

# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Chemical Constituents of *Anacolosa frutescens*.

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### ABSTRACT

Chemical investigation of the dichloromethane extract of the leaves of *Anacolosa frutescens* afforded 3-acetylaleuritolic acid (**1**),  $\beta$ -amyryn (**2**) and a mixture of monounsaturated and saturated fatty acids. The structures of **1** and **2** were identified by comparison of their <sup>13</sup>C NMR data with those reported in the literature.

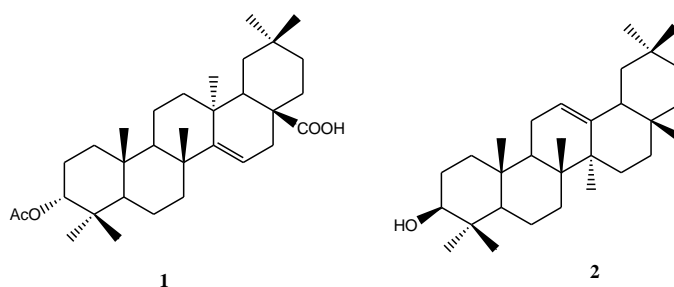
**Keywords:** *Anacolosa frutescens*, Olacaceae, 3-acetylaleuritolic acid,  $\beta$ -amyryn

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## INTRODUCTION

*Anacolosia frutescens* (Blume) Blume, commonly known as galonut, is a shrub or small tree which is a native of tropical Asia [3]. The fruits including the seeds are used as food [1], while the wood is used for house posts [4]. There are no reported studies on the chemical constituents and biological activities of *A. frutescens*. However, its congener, *A. pervilleana* was recently studied and found to exhibit antiviral properties. *A. pervilleana* afforded anacolosine, octadeca-9,11,13-triynoic acid, (13*E*)-octadec-13-en-9,11-diynoic acid, (13*E*)-octadec-13-en-11-ynoic acid, lupenone,  $\beta$ -amyrone, and (*S*)-sambunigrin. Lupenone and  $\beta$ -amyrone showed a moderate activity against CHIKV ( $EC_{50}$  77 and 86  $\mu$ M, respectively) and the acetylenic acids exhibited  $IC_{50}$  values around 3  $\mu$ M in the DENV RdRp assay [3].

We report herein the isolation and identification of the triterpenes, 3-acetylaleuritic acid (**1**),  $\beta$ -amyryn (**2**), and a mixture of monounsaturated and saturated fatty acids from the leaves of *A. frutescens*. To the best of our knowledge this is the first report on the isolation of these compounds from *A. frutescens*.



## MATERIALS AND METHODS

### General experimental procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in  $CDCl_3$  at 600 MHz for  $^1H$  NMR and 150 MHz for  $^{13}C$  NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F<sub>254</sub> and the plates were visualized by spraying with vanillin/ $H_2SO_4$  solution followed by warming.

### Sample Collection

The leaves of the plant were collected at Barangay Tambo Balagbag, Indang, Cavite in January, 2013. The leaves were identified as *Anacolosia frutescens* (Blume) Blume at the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman, Quezon City.

### Isolation

The air-dried leaves (1.22 kg) of *A. frutescens* were ground in a blender, soaked in  $CH_2Cl_2$  for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (65.9 g) which was chromatographed using increasing proportions of acetone in  $CH_2Cl_2$  at 10% increment. The 20% acetone in  $CH_2Cl_2$  fraction was rechromatographed (8  $\times$ ) using petroleum ether to afford a mixture of monounsaturated and saturated fatty acids (8 mg). The 40% acetone in  $CH_2Cl_2$  fraction was rechromatographed (6  $\times$ ) using 10% EtOAc in petroleum ether to yield **2** (5 mg) after washing with 2.5% EtOAc in petroleum ether. The 50% acetone in  $CH_2Cl_2$  fraction was rechromatographed (5  $\times$ ) using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether (2  $\times$ ) to afford **1** (3 mg).

### 3-Acetylaleuritic acid (**1**)

Colorless solid.  $^{13}C$  NMR (150 MHz,  $CDCl_3$ ):  $\delta$  37.37 (C-1), 23.45 (C-2), 80.00 (C-3), 37.66 (C-4), 55.58 (C-5), 18.69 (C-6), 40.77 (C-7), 39.00 (C-8), 49.04 (C-9), 37.91 (C-10), 17.30 (C-11), 33.65 (C-12), 37.31 (C-13), 160.59 (C-14), 116.72 (C-15), 31.36 (C-16), 51.33 (C-17), 41.48 (C-18), 35.32 (C-19), 29.27 (C-20), 33.31 (C-21), 30.71 (C-22),

27.94 (C-23), 16.58 (C-24), 15.61 (C-25), 26.13 (C-26), 22.39 (C-27), 183.56 (C-28), 31.89 (C-29), 28.68 (C-30), 171.00, 21.30 (OAc).

### ***β*-Amyrin (2)**

Colorless solid. <sup>13</sup>C NMR: 38.77 (C-1), 27.22 (C-2), 79.03 (C-3), 38.57 (C-4), 55.15 (C-5), 18.37 (C-6), 32.49 (C-7), 39.78 (C-8), 47.62 (C-9), 36.94 (C-10), 23.52 (C-11), 121.71 (C-12), 145.19 (C-13), 41.71 (C-14), 26.14 (C-15), 26.14 (C-16), 32.64 (C-17), 47.22 (C-18), 46.82 (C-19), 31.08 (C-20), 34.72 (C-21), 37.13 (C-22), 28.09 (C-23), 15.49 (C-24), 15.58 (C-25), 16.79 (C-26), 25.99 (C-27), 28.39 (C-28), 33.33 (C-29), 23.68 (C-30).

## **RESULTS AND DISCUSSION**

Silica gel chromatography of the dichloromethane extract of the leaves of *A. frutescens* afforded **1-2** and a mixture of monounsaturated and saturated fatty acids. Their structures were identified by comparison of their <sup>1</sup>H and <sup>13</sup>C NMR data with those reported in the literature for 3-acetylaleuritolic acid (**1**) [4], *β*-amyrin (**2**) [5] and a mixture of monounsaturated and saturated fatty acids.

Triterpene **1** exhibited antimicrobial activity against *S. aureus* and *S. typhimurium* [6]; significant inhibitory activity on vitality of adult male worms of *O. gutturosa* [7]; strong inhibition of DNA topoisomerase II and high cytotoxicity against human lung carcinoma A549 cells [8]. On the other hand, *β*-amyrin (**2**) was reported to possess anti-inflammatory activity [9, 10] and analgesic property [11, 12].

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