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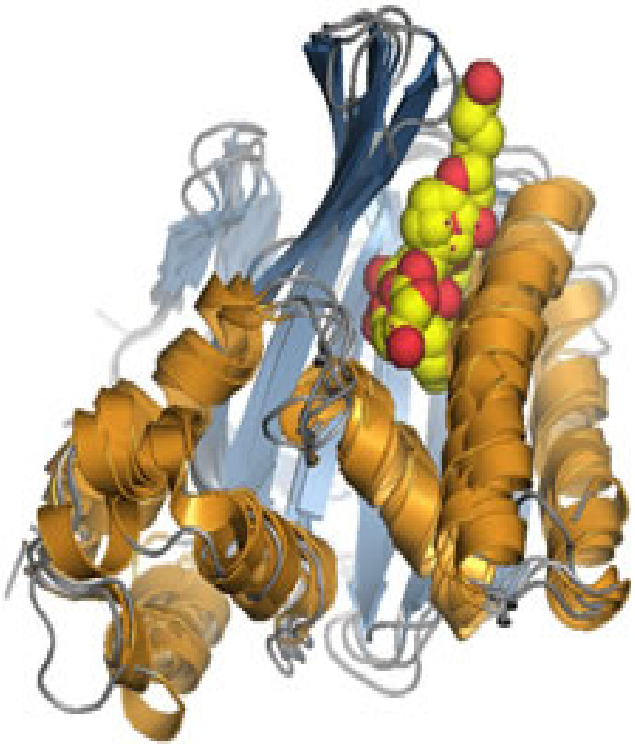

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The Coumarin from *Euphorbia guyoniana* Boissier and Reuter

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Abstract. *Esculetin is a phenolic compound that is found in various natural plant products and induces apoptosis in several types of human cancer cells. However, the underlying mechanisms of its action are not completely understood. In the present work a phytochemical study of ethyl acetate extracted from the aerial parts of Euphorbia guyoniana plant endemic of Algeria, led to the isolation of a compound (esculetin). The purification of this compound was by column chromatography and TLC. The identification of the structure was used by usual spectroscopic methods such as ESI-MS, NMR (1D and 2D). Coumarins Euphorbia guyoniana were not isolated earlier.*

***Key Words*:** Euphorbiaceae, *Euphorbia*, *guyoniana*, coumarin and esculetin

1. Introduction

Euphorbia guyoniana Boissier and Reuter is an endemic plant of the Saharan plant growing in sandy and desert habitat, in the south of Algeria. This plant belongs to the Euphorbiaceae family; *Euphorbia* species are well-known to be generally toxic, skin irritants *Euphorbia* Guyoniana traditionally used to treat snakebites **(Quezel, 1962; Bellakhdar, 1997).**

This endemic plant of Algeria, is located in sandy regions, pre-desert and in the north of Sahara. It is a powerful herb in length and creeping underground stem, bright green 30 Cm to 100 Cm in height. *Euphorbia guyoniana* has a local name "lebina". It was previously worked on by **(Ahmed, 2006**) and he reported the isolation of diterpenoids isolated from the aerial parts and by **(Haba, 2007)** who reported the isolation of triterpenoids from the roots. The purposes of this work were developed and evaluated efficient and simple procedures for extraction of secondary metabolites.

Coumarins are widespread in some families; they are almost in all parts of plants and especially in fruits and seeds. Coumarins are often glycosides (**Bruneton, 2009**).

Esculetin (6, 7-dihydroxycoumarin) is a naturally occurring coumarin derivative that is found in various natural plant products and was reported to have beneficial pharmacological and biochemical activities (**Park, 2008)** and chemotherapeutic activities against several types of cancers (**Kok, 2009).**

2. Materials and Methods

General Experimental Procedures

The solvents were analytical grade quality, purchased from Sigma-Aldrich Acros Organic and VWR-Prolabo. Water was produced in laboratory of Pharmacognosy, Faculty of Pharmacy, University of Montpellier 1, France (MilliQ-water 18.2 MΩ (ELGA-II-MK, 230V ac-50 Hz-60 VA). Column silica powder and TLC silica F_{254} plates from Merck, and developed in mobile phase below. Components were visualized under ultra-violet light (λ 254 and 365 nm) and detected by Natural product (2-aminoethyl-phenyl-borate) 0.1% in methanol and PEG 5% (polyethylene-glycol-3000, Fluka) in ethanol. FT-IR measurements were carried out at room temperature on a Perkin- Elmer "Spectrum 100" FT-IR Spectrometer (America), all the samples were deposited as a solid after lyophilization. But Mass spectra were performed with an apparatus LCQ Advantage "Thermo finnigan" ESI in negative and positive mode. NMR spectra obtained at 22°C on Brüker Advance 500 operating at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C. Chemicals shifts (δ) are given in ppm, and coupling constants (*J*) are reported in Hz. Simples were dissolved in deuterated solvent (CD_3OD).

Plant Material

The seeds of *Euphorbia guyoniana* Boiss. and Reut. were collected in April 2008 from Oued-Souf region, in the south eastern part of Algeria. This plant identified by Dr A. Chahma Department of Agriculture, Faculty of Science, University Kasdi Merbah Ouargla, Algeria.

Extraction and Isolation

142 g from seeds was ground into fine powder, then delipited with hexane (24 hours of maceration). The powder was dried and then degreased by dichloromethane 24 hours. The dried powder of seeds was extracted successively with solvent of increasing polarity: ethyl acetate (2 x 1,4 L) and n-butanol (2 x 1,4 L) 24 hours for each extract, the pooled solvents were removed under reduced pressure (40°C maximum) to yield (572 mg) of EtOAc and (32 mg) of n-butanol. Each step was followed by thin layer chromatography. In this study we are interested in ethyl acetate extract.

The ethyl acetate extract (572 mg) dissolved in methanol is mixed with a small amount of silica gel; the mixture was dried in vacuum and then evaporated to spraying of a homogeneous powder. The latter was deposited on the silica gel column (0.9 x 25 Cm, 70 - 230 mesh) previously prepared. The ethyl acetate extract was subjected to a first fractionation, using the flowing gradients dichloromethane / ethyl acetate up to 100 % EtOAc and then followed by the gradient of ethyl acetate / methanol up to 100 % MeOH to collect 13 fractions noted F1-F13.

Chromatographic analysis of the different fractions of the extract has noted that the fractions F6, F7, F8, F9 and F10 are not pure, but they have a major product as green fluorescent spot. It's R_f = 0.36 in the following system elution:

The system: toluene/ethyl formiate/ formic acid (v/v/v: 5/4/1)
Revealing reagent Np/PEG (**wagner, 2001**).

Fractions F6, F7, F8, F9 and F10 are collected for these fractions which contain the same major product. 173.6 mg of this mixture of ethyl acetate seeds fractions (ACG-F7) was chromatographed on second silica gel column (70 - 230 mesh) using dichloromethane, gradient of dichloromethane / methanol up to collect nine fractions of 100 ml volume.

The column is followed by thin layer chromatography, this column also allowed us to obtain a pure fraction as colorless crystals in long needles (ACG-C2) 22.8 mg (**compound I**) after evaporating solvent.

3. Results and Discussions

Identifications of the compound isolated from the ethyl acetate extract

After purification of the crystals the compound **I** (fig.1) was obtained as colourless needles (fig.2). The molecular formula was determined as $C_9H_6O_4$. The analysis of mass spectrometry was performed by high-resolution electrospray (ESI-MS). The spectrum obtained shows us a molecular ion at m / z 177.00 $[M - H]^-$. In the ^1H NMR spectrum (500 MHz, CD_3OH), showed two singlet signals at δ 6.65 (1H, s) and 6.93 (1H, s) and two doublets at δ 6.18 (*d, J*= 9.35Hz, 1H) and 7.78 (*d, J*= 9.35 Hz, 1H). ^{13}C NMR spectrum (125.75 MHz, CD_3OH) displayed signal at δ 164.30 of carbon carbonyl, four carbon of methine at δ 112.46, 146.08, 103.60 and 112.98 ppm, two hydroxylated carbons at δ 112.77, 144.58 ppm and two quaternary carbon atoms at δ 152.04, 150.47 ppm. ^1H NMR, ^{13}C NMR-J mod., HSQC and HMBC identified that the compound **I** is the esculetin (**El-Bassuony1, 2006; Gherraf, 2006)** table 1**.**

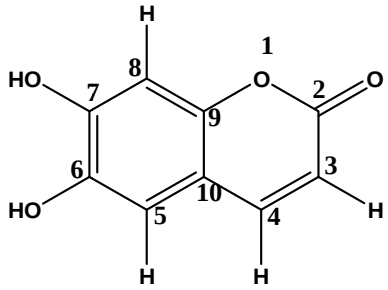

Fig.1: 6,7-Dihydroxycoumarin(Esculetin) structure

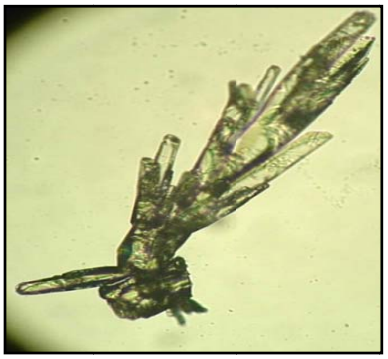

Fig.2: Esculetin cristal

Table 1: ^{1}H NMR and ^{13}C NMR data of compound I (500 MHz in methanol CD_3OD deuterated and the coupling constant *J* (Hz).

Crystals = Esculetin (compound I)				
Position	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC (H to C)	HSQC (H to C)
2	-	164.30	-	-
3	6.18 (*d*, *J* = 9.35)	112.46	C2	C3
4	7.78 (*d*, *J* = 9.35)	146.08	C2, C8, C9	C4
5	6.75 s	103.60	C6, C7, C9	C5
6	-	144.58	-	-
7	-	112.77	-	-
8	6.93 s	112.98	C4, C5, C9	C8
9	-	152.04	-	-
10	-	150.54	-	-

Conclusion

The study of the seeds of Euphorbia guyoniana by column chromatography on silica gel isolated coumarin is not previously obtained from this plant. Our results showed that the compound obtained and identified by the usual spectroscopic methods (1D, 2D) is Escultine form of colorless needle crystals.

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