

## Investigación

Chemical Composition of the Epicuticular Wax of *Cnidoscolus aconitifolius*Fabiola Escalante-Erosa,<sup>1</sup> Ilka Ortegón-Campos,<sup>1</sup> Víctor Parra-Tabla,<sup>2</sup> and Luis M. Peña-Rodríguez<sup>1\*</sup><sup>1</sup> Grupo de Química Orgánica, Unidad de Biotecnología, Centro de Investigación Científica de Yucatán and <sup>2\*</sup>Departamento de Ecología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mérida, Yucatán, México 97200

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**Abstract.** Purification of the wax extract from leaves of *Cnidoscolus aconitifolius* (Miller) I.M. Johnston resulted in the isolation and identification of three triterpenoids: amyrenone (1),  $\beta$ -amyrin acetate (2) and  $\alpha$ -amyrin acetate (3); additionally two separate fractions containing a mixture of  $\beta$  and  $\alpha$  amyrin (4 and 5) and an unidentified number of alkanes, were also obtained. Metabolites were identified by comparing their spectral data with those from authentic samples and/or with those reported in the literature.

**Keywords:** *Cnidoscolus aconitifolius*, epicuticular wax, triterpenoids, amyrenone,  $\alpha$ -amyrin,  $\beta$ -amyrin.

**Resumen.** La purificación del extracto ceroso de las hojas de *Cnidoscolus aconitifolius* (Miller) I.M. Johnston resultó en el aislamiento e identificación de tres triterpenoides: amirenona (1), acetato de  $\beta$ -amirina (2) y acetato de  $\alpha$ -amirina (3); además de dos fracciones adicionales conteniendo una mezcla de  $\beta$  y  $\alpha$  amirina (4 y 5) y un número desconocido de alcanos. Los metabolitos fueron identificados por comparación de sus datos espectroscópicos con los de los estándares respectivos y/o con los reportados en la literatura.

**Palabras clave:** *Cnidoscolus aconitifolius*, cera epicuticular, triterpenos, amirenona,  $\alpha$ -amirina,  $\beta$ -amirina.

## Introduction

Most higher plants are covered by a continuous wax layer that protects plant cells from damage by environmental factors such as drought and UV radiation [1]; this wax layer also acts as a first line of defense against insects and microbial pathogens [2,3].

*Cnidoscolus aconitifolius* (Euphorbiaceae) is a shrub widely distributed in the Yucatan peninsula, where is commonly used for both medicinal and cooking purposes [4]. Even though this plant presents a variety of defense mechanisms (e.g. presence of irritant thorns in the leaves, production of irritating latex in the stems, and secretion of ant-attracting floral nectar), it has been observed that some populations of *C. aconitifolius* are more affected by herbivore attack than others, with differential levels that can vary between 0 and 80% (Víctor Parra-Tabla, personal communication).

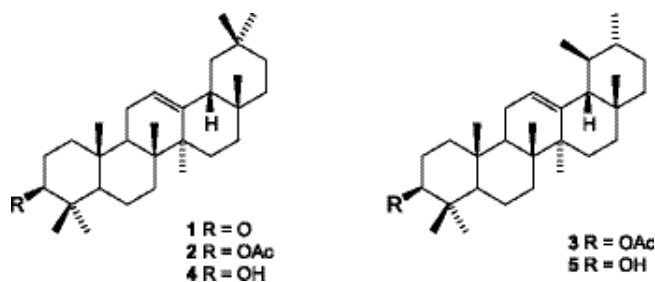
Morphological and chemical studies carried out on epicuticular waxes of some higher plants have been used to correlate the nature and chemical composition of the wax, with the susceptibility of the plant to insect attack [5]. Although a number of phytochemical studies have been carried out on various *Cnidoscolus* species, reporting the isolation and identification of triterpenes from *C. elaeagnifolius* [6] and *C. multilobus* [7], sterols from *C. chayamansa* [8], fatty acids from *C. stimulosus* [10], cyanogenic glycosides from *C. texanus* [9], and a number of terpenoids from *C. urens*; to date there are no reports describing the chemical composition of the epicuticular wax of *C. aconitifolius*. We wish to report herein on the identification of amyrenone (1),  $\beta$ -amyrin acetate (2), and  $\alpha$ -amyrin acetate (3), together with the characterization of two fractions

containing a mixture of  $\beta$  and  $\alpha$  amyrin (4 and 5) and a mixture of an unknown number of alkanes, from the epicuticular wax extract of *C. aconitifolius*.

## Results and Discussion

Successive chromatographic purifications of the dichloromethane extract from the leaves of *C. aconitifolius* produced five fractions (A-E), all of them showing a single spot on TLC. However, a GC analysis of the least polar fraction A showed that it contained at least two main components ( $t_R$  7.25 and 9.78 min), both showing a fragmentation pattern characteristic of aliphatic hydrocarbons. These components were not identified.

The single component in fraction B was identified as amyrenone (1) by comparing its spectroscopic data (MS, <sup>1</sup>H and <sup>13</sup>C NMR) with those reported in the literature [9]. Similarly, the two pure metabolites present in fractions D and E were identified as  $\beta$ -amyrin acetate (2) and  $\alpha$ -amyrin acetate (3), respectively, by both comparing their MS fragmentation



patterns with those included in the database of the apparatus and by direct comparison on TLC with authentic samples.

Finally, a GC-MS analysis of fraction C showed the presence of two main components ( $t_R$  11.75 and 12.92 min), which were identified through their fragmentation patterns as the ubiquitous mixture of  $\beta$  and  $\alpha$ -amyrin (**4** and **5**).

The presence of amyirin-type triterpenes in epicuticular waxes is well documented, particularly in studies correlating the nature and chemical composition of waxes, with the susceptibility of the plant to insect attack [2,3,5]. The amyirins have been identified as the main components of the wax of *Brassica oleracea* and they have been recognized as the metabolites responsible for the repellent effect of the plant against *Plutella xilostella* aphids [11].

The role of the amyirins isolated from the epicuticular wax of *C. aconitifolius* in the plant-herbivore interaction is currently under evaluation; the results of this investigation will be published in due course.

## Experimental

### General

GC-MS analyses were carried out in a Hewlett Packard 5890 gas chromatograph coupled to a 5971 mass selective detector (GC conditions: HP column Ultra 2; flow rate: 1 mL/min; oven temperature 280 to 300 °C; gradient 5 °C/min; injector temperature: 290 °C; detector temperature: 300 °C). Analytical TLC analyses were performed on aluminum-backed silica gel 60 F<sub>254</sub> plates (E. M. Merck, 0.20 mm thickness). Flash column chromatography purifications were run according to published procedures [12].

### Plant material

Leaves were collected in March 2001 from plants of *Cnidoscolus aconitifolius* growing in the surrounding areas of the School of Biology and Veterinary Medicine, in Xmatkuil, a suburb of Mérida, Yucatán, México. Specimens were identified by one of us (Victor Parra-Tabla) and a sample has been deposited at the herbarium of the School of Biology and Veterinary Medicine of the University of Yucatán.

### Wax extraction

Approximately 15-20 freshly-cut leaves were dipped in CH<sub>2</sub>Cl<sub>2</sub> (1 L) for 20 seconds. The solvent was evaporated to dryness under reduced pressure to produce 533.3 mg of crude wax extract. Flash cc purification of the wax extract using a gradient elution with Hx/CH<sub>2</sub>Cl<sub>2</sub> yielded five major fractions

(A-E), each showing a single component on TLC. GC-MS analyses of fractions B, D and E confirmed the presence of a single component in each of them. The purified metabolites were identified as amyrenone (**1**,  $t_R$  11.17 min, 31.3 mg),  $\alpha$ -amyrin acetate (**2**,  $t_R$  13.73 min, 3.3 mg) and  $\beta$ -amyrin acetate (**3**,  $t_R$  14.88 min, 67.4 mg) respectively; GC analyses of fractions A and C clearly indicated the presence of at least two main components in each fraction ( $t_R$  7.25 and 9.78 min in A; and  $t_R$  11.95 and 12.92 min in C). The alkane metabolites in fraction A were not fully identified; the two components in fraction C were identified as  $\beta$  and  $\alpha$  amyirin (**4** and **5**, respectively).

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