Phytochemistry Letters 14 (2015) 203–208

Contents lists available at ScienceDirect

Phytochemistry Letters

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

New immunomodulatory steroidal alkaloids from *Sarcococa saligna*

Naveed Iqbal[a], Achyut Adhikari[a], Nayab Kanwal[a], Omer Mohamed Abdalla[b], M. Ahmed Mesaik[b,c], Syed Ghulam Musharraf[a,b,*]

[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 (ICCBS), University of Karachi, Karachi 75270, Pakistan
[c] Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 2 October 2015
Accepted 10 October 2015
Available online xxx

Keywords:
Sarcococca saligna
Steroidal alkaloids
Immunomodulatory activity

ABSTRACT

Two new steroidal alkaloids, (20 *S*)-(bennzamido)-3β-(*N*,*N*-dimethyamino)-pregnane (**1**), and (20 *S*)-(bennzamido)-pregnane-3-one- (**2**), and two known steroidal alkaloids, pachysanaximine A (**3**) and 3β, 20α-diacetamido-5α-pregnane (**4**) were isolated from the whole plant of *Sarcococca saligna*. The structures of these compounds were identified with the help of spectroscopic techniques while spectra for known compounds were compared with spectra reported in literature. The immunomodulatory potential of the new compounds were found to be significant and dose dependent. Compound **1** showed inhibition of T cells proliferation at 10 μg/mL (95%), and inhibition of IL-2 production with an IC50 = 1.6 μg/mL.

1. Introduction

Sarcococca saligna (syn. *S. pruniformis*) (D. Don.) Muell. is a member of the family Buxaceae and genus *Sarcococca*. It has 3–5 meter tall green shrub with green branches. The species *S. saligna* is found in a wide area stretching from Taiwan in the East to Afghanistan in the West including China, India, Nepal and Pakistan. *S. saligna*is found in the northern regions of Pakistan at altitudes of 260 to 300 m particularly in Malakand and Hazara divisions of Khyber Pakhtunkhwa province (Hua, 2008; Jafri, 1966).

S. saligna has been used as a remedy for various diseases from very early times (Gilani et al., 2005). Methanolic extracts of *S. saligna* has shown polygalacturonase inhibition activity and dichloromethane extract has shown cytotoxicity properties (Poudel et al., 2003). The ethanolic extract of *S. saligna*has antifungal activity (Mollazadeh et al., 2010). The petroleum ether and ethyl acetate extract of *S. saligna*showed significant anti-hyperglycemic activity (Kant et al., 2011). Crude extracts of this plant and its purified compounds are potential AChE and BChE inhibitors and have also shown antibacterial activities (Atta ur Rahman et al., 1998; Naeem et al., 2005). This plant is also used for relief of muscular pain, as a cardio-suppressant, vasodilator and tracheal relaxant (Ghayur and Gilani, 2006).

Most biological activities of the plant are attributed to the presence of steroidal alkaloids. So far family *Buxus* has yielded 256 new alkaloids, out of which more than 100 new steroidal alkaloids have alone been reported from genus *Sarcococca* (Yan et al., 2011a). *S. saligna*is known to have steroidal alkaloids and numerous steroidal alkaloids have already been reported from various phytochemical investigations (Atta ur Rahman et al., 2004a; Devkota et al., 2007a; Gilani et al., 2005; Khalid et al., 2004; Yan et al., 2011b). The steroidal alkaloids isolated from *Sarcococca* species have shown good antileishmanial potential (Choudhary et al., 2010; Devkota et al., 2007a) while some of them have shown spasmolytic and cholinesterase inhibiting activities (Devkota et al., 2007b; Khalid et al., 2004).

This paper describes the isolation and characterization of four steroidal alkaloids from *S. saligna*(Fig. 1.). Steroidal alkaloids **1** and **2** have benzoyl group attached to C-20 amino group instead of C-3 amino group, as in many reported alkaloids. Moreover, compounds **1** showed good suppressing immunumodulatory activities during T-cell culture proliferation and Interleukin 2 (IL-2) inhibition assays.

2. Experimental

Melting points of purified compounds were determined on a Yanaco MP-S3 micro melting point apparatus and Büchi 535 melting point apparatus and were uncorrected. Optical rotations were measured on JASCO DIP-360 polarimeter. Solvents used were methanol and chloroform. The UV absorptions were recorded in

* Corresponding author. Fax: +92 321 4819018 9.
E-mail address: musharraf1977@yahoo.com (S.G. Musharraf).

http://dx.doi.org/10.1016/j.phytol.2015.10.009
1874-3900/

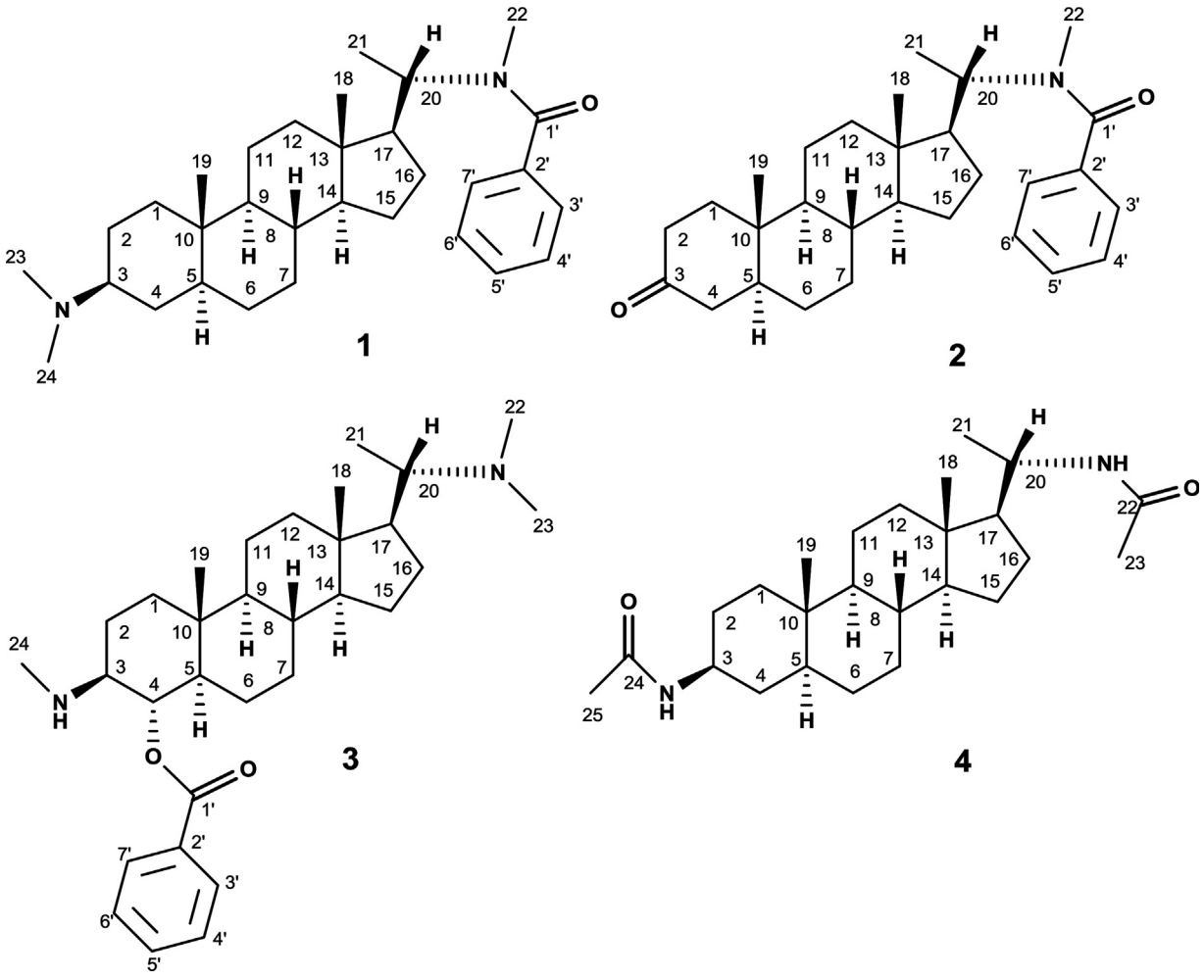

Fig. 1. Structure of compounds **1–4**.

MeOH and $CHCl_3$ on Hitachi UV 3200 spectrophotometer. Infrared (IR) spectra were recorded on a Shimadzu IR 460 (Shimadzu Corporation Koyota, Japan) in $CHCl_3$ solution.

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker Avance-NMR (400, and 500 MHz). Multiplicities of carbon signals were determined by using DEPT 90° and 135°. COSY-45° experiment was used to determine homonuclear ^1H–^1H correlation. Hetero nuclear one-bond ^1H–^{13}C scalar coupling were studied through HSQC experiment. Multiple bonds hetero nuclear ^1H–^{13}C correlations were studied through HMBC experiment. EI-MS and HREI-MS were recorded on JEOL JMS-600H mass spectrometer (Japan). Column chromatography was carried out using silica gel (E. Merck, type 60, 70–230 mesh as adsorbent). Purity of the samples was checked on the precoated TLC cards (silica gel). TLC plates were viewed under ultraviolet light at 254 and 366 nm. Dragendorff's spraying reagent was used as a spraying reagent for staining the alkaloids on TLC.

2.1. Processing and extraction of plant material

Whole plant of *S. saligna* (D. Don) Muell. was collected from Miandam area of Swat District, Khyber Pakhtunkhwa province, Pakistan. Plant material was identified at the Department of Botany, University of Karachi, Karachi, Pakistan. The voucher specimen (85854) was deposited at the Herbarium, University of Karachi.

Whole plant of *S. saligna*(about 25 kg) was dried in shade and soaked in 80% MeOH:H_2O for 15 days at room temperature. The solvent was evaporated with the help of rotary evaporator and crude extract (A, 1.5 kg) of *S. saligna* was obtained. Extract "A" (1.5 kg) was suspended in cold distilled water and completely defatted with petroleum ether (3 × 10 L) that resulted "petroleum ether extract" (AP, 170 g). The "defatted aqueous fraction" B that was left behind was then extracted with dichloromethane (3 × 20 L) to obtain another fraction in nearly neutral conditions called the "neutral fraction" (SSA, 195 g) and an aqueous fraction C. The fraction C was then made alkaline by adding ammonia solution to it (pH 9–10). After basifying, it was extracted with dichloromethane (DCM) (3 × 20 L) to obtain "alkaline fraction" (SSB, 183 g) and aqueous fraction D.

The alkaline fraction SSB was subjected to alumina gel (basic) vacuum liquid chromatography (VLC) which produced several fractions, A1–A7. These fractions were eluted with a solvent system of hexane/acetone. At start, the column was eluted with pure hexane and then the polarity of the solvent system was increased by gradually increasing the amount of acetone in the solvent system mixture. The six sub fractions (A1, 9.4 g; A2, 13.5 g; A3, 51.5 g; A4, 11.0 g; A5, 9.2 g and A6, 11.1 g) were obtained using hexane: acetone (9.0: 1.0, 8.5: 1.5, 7.8: 2.2, 7.4: 2.6, 3.0: 7.0, 0: 100, respectively) solvent system, while the fraction A7 (10.6 g) was obtained using acetone: methanol (0.5:9.5) solvent system. The sub fraction A4 was subjected to silica gel chromatography using