Sterols and Triterpenes From the Fruit of *Annona muricata* Linn.

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Annona muricata Linn., commonly known as guyabano, is a well-known medicinal tree. The bioactivities of *A. muricata* are varied, but the commonly isolated compounds are acetogenins which exhibit anticancer properties. This study reports on the isolation of the sterols: β-sitosterone (1), β-sitosteryl fatty acid ester (2) and β-sitosterol (3); and the triterpenes: β-amyrin (4), β-amyrin (5) and squalene (6) from the dichloromethane extract of the freeze-dried fruit of *Annona muricata* Linn. Compounds 1-6 were isolated by silica gel chromatography and identified by NMR spectroscopy. These compounds were reported to possess diverse bioactivities.

KEYWORDS: *Annona muricata*, Annonaceae, sitosterone, β-sitosteryl fatty acid ester, β-sitosterol, β-amyrin, β-amyrin, squalene

INTRODUCTION

Annona muricata Linn. of the family Annonaceae, commonly known as *guyabano,* is a well-known medicinal tree with anti-bacterial (Oberlies et al., 1997), antiviral (Padma et al., 1998; Betancur-Galvis et al., 1999), molluscicidal (Dos Santos &

Sant'Ana, 2001), anti-oxidative stress (Adewole & Caxton-Martins, 2006) and diuretic properties (Quisumbing, 1951). The bioactivities of *A. muricata* are varied, but the commonly isolated compounds are acetogenins which are known for their anticancer properties. Annohexocin, a mono-THF annonaceous acetogenin from the leaves of *A. muricata*, showed significant inhibitory effect against six human cancer cell lines: lung, breast, colon, pancreatic, kidney carcinoma and prostate adenocarcinoma (Zeng et al., 1995). Muricoreacin and murihexocin C, acetogenins from the leaves of *A. muricata*, exhibited significant cytotoxicities against six human tumor cell lines with selectivities to the prostate adenocarcinoma (PC-3) and pancreatic carcinoma (PACA-2) cell lines (Kim et al, 1998a). Annomuricine and muricapentocin showed significant cytotoxicities against six types of human tumors, with selectivity to pancreatic carcinoma (PACA-2) and colon adenocarcinoma (HT-29) cell lines (Kim et al., 1998b).

In our earlier study, we reported the isolation of three acetogenins: *cis*-annoreticuin and sabadelin from the fruit; and annoreticuin-9-one from the seeds of *A. muricata* (Ragasa et al., 2012). Annoreticuin-9-one was earlier reported to exhibit cytotoxic activities against the human pancreatic tumor cell line (PACA-2), human prostate adenocarcinoma (PC-3) and human lung carcinoma (A-549) (Craig Hopp et al., 1997),

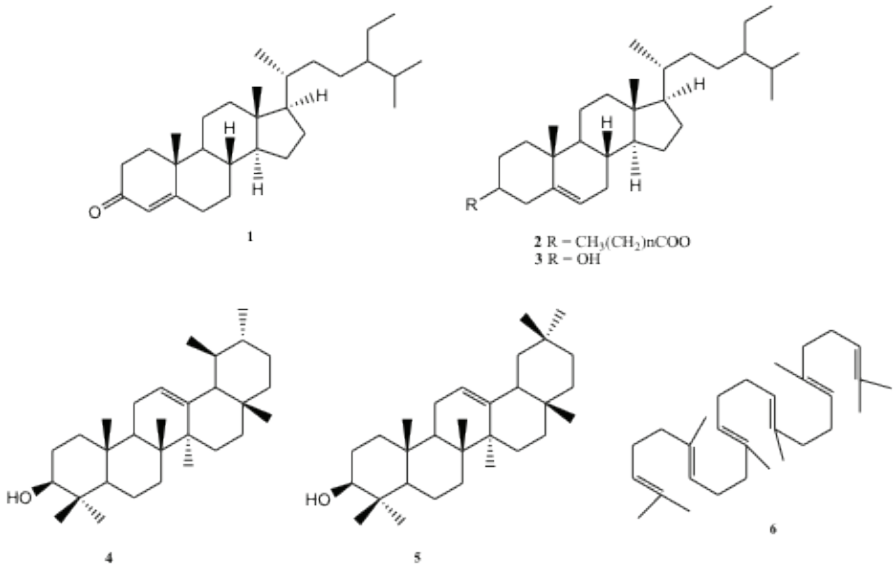

Figure 1. **The sterols: β-sitosterone (1), β-sitosteryl fatty acid ester (2) and β-sitosterol (3), and triterpenes: α-amyrin (4), β-amyrin (5), and squalene (6) from the freeze-dried fruit of *Annona muricata*.**

while *cis*-annoreticuin exhibited cytotoxicity against human hepatoma carcinoma cell line (Hep G2) (Liaw et al., 2004).

This article reports on the isolation of the sterols: β-sitosterone (1), β-sitosteryl fatty acid ester (2) and β-sitosterol (3); and the triterpenes: α-amyrin (4), β-amyrin (5), and squalene (6) from the fruit of *Annona muricata* (Fig. 1). To the best of our knowledge, this is the first report on the isolation of 1-6 from *A. muricata*.

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H 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_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 followed by warming.

Plant Material

The *Annona muricata* Linn. fruits were collected from Painaan, Rizal, Philippines in September 2012. The specimens of the plant were authenticated at the Bureau of Plant industry, Quirino Avenue, Manila, Philippines.

Extraction and Isolation

The flesh of the *A. muricata* fruit (3 kg) was separated from the seeds and fruit peel, and then freeze-dried. The freeze-dried flesh of the fruit (550 g) was 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 (5 g).

The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in CH2Cl2 (10% increment) as eluents. A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extract. Five milliliter fractions were collected. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass

column of 30.5 cm height and 1.3 cm internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

The CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed using petroleum ether as eluent. The less polar fractions were rechromatographed (3x) in petroleum ether to afford **6** (6 mg). The more polar fractions were rechromatographed (2x) in petroleum ether to afford **1** (4 mg). The 10% and 20% acetone in CH_2Cl_2 fractions were combined and rechromatographed (2x) using 1% EtOAc in petroleum ether, followed by 2.5% EtOAc in petroleum ether and finally, 5% EtOAc in petroleum ether to afford **2** (9 mg). The 30% and 40% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether, followed by 7.5% EtOAc in petroleum ether and finally, 10% EtOAc in petroleum ether to afford **3** (12 mg) and a mixture of **4** and **5** (8 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

The dichloromethane extract of the freeze-dried fruit of *Annona muricata* afforded the sterols: β-sitosterone (**1**), β-sitosteryl fatty acid ester (**2**) and β-sitosterol (**3**); and the triterpenes: α-amyrin (**4**), β-amyrin (**5**), and squalene (**6**) by silica gel chromatography. The structures of 1 and 2 were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR data with those reported in the literature for β-sitosterone (Prachayasittikul et al., 2009) and β-sitosteryl fatty acid ester (Julien-David et al., 2008), respectively. The structures of 3-6 were identified by comparison of their ^{13}C NMR data with those reported in the literature for β-sitosterol (Kojima et al., 1990), α-amyrin (Mahato & Kundo,, 1994), β-amyrin (Mahato & Kundo, 1994), and squalene (Brown & Martens, 1977), respectively.

Literature search revealed that the compounds isolated from *Annona muricata* were reported to possess diverse bioactivities. β-Sitosterone exhibited significant hypoglycemic (Alexander-Lindo et al., 2007), antiarrhythmic (Hotta et al., 2003) and pronounced antitubercular (Saludes et al., 2002) activities. β-Sitosterol had been shown to inhibit proliferation and induce apoptosis in human solid tumors such as colon and breast cancers (Park et al., 2007). α-Amyrin

and β-amyrin were reported to possess anti-inflammatory (Recio et al., 1995; Madeiros et al., 2007) and analgesic properties (Otuki et al., 2005; Soldi et al., 2008). Squalene had shown cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties (Farvin et al., 2006). Furthermore, this triterpene significantly suppresses colonic ACF formation and crypt multiplicity which strengthens the hypothesis that it possesses chemopreventive activity against colon carcinogenesis (Rao et al., 1998).

CONCLUSION

Compounds **1-6** which were isolated for the first time from *A. muricata* were reported to possess diverse biological activities. β-Sitosterone exhibited significant hypoglycemic, antiarrhythmic and antitubercular activities, while β-sitosterol inhibited proliferation and induced apoptosis in human solid tumors. α-Amyrin and β-amyrin were reported to possess anti-inflammatory and analgesic properties, while squalene showed cardioprotective effect and significantly suppressed colonic ACF formation and crypt multiplicity.

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