Phytochemistry Letters 5 (2012) 490–495

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

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

New ursane-type triterpenes from the root bark of *Calotropis procera*

Sabrin R.M. Ibrahim [a], Gamal A. Mohamed [b,e], Lamiaa A. Shaala [c], Laetitia Moreno Y. Banuls [d], Gwendoline Van Goietsenoven [d], Robert Kiss [d], Diaa T.A. Youssef [e,*]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt
[b] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt
[c] King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Laboratoire de Toxicologie, Faculté de Pharmacie, Université Libre de Bruxelles, Bruxelles, Belgium
[e] Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 31 March 2012
Accepted 23 April 2012
Available online 8 May 2012

Keywords:
Calotropis procera
Asclepiadaceae
Calotroprocerol A
Calotroproceryl acetate A
Calotroprocerone A
Calotroproceryl acetate B
Cancer growth inhibitory activity

ABSTRACT

As a part of our continuing interest in identifying anticancer drug leads from natural sources, we have investigated the *in vitro* growth inhibitory effects of the hexane fraction of the root bark of *Calotropis procera* (Ait) R. Br. (Asclepiadaceae). This study reports the isolation and structure elucidation of four new ursane-type triterpenes named calotroprocerol A (**1**), calotroproceryl acetate A (**2**), calotroprocerone A (**3**) and calotroproceryl acetate B (**4**) in addition to five known compounds including pseudotaraxasterol acetate (5), taraxasterol (**6**), calotropursenyl acetate B (**7**), stigmasterol (**8**) and (*E*)-octadec-7-enoic acid (**9**). Their structures were established on the basis of 1D and 2D NMR studies (^{1}H–^{1}H COSY, HSQC, and HMBC) and HRMS spectral data. The *in vitro* growth inhibitory activity of the isolated compounds was evaluated against three human cancer cell lines including the A549 non-small cell lung cancer (NSCLC), the U373 glioblastoma (GBM) and the PC-3 prostate cancer cell lines.

1. Introduction

The *Calotropis* (Asclepiadaceae) genus is a glabrous or hairy laticiferous small tree commonly known as the "Swallow Wort" or "Milk Weed", and it is distributed in the tropical and subtropical regions of Asia and Africa (Ansari and Ali, 2001; Mijatovic et al., 2007a,b, 2008a,b). It is known to possess several medicinal properties. Different parts of the plant are used in the treatment of various diseases in folk medicine, such as leprosy, ulcers, tumors, snake bites, malaria and piles, in addition to diseases of the spleen, liver and abdomen (Mijatovic et al., 2007a,b, 2008a,b; Bharti et al., 2010; Iqbal et al., 2005). *Calotropis procera* possesses different biological activities including the following: anti-inflammatory, analgesic, antitumor, antidiarrheal, hepatoprotective, antiulcer, anthelmintic, insecticidal, antioxidant, antibacterial, and spasmolytic activities (Bharti et al., 2010; Iqbal et al., 2005; Iwalewa et al., 2005; Moustafa et al., 2010; Van Quaquebeke et al., 2005; Begum et al., 2011; Sharma et al., 2011; Yesmin et al., 2008). Reported constituents of *C. procera* in the literature include cardenolides (Moustafa et al., 2010; Van Quaquebeke et al., 2005; Hanna et al., 1999), triterpenes (Ansari and Ali, 2001; Alam and Ali, 2009), flavonoids (Moustafa et al., 2010; Shaker et al., 2010; Heneidak et al., 2006), sterols (Ansari and Ali, 2001; Alam and Ali, 2009), and fatty acids (Ansari and Ali, 2001; Khanzada et al., 2008).

We have identified a novel cardenolide, 2″-oxovoruscharin, in *C. procera* samples growing in Burkina Faso (Van Quaquebeke et al., 2005) from which we hemisynthesized 19-hydroxy-2″-oxovoruscharin (Van Quaquebeke et al., 2005). This cardenolide displays marked anticancer activity both *in vitro* and *in vivo* by targeting the alpha-1 or alpha-3 Na^+/K^+-ATPase subunits in various cancer types including non-small cell lung cancer (NSCLC) (Mijatovic et al., 2007a,b), glioblastoma (GBM) (Lefranc et al., 2008) and melanoma (Mathieu et al., 2009).

C. procera is known as "Usher" or "Oshar", and it widely grows in the Egyptian desert in Sinai (Elgamal et al., 1999). Therefore, we investigated whether *C. procera* samples growing in Egypt contained potential anticancer compounds.

Bioassay-directed fractionation of the non-polar *n*-hexane fraction of a methanolic extract of the root bark of *C. procera* using three human cancer cell lines (A549 NSCLC, U373 GBM and PC-3 prostate cancer models) led to the isolation of four new ursane-type triterpenes including calotroprocerol A (**1**), calotroproceryl acetate A (**2**), calotroprocerone A (**3**), and calotroproceryl acetate B (**4**) in addition to five known compounds pseudotaraxasterol acetate (**5**) (Reynolds et al., 1986), taraxasterol (**6**) (Reynolds et al., 1986), calotropursenyl acetate B (**7**) (Ali et al.,

* Corresponding author. Tel.: +966 548535344; fax: +966 26951696.
E-mail addresses: dyoussef@kau.edu.sa, diaa22@yahoo.com (Diaa T.A. Youssef).

1874-3900/$ – see front matter © 2012 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.phytol.2012.04.012

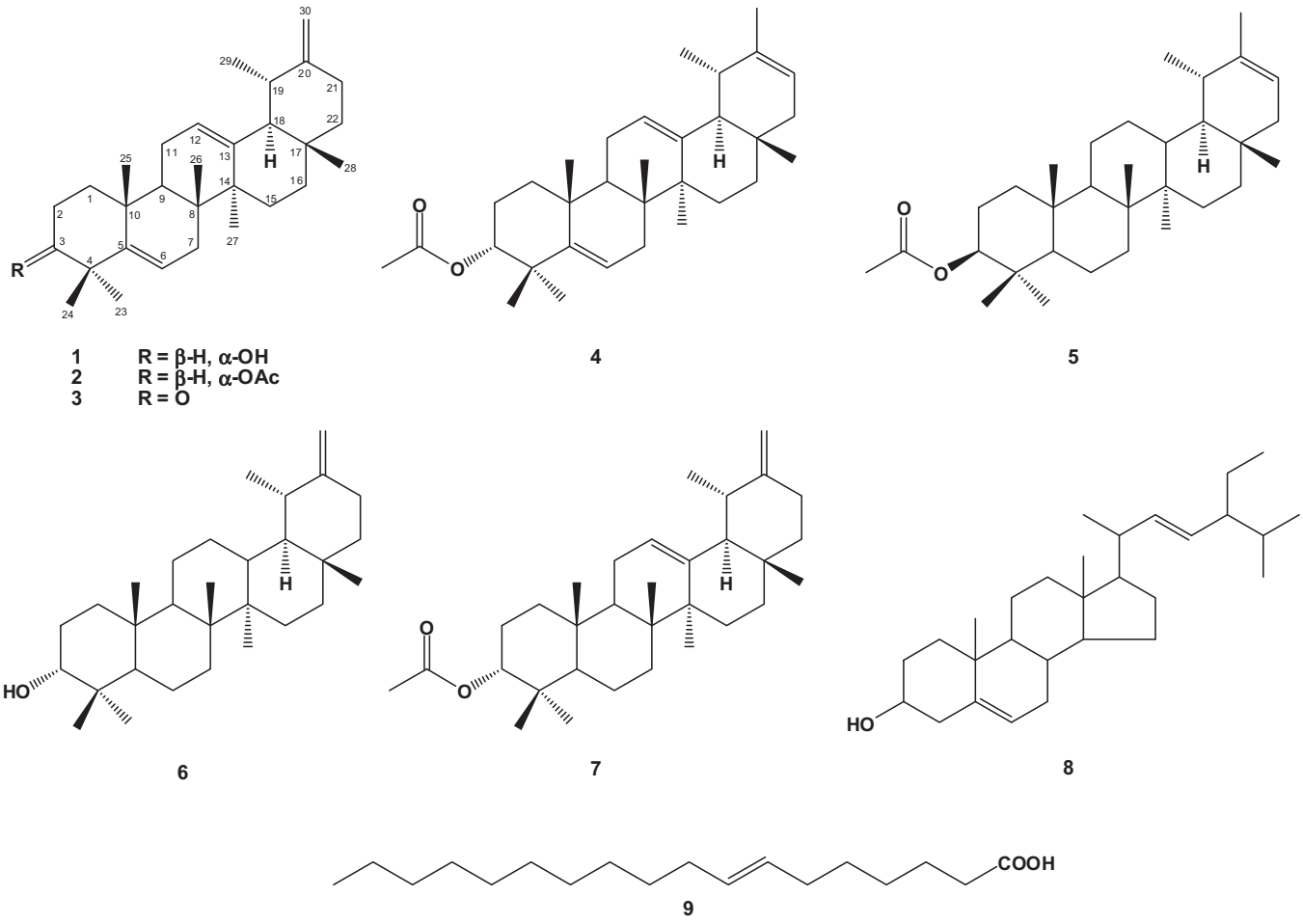

Fig. 1. Structures of the isolated compounds.

1998), stigmasterol (**8**) (Mohamed and Ibrahim, 2007) and (*E*)-octadec-7-enoic acid (**9**) (Dembitskii et al., 1991; Chen et al., 1995) (Fig. 1). This study reports the full account of the structure characterization of the isolated terpenoidal compounds using spectral means, especially 1D and 2D NMR studies and high resolution MS data. In addition, the *in vitro* growth inhibitory activity of the isolated compounds against three cancer cell lines (A549 NSCLC, U373 GBM and PC-3 prostate cancer) was also evaluated.

2. Results and discussion

Calotroprocerol A (**1**) was obtained as colorless needles. It produced a positive Liebermann–Burchard reaction (Reinhold, 1935). The molecular formula was $C_{30}H_{46}O$ on the basis of HREIMS together with ^{13}C NMR and HSQC spectral data. The IR spectrum indicated the presence of a hydroxyl group (3510 cm^{-1}) and double bond (1615 cm^{-1}) absorption bands. The MS of **1** was similar to pentacyclic triterpenes of the ursane series in which rings B and C are unsaturated (Shiojima et al., 1992; Ogunkoya, 1981; Begum et al., 1997). The retro Diels–Alder fragmentation of **1** generated the base peak at *m*/*z* 220 (Fig. 2). The ion peaks at *m*/*z* 152, 220, 233 and 257 indicated the existence of olefinic moieties in rings B and C at C-5/C-6 and C-12/C-13 (Shiojima et al., 1992; Ogunkoya, 1981; Begum et al., 1997; Siddiqui et al., 1986). The saturated nature of rings D and E was inferred from the fragment ion peaks at *m*/*z* 54, 67, 82, 121, and 136 (Fig. 2). The ^1H NMR spectrum of **1** showed two downfield protons at δ_H 5.18 (t; J = 3.6 Hz) and 5.13 (t; J = 3.6 Hz) assigned to H-6 and H-12, respectively. Two one-proton doublets at δ_H 4.61 and 4.59 with a coupling constant of 2.0 Hz were associated with the C-30 exocyclic methylene group. The signal at δ_H 3.22 (dd; J = 5.3; 4.1 Hz) was ascribed to C-3β carbinol proton (Ali et al., 2000; Mahato and Kundu, 1994). A doublet proton at δ_H 2.06 (J = 6.9 Hz) was ascribed to the 18α proton (Ali et al., 1998, 2000). In addition, the ^1H NMR spectrum showed signals for seven methyl groups, six of which were positioned at quaternary carbons corresponding to the singlets at δ_H 1.00 (H_3-23), 0.81 (H_3-24), 1.00 (H_3-25), 0.92 (H_3-26), 1.02 (H_3-27), and 0.80 (H_3-28). The H_3-29 methyl appeared as a three-proton doublet at δ_H 0.97 (J = 6.3 Hz) supporting the ursane-type carbon framework of the molecule. The ^{13}C NMR spectrum of **1** showed 30 carbon signals corresponding to those of the ursane-type molecule (Ali et al., 1998, 2000). The signals of vinylic carbons appeared at δ_C 145.2 (C-5), 121.7 (C-6), 124.4 (C-12), and 139.6 (C-13), and the signals of exocyclic methylene appeared at δ_C 154.7 (C-20) and 107.2 (C-30). The HSQC and HMBC spectra were utilized to assign the positions of the carbons and protons in **1** (Table 1). The double bonds were established at C-5/C-6 and C-12/C-13, which was established from the HMBC correlations of H_3-23 and H_3-24 with C-5 and H_3-23/C-6, H_3-24/C-6, H_3-25/C-5, and H_3-27/C-13 (Fig. 3). The exocyclic methylene group was assigned at C-20 based on the HMBC correlations of H_3-29/C-20 and H_2-30/C-19. In addition, the hydroxyl group was assigned to the C-3 position based on the HMBC correlations of H_3-23 and H_3-24 with C-3. Thus, from these findings, the structure of calotroprocerol A was assigned as ursa-5,12,20(30)-trien-18α*H*-3β-ol.

Calotroproceryl acetate A (**2**) was isolated as colorless needles. It responded positively to the Liebermann–Burchard test of