	CH	1, 4	3
3	7.31, m	129.4	CH		2
4	7.43, m	134.2	CH		3, 5
5	7.31, m	129.4	CH		4, 6
6	7.98, d, ($J_{5,6}$ = 7.6)	130.9	CH		5
7	–	167.2	C=O		
1′	5.29, m	100.7	CH		2′
2′	5.28, m	75.2	CH	1′, 3′, 7	1′, 3′
3′	3.78, m	76.0	CH		2′, 4′
4′	3.57, m	71.6	CH		3′, 5′
5′	3.57, m	78.5	CH		4′, 6′
6′	H_a = 3.96, dd, (J = 11.2, 2.4); H_b = 3.78, overlap	62.4	CH_2		5′
1″	–	156.7	C		
2″	–	125.9	C		
3″	7.03, m,	123.7	CH		4″
4″	7.31, overlap	130.2	CH		3″, 5″
5″	7.31, overlap	131.0	CH		4″, 6″
6″	7.30, m	116.6	CH	2″, 3″, 6′	5″
7″	H_a = 5.20, d, (J = 12.4); H_b = 5.15, d, (J = 12.4)	63.0	CH_2	2″,4″, 7‴	Geminal coupling
1‴	–	112.1	C		
2‴	–	162.6	C		
3‴	6.88, d, ($J_{3‴,\ 4‴}$ = 8.4)	118.2	CH	1‴	4‴
4‴	7.41, m	136.6	CH		3‴, 5‴
5‴	6.73, m	120.2	CH	1‴, 3‴	4‴, 6‴
6‴	7.55, d, ($J_{5‴,6‴}$ = 8.0)	130.5	CH		5‴
7‴	–	170.8	C=O		