

Sapogenin from Leaves of *Amaranthus cruentus*

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SUMMARY. The nutritional quality and haemolytic activity of *Amaranthus cruentus* leaves is well-known. Sapogenins present in these leaves were identified by the separation technique of Peñafiel and Díaz de Villar. After purification by silica gel column chromatography 0.47 g of oleanolic acid was obtained (yield: 22.3%). The structure of oleanolic acid was determined by the combination of IR, ¹H/¹³C NMR and MS spectroscopy. The glycone moiety (rhamnose) was identified by comparison (paper chromatography) with an authentic sample.

RESUMEN. "Sapogeninas de hojas de *Amaranthus cruentus*". Conocida la calidad nutricional de las hojas de *Amaranthus cruentus* y habiéndose detectado alta actividad hemolítica, se buscaron las sapogeninas presentes. La técnica de separación usada fue la de Peñafiel y Díaz de Villar, obteniéndose luego de purificación a través de cromatografía en columna de gel de sílice 0,47 g de ácido oleanólico (rendimiento: 22,3%). La estructura del aglicón se determinó mediante la combinación de espectroscopía IR, ¹H/¹³C NMR y MS. La glicona detectada por comparación con una muestra auténtica (cromatografía en papel) fue ramosa.

INTRODUCTION

Amaranthus cruentus is a plant of a well-known nutritional quality and its grain has intensively been studied. Its chemical composition, amino acid content and protein quality renders it useful for human feeding or as forage¹⁻⁴. The most widely used part of *A. Cruentus* is the seed, though the utilisation of leaves has been recently recommended as an alternative to spinach, cabbage and lettuce, among others.

Saponins have been implicated in reduced animal growth and performance⁵. Mostly, these substances are bitter, exhibit activity on the superficial tension and are highly haemolytic. These glycosides lack toxic effects for man, probably because they are not absorbed in the gastrointestinal tract and, besides, the occurrence of saponins do decrease the *Amaranthus* nutritional quality since they can be eliminated with the cooking water⁶.

KEY WORDS: *Amaranthus cruentus*, Oleanolic acid, Sapogenins.

PALABRAS CLAVE: Acido oleanólico, *Amaranthus cruentus*, Sapogeninas.

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It has been demonstrated that some saponins exert definite pharmacological actions, turning the research more interesting. The saponins isolated from *Panax ginseng* are effective anti-tumoral agents in the stomach cancer treatment ⁷. The saponins from the chinese carnation shows analgesic and antihepatotoxic activities ⁸ and those from *Calendula arvensis* are responsible for this plant haemolytic properties. An anti-inflammatory response has also been observed in carrageen-induced oedemas ⁹. The anti-fungal activity on plant pathogen fungi of a saponin isolated from alfalfa root has also been reported ¹⁰. Other saponins have been found to prevent the hipercholesterolemia by inhibiting the cholesterol intestinal absorption ^{11, 12}.

In a previous study the nutritional quality of the *A. cruentus* and *A. mangazzianus* was compared with that of chard, the antinutrients were determined, resulting a higher haemolytic activity in the *A. cruentus* leaves ¹³. The aim of the present study is to detect and identify the sapogenins present in *A. cruentus* leaves.

EXPERIMENTAL

Materials

Leaves from *Amaranthus cruentus* var. Don Guiem and var. Don Juan were employed. The material was dried in a forced-air stove at 60 °C during 48 h. The obtained product was ground in an electric mill, obtaining the powder which was here used.

Methods

For the sapogenin separation the technique of Peñafiel and Díaz de Villar ¹⁴ was followed. The quantities were prepared starting from 45 g of leaves, then the hydrolysis was performed, resulting 2.1 g of sapogenin extracts in organic phase. This extract was purified through a Silica gel column (MN-Kieselgel 60, 0.063-0.2 mm/70-230 mesh ASTM), using as eluent hexane and hexane-ethyl acetate mixture in different proportions until reaching pure ethyl acetate. TLC was applied to each portion (Silicagel 60 F₂₅₄ Merck). A pure white product (0.47 g; yield: 22.3%) was obtained from the fractions hexane:ethyl acetate 3:7, the structure of which was identified by spectroscopic methods and compared with an authentic sample.

The melting point was determined. The IR spectra was performed on a Beckman IR-10 spectrophotometer. The mass spectra EIMS were collected at 70 eV. The ¹HNMR (CDCl₃ /MeOD), ¹³CNMR and DEPT (CDCl₃) spectra were obtained with a 200 MHz spectrometer in 1H and 50.3 MHz in ¹³C.

After obtaining the aqueous extract from hydrolysis, paper chromatography with butanol:acetic acid:water (2:1:1) as running solvent and revealed with Patrich reagent against controls was used.

RESULTS AND DISCUSSION

The spectroscopic study and the physical properties determined that the sapogenin isolated from *A. cruentus* leaves was oleanolic acid: m.p.: 302-304 °C (lit. ref. 300-4 °C); IR. KBr cm⁻¹: 3600-3200 br (OH), 3000, 1700 (C=O), 1650 (C=C).EIM m/z (%): 456 M⁺ , (5); 438 M- H₂O (8), 411 M-COOH (11), 248 (91), 207 (20), 203

C	δ	C	δ
1	38,3	16	23,2 ^b
2	26,5	17	46,2
3	78,5	18	41,0
4	38,5	19	45,7
5	55,0	20	30,4
6	18,1	21	33,6
7	32,5 ^a	22	32,3 ^a
8	39,0	23	27,7
9	47,4	24	15,3
10	36,8	25	15,0
11	22,8 ^b	26	16,6
12	122,1	27	25,6
13	143,6	28	188,8
14	41,5	29	32,8
15	27,4	30	23,2

Table 1. ¹³C NMR chemical shifts of oleanolic acid. ^a, ^b Interchangeables signals

(100), 189 (22), 133 (28). ¹H NMR (CDCl₃ /MeOD): 0,77 (3H, s), 0,81 (3H, s), 0,81 (3H,s), 0,92 (3H, s), 0,93 (3H, s), 0,94 (3H, s), 0,99 (3H, s), 1,16 (3H, s), 2,82 (1H, dd, J= 12,5; 3Hz, H-18), 3,42 (1H, dd J = 7,8; 8,1 Hz., H-3), 5,3 (1H, t, J = 3,4 Hz., H-12). ¹³C NMR chemical shifts^{14,15} are showed in Table 1.

Paper chromatography showed a spot with the same R_f than the rhamnose control. According to the obtained results it can be concluded that in the *A. cruentus* leaves the sapogenin found was one corresponding to a pentacyclic triterpene: oleanolic acid, being rhamnose the genin

Numerous biological properties of oleanolic acid have been reported. Oleanolic acid has been marketed in China as an oral drug for human liver disorders. The mechanism of hepatoprotection by this compound may involve the inhibition of toxicant activation and the enhancement of the body defence systems. Oleanolic acid has also been long-recognised to have anti-inflammatory and anti-hyperlipidemic properties in laboratory animals¹⁷. Furthermore, oleanolic acid plays a role in anti-cancer treatment and has the ability to decrease undesirable radiation damage to the haematopoietic tissue after radiotherapy¹⁸.

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