SHORT REPORT

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Rec. Nat. Prod. 7:2 (2013) 114-118

records of natural products

Maroccanin: A New γ-lactone and Other Constituents from *Centaurea maroccana* Ball. (Asteraceae)

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(Received June 5, 2012; Revised December 20, 2012; Accepted January 31, 2013)

Abstract: A new γ-lactone named maroccanin (**1**) together with 5 known compounds : scopoletin (**2**); 8α-[2'-hydroxymethyl-2'-butenoyloxy]-dehydromelitensin (**3**); 11,13-dehydromelitensin (**4**); 8α-[2'-hydroxymethyl-2'-butenoyloxy]-sonchucarpolide (**5**) and 5Hα,6Hβ,7Hα,-15-hydroxy-8α-(1',2'-dihydroxyethyl-acryloxy)-elema-1(2),3(4),11(13)-trien-6,12-olide (**6**), were isolated from the chloroform soluble part of the aqueous-MeOH extract of the flowering aerial parts of *C. maroccana* Ball. (Asteraceae), an endemic species of the North of the Sahara. The structures were established by chemical and spectral analysis, mainly HR-ESIMS, HREIMS,UV and NMR experiments (COSY, NOESY, HSQC and HMBC). The compounds **3** and **5** are new for the genus *Centaurea*.

Keywords: Maroccanin; Sesquiterpene lactones; *Centaurea maroccana*; Asteraceae.

1. Plant Source

The species of the genus *Centaurea* are known for their antidiabetic, antidiarrhetic, antirheumatic, anti-inflammatory, colagog, choleretic, digestive, stomachic, diuretic, menstrual, astringent, hypotensive, antipyretic and antibacterial effects by public medicals [1]. These species have been object of many phytochemical investigations which showed their wealth of bioactive

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 3/15/2013 EISSN: 1307-6167

secondary metabolites in particular flavonoids [2, 3] and sesquiterpene lactones [4-7]. As a part of our ongoing program of research of new molecules with potential biological activity from this genus [8-12], we report our results on *C. maroccana* Ball., an endemic species of the north of the Sahara [13]. The present work which concerned the chemical constituents of the chloroform soluble part of the aqueous-MeOH extract of the leaves and flowers of *C. maroccana* Ball., led to the isolation of a new γ-lactone named maroccanin (**1**) together with 5 known compounds: a coumarin and 4 sesquiterpene lactones (Figure 1).

Flowering aerial parts of *Centaurea maroccana* Ball. were collected from the area of Biskra in the South of Algeria in April 2002 and authenticated by Prof. M. Kaabeche (Biology Department, University of Setif, Algeria). A voucher specimen (CCM12/04/02) has been deposited in the Herbarium of the Valorization of Natural Resources and Synthesis of Bioactive Substances Laboratory, Mentouri University, Constantine.

2. Previous Studies

In previous studies, we reported some compounds from the flowering aerial parts of this plant [14, 15].The purpose of the present work was the isolation and the structure elucidation of further constituents

3. Present Study

Air-dried leaves and flowers (2700 g) of *C. maroccana* were macerated at room temperature with MeOH–H_2O (80:20, v/v) for 24 h, three times. After filtration, the filtrate was concentrated (1200 ml) and dissolved in H_2O (1100 ml) under magnetic agitation. The resulting solution was filtered and successively extracted with $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered and concentrated *in vacuo* at room temperature to obtain the following extracts: chloroform (12,5 g), EtOAc (20 g) and *n*-butanol (40 g).

A part of the chloroform extract (9 g) was fractionated by CC on silica gel using chloroform with increasing percentages of acetone to yield 50 fractions (1–50) obtained by combining the eluates on the basis of TLC analysis. Fraction 10 (67 mg) and fraction 11 (45.5 mg) (99:1) were submitted to preparative TLC (benzene / EtOAc 5:1) and (chloroform / acetone, 9:1) respectively, to give the new compound **1** (10 mg) named maroccanin and scopoletin (**2**) (20 mg) [16] respectively. Fraction 27 (120 mg) (90:10), gave after purification on preparative plates of silica gel (chloroform / acetone, 6:1) 8α-[2'-hydroxymethyl-2'-butenoyloxy]-dehydromelitensin (**3**) (6.1 mg) [17-19] and 11,13-dehydromelitensin (**4**) (13.2 mg) [20]. Fraction 40 (200 mg) (80:20) was rechromatographed on a silica gel column using chloroform / acetone (4:1) as eluent to yield 4 sub-fractions. Sub-fraction 1 gave after purification on preparative plates of silica gel (benzene / acetone, 1:1) 8α-[2'-hydroxymethyl-2'-butenoyloxy]-sonchucarpolide (**5**) (10 mg) [18]. Fraction 41 (150 mg) (70:20), was rechromatographed on a silica gel column using chloroform / EtOAc, 3:1) to yield five sub-fractions. Sub-fraction 4 gave after purification on preparative TLC (silica gel, benzene / EtOAc, 2:1) 5Hα,6Hβ,7Hα,-15-hydroxy-8α-(1',2'-dihydroxyethyl-acryloxy)-elema-1(2),3(4),11(13)-trien-6,12-olide (**6**) (20 mg) [21,22].

*Maroccanin **(1)**:* Colourless oil; $[\alpha]_D^{20} = -66.5$ (c = 0.0035, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.27 (3H, s, H-10), 1.70 (1H, m, H-5b), 2.24 (3H, s, H-14), 2.30 (1H, m, H-5a), 2.48 (1H, m, H-6b), 2.51 (1H, brd, J = 6.1 Hz, H-7), 2.60 (1H, m, H-6a), 2.81 (1H, brq, J = 9.4 Hz, H-4), 3.07 (1H, dd, J = 9.4, 1.5 Hz, H-3), 4.83 (1H, d, J = 1.5 Hz, H-2), 6.22 (1H, d, J = 16.1, H-12), 6.54 (1H, dd, J = 16.1, 6.1 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 9.4 (CH_3, C-10), 21.5 (CH_2, C-5), 27.2 (CH_3, C-14), 40.4 (CH_2, C-6), 41.7 (CH, C-4), 54.1 (CH, C-7), 55.2 (CH, C-3), 55.3 (C, C-8), 81.9 (CH, C-2), 136.0 (CH, C-12), 137.6 (CH, C-11), 177.5 (C, C-9), 197.0 (C, C-13), 213.0 (C, C-1) ; HRESI-MS: *m/z* 271.0940 (calcd. 271.0946 for $C_{14}H_{16}O_4Na$).

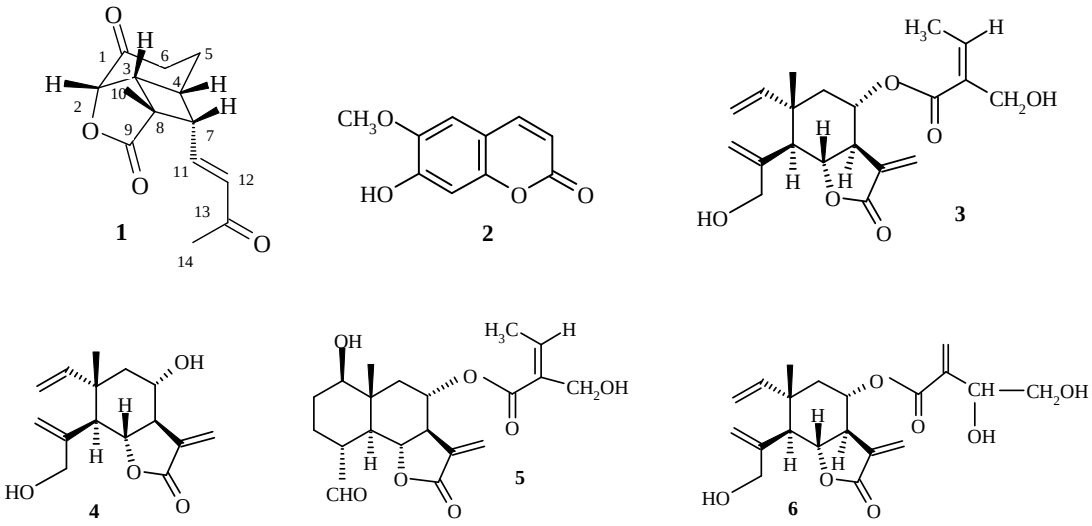

Figure 1. Structures of compounds (**1-6**)

The new compound **1** was obtained as oil. The positive mode HRESI-MS spectrum presented [M+Na] ion at *m/z* 271.0940 (100%) according to the molecular formula $C_{14}H_{16}O_4$ (calculated for $C_{14}H_{16}O_4Na$: 271.0946) indicating a compound containing 7 unsaturations.

The ^1H, ^{13}C NMR, DEPT and HSQC spectra led to the presence of 2 CH_3, 2 CH_2, 6 CH from which 2 ethylenic and 1 oxygenated (δ_C 81.9; δ_H 4.83, d, J = 1.5 Hz) and 4 quaternary carbon atoms from which a carbonyl of a ketone at δ_C 213.0, a carbonyl of a conjugate ketone at δ_C 197.0 and a characteristic carbonyl of a γ-lactone at δ_C 177.5. The presence of the five membered cycle of the γ-lactone in this compound was confirmed by the observed correlation between this last carbonyl and the proton of the oxygenated CH group (δ_H 4.83) in the HMBC spectrum (Figure 2), the observed correlation between the proton of this CH (H-2) and the proton of the CH at δ_H 3.07 (dd, J = 9.4, 1.5 Hz) in the COSY spectrum and the observed correlations between the carbon of this last CH (C-3 at δ 52.2), the carbon of the lactone carbonyl and the protons of the CH_3 group at δ_H 1.27 (s, δ_C 9.4). The multiplicity of the signal of this CH_3 group suggested the presence of the quaternary carbon atom (δ 55.3) between C-3 and the carbonyl of the lactone.

The examination of the COSY spectrum showed correlation between H-3 and the proton of the CH group at δ 2.82 (br. q, J = 9.4, H-4), correlations between H-4 and the diastereotopic protons of the CH_2 group at δ 2.30 (m, H-5a) and δ 1.70 (m, H-5b) and correlations between these last two protons and the diastereotopic protons of the other CH_2 group of this molecule (δ 2.60, m, H-6a, δ 2.48, m, H-6b). In the HMBC spectrum, H-6a, H-6b and H-3 clearly showed correlations with the carbon of the ketone at δ 213.0. These observations led to the presence of a 2,3,4-trisubstitued cyclohexanone in this compound. According to this result, the C-2 and C-3 positions were occupied by the γ-lactone wile the position C-4 was substituted by a C_5 chain which began by a CH group (δ_H 2.51 (br. d, J = 6.1 Hz, δ_C 54.1) according to the HMBC correlations observed between the carbon of this group (C-7) and H-3, H-4 and H_2-5. Moreover, the proton of this group (H-7) correlated with the carbonyl of the lactone in the HMBC spectrum suggesting the presence of a bond between C-7 and the C_q at δ 55.3 (C-8). In the COSY spectrum H-7 showed correlation with the ethylenic proton at δ_H 6.54 (dd, J = 16.1, 6.1 Hz, H-11, δ_C 137.6), which correlated with the second ethylenic proton (H-12, δ_H 6.22, d, J = 16.1 Hz, δ_C 135.1). In the HMBC spectrum H-11 showed correlations with the carbonyl of the conjugated ketone (C-13) and the carbon of the methyl group (C-14) at δ_C 27.2 (δ_H 2.25, s).

The stereochemistry of the chiral centers was assigned on the basis of the NOESY spectrum analysis.The NOESY interactions CH_3-10/H-3, CH_3-10/H-4, CH_3-10/H-7, H-3/H-7 and H-2/H-7 indicated a β-orientation for H-2, H-3, H-4, H-7 and CH_3-10 (Figure 3).

All these data led to the new molecule named maroccanin **1** as illustrated in Figure 1. Compounds **3** and **5** are reported for the first time from the genus *Centaurea.*

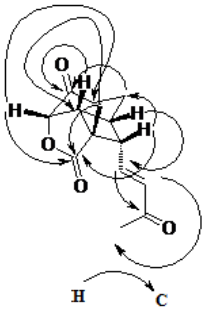

Figure 2. Important HMBC correlations for maroccanin (**1**)

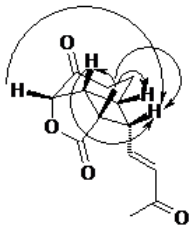

Figure 3. Important NOESY interactions for maroccanin (**1**)

Acknowledgements

This work was supported by Algerian FNR program (PNR 8u250/4211, ATRST contract 26/70) and by the NATO Science project (Valorisation of Phytochemical Natural Resources in Algeria).

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