Chemical constituents of *Annona muricata*

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ABSTRACT

Chemical investigations of the freee-dried seeds of *Annona muricata* led to the isolation of murisolin (1), annoreticuin-9-one (2), cis-annoreticuin (3), sabadelin (4), β-sitosterol (5), stigmasterol (6), and triglyceride (7); while the fruits afforded 2-5. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy, while the structures of 2-7 were identified by comparison of their ¹³C NMR data with those reported in the literature.

Keywords: *Annona muricata*, Annonaceae, murisolin, annoreticuin-9-one, cis-annoreticuin, sabadelin

INTRODUCTION

*Annona muricata* Linn. of the family Annonaceae, commonly known as *guyabano*, is a well-known medicinal tree with anti-bacterial [1], antiviral [2, 3], molluscidal [4], anti-oxidative stress [5] and diuretic properties [6]. The ethanolic extract of the stem bark of *Annona muricata* demonstrated inhibitory activity against cytopathic effects of Herpes simplex virus-1 (HSV-1) [2]. The dry ethanolic extract of *A. muricata* leaf has molluscidal properties against *Biomphalaria glabrata*. [4]. *A. muricata* aqueous leaf extract treatment diminished and/or prevented pancreatic oxidative damage produced by Streptozotocin and showed antioxidant activity [5]. The juice of the ripe fruit is said to be a diuretic, while a decoction of powdered immature fruits is a dysentery remedy [6]. Aqueous extracts of the skin of *A. muricata* exhibited antibacterial effect against *Staphylococcus aureus* and *Vibrio cholera* [7].

The leaves, root and stem barks of *A. muricata* afforded seven isoquinoline alkaloids: reticuline, coclaurine, coreximine, atherosperminine, stepharine, anomurine and anomuricine [8]. The essential oil of the fresh fruit pulp of *A. muricata* yielded 2-hexenoic acid methyl ester (23.9%), 2-hexenoic acid ethyl ester (8.6%), 2-octenoic acid methyl ester (5.4%), 2-butenoic acid methyl ester (2.4%), β-caryophyllene (12.7%), 1,8-cineole (9.9%), linalool (7.8%), α-terpineol (2.8%), linalyl propionate (2.2%), and calarene (2.2%) [9]. The seeds of *A. muricata* afforded annomuricatin A [10].

Annohexocin, a mono-THF annonaceous acetogenin from the leaves of *A. muricata* exhibited significant inhibitory effects against six human cancer cell lines: lung, breast, colon, pancreatic, kidney carcinoma with selectivity for the prostate adenocarcinoma, PC-3 (ED₅₀ = 0.0195 µg/mL) [7]. Muricoreacin and murihexocin acetogenins from the
leaves of *A. muricata* showed significant cytotoxicities against six human tumor cell lines with selectivities to the prostate adenocarcinoma (PC-3) and pancreatic carcinoma (PACA-2) cell lines [11].

Fig. 1. Acetogenins from *Annona muricata* seeds and fruits: murisolin (1), annoreticuin-9-one (2), *cis*-annoreticuin (3), and sabadelin (4)

In an earlier study, we reported the isolation of annoreticuin-9-one from the seeds of *A. muricata*, while the fruits yielded *cis*-annoreticuin and sabadelin [12]. In another study, we reported the isolation of β-sitosterone, β-sitosteryl fatty acid ester, β-sitosterol, α-amyrin, β-amyrin, and squalene from the fruits [13].
We report herein the isolation and identification of the acetogenins: murisolin (1), annoreticin-9-one (2), cis-annoreticin (3), and sabadelin (4) (Fig. 1), as well as a mixture of β-sitosterol (5) and stigmasterol (6) in a 3:1 ratio, and triglyceride (7) from the seeds; while the fruits yielded 2-5. To the best of our knowledge this is the first report on the isolation of 1, 6 and 7 from a local collection of Annona muricata and the first report on the isolation of 3 and 4 from the seeds and 2 from the fruits.

MATERIALS AND METHODS

General Experimental Procedure
NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl₃ at 600 MHz for ²H NMR and 150 MHz for ¹³C 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 F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

Sample Collection and Extraction
The fruits of Annona muricata which were collected from Davao, Philippines were bought from Q-Mart, Quezon City, Philippines in July 2013. The seeds were separated from the fruit, ground in a blender, and then freeze-dried. The freeze-dried seeds (200 g) and fruits (500 g) were separately soaked in CH₂Cl₂ (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. 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 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation
The crude seeds extract was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The 10% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to afford 7 (105.4 mg). The 30% acetone in CH₂Cl₂ fraction was rechromatographed (4 ×) in 12.5% EtOAc in petroleum ether to afford 4 (2.5 mg) after washing with petroleum ether. The 40% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) in 15% EtOAc in petroleum ether to afford a mixture of 5 and 6 (15.5 mg) after washing with petroleum ether. The 60% and 70% acetone in CH₂Cl₂ fractions were combined and rechromatographed (5 ×) in CH₂CN:Et₂O:CH₂Cl₂ (1:1:8, v/v) to afford 1 (4.3 mg) after trituration with petroleum ether. The 80% and 90% acetone in CH₂Cl₂ fractions were combined and rechromatographed (4 ×) in CH₂CN:Et₂O:CH₂Cl₂ (1.5:1:5.7, v/v) to afford 2 (3.2 mg) after trituration with petroleum ether. The 100% acetone fraction was rechromatographed by gradient elution in CH₂CN:Et₂O:CH₂Cl₂ (0.5:0.5:9, v/v), followed by CH₂CN:Et₂O:CH₂Cl₂ (1:1:8, v/v), followed by CH₂CN:Et₂O:CH₂Cl₂ (1.5:1:5.7, v/v) and finally CH₂CN:Et₂O:CH₂Cl₂ (2.5:2.5:5, v/v) to afford 3 (2.1 mg) after trituration with petroleum ether.

The crude fruits extract was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment. The 30% acetone in CH₂Cl₂ fraction was rechromatographed (4 ×) in 12.5% EtOAc in petroleum ether to afford 4 (21.8 mg) after washing with petroleum ether. The 50% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) in 15% EtOAc in petroleum ether to afford 5 (3.4 mg) after washing with petroleum ether. The 90% acetone in CH₂Cl₂ fractions were combined and rechromatographed (4 ×) in CH₂CN:Et₂O:CH₂Cl₂ (1.5:1:5.7, v/v) to afford 2 (1.2 mg) after trituration with petroleum ether. The 100% acetone fraction was rechromatographed by gradient elution in CH₂CN:Et₂O:CH₂Cl₂ (0.5:0.5:9, v/v), followed by CH₂CN:Et₂O:CH₂Cl₂ (1:1:8, v/v), followed by CH₂CN:Et₂O:CH₂Cl₂ (1.5:1:5.7, v/v) and finally CH₂CN:Et₂O:CH₂Cl₂ (2.5:2.5:5, v/v) to afford 3 (1.5 mg) after trituration with petroleum ether.

Murisolin (1): ¹H NMR (600 MHz, CDCl₃) δ 1.23-1.40 (m, CH₃), 1.43 (d, J = 7.2 Hz, H-35), 1.48, 1.34 (H-6), 1.65, 1.98 (m, H-17, H-18), 2.52, 2.38 (m, H-3), 3.38 (m, H-15, H-20), 3.78 (m, H-16, H-19), 3.82 (m, H-4), 7.16 (d, J = 1.2 Hz, H-33), 5.04 (qd, 1H, J = 7.2, 1.2 Hz, H-34), 0.88 (t, J = 7.2 Hz, H-35); ¹³C NMR (150 MHz, CDCl₃) δ
RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the freeze-dried seeds of *A. muricata* yielded the acetogenins: murisolin (1) [14,15], annoreticuin-9-one (2), cis-annoreticuin (3), and sabadelin (4) [12], as well as a mixture of β-sitosterol (5) [16] and stigmasterol [6] [17] in a 3:1 ratio, and triglyceride (7) [18] from the seeds; while the fruits afforded cis-annoreticuin (18). The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of its $^1$H and/or $^{13}$C NMR data with those reported in the literature [14, 15]. The structures of 2-7 were identified by comparison of their $^{13}$C NMR data with those reported in the literature [12, 16-18]. The 3:1 ratio for the mixture of 5 and 6 was deduced from the the integrations of the $^1$H NMR resonances for the olefinic protons of 5 at $\delta$ 5.32 (dd, $J = 1.8, 5.4$ Hz, H-6) and 6 at $\delta$ 5.32 (dd, $J = 1.8, 5.4$ Hz, H-6), 5.13 (dd, $J = 8.4, 15.0$ Hz, H-22) and 5.00 (dd, $J = 8.4, 15.0$ Hz, H-22). The triglyceride is made up of diunsaturated, monounsaturated and saturated fatty acids as deduced from the integrations of the resonances for the methyl protons at $\delta$ 0.87, 0.86 (t, $J = 6.6$ Hz) for the three fatty acids and the resonances at $\delta$ 2.75 (double allylic methylene), 2.00 (allylic) and 5.32 (olefinic) protons for the diunsaturated fatty acid; and $\delta$ 2.00 (allylic) and 5.32 (olefinic) protons for the monounsaturated fatty acid. The diunsaturated fatty acid could be linoleic acid, while the monounsaturated fatty acid could be oleic acid based on the integration for the methylene protons at $\delta$ 1.23-1.35.

Although no biological activity tests were conducted on the isolated compounds, except for 4, literature search revealed that these have diverse biological activities as follows.

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A study reported that murisolin (1) exhibited inhibitory action with bovine heart mitochondrial complex I [19]. It showed potent and selective cytotoxicities among six human tumor cell lines [20]. Another study reported that 1 from the seeds of *A. muricata* exhibited cytotoxic activity [21]. It also showed cytotoxicity against human hepatoma cells, Hep G2 [22] and human tumor cell lines [23].

Annoreticuin-9-one (2) was first isolated from the fruits of *A. reticulata*. This acetogenin was also isolated from *A. squamosa* and reported to exhibit cytotoxic activities against the human pancreatic tumor cell line, PACA-2, with ED$_{50}$ = 2.4 × 10$^{-4}$ µg/mL; human prostate adenocarcinoma, PC-3, with ED$_{50}$ = 9.8 × 10$^{-3}$ µg/mL; human lung carcinoma, A-549, with ED$_{50}$ = 2.7 × 10$^{-2}$ µg/mL, and brine shrimp lethality test, BST, with LC$_{50}$ = 2.4 × 10$^{-3}$ µg/mL [24]. Another study reported that 2 exhibited significant cytotoxicities against Hep 2,2.15 (human hepatoma cell transfected HBV), Hep G2 (human hepatoma cell), KB (human nasopharyngeal carcinoma) and CCM2 (human colon tumor cell) cell culture systems with ED$_{50}$ values of 5.40 × 10$^{-3}$, 5.40 × 10$^{-4}$, 3.10, and 1.00 × 10$^{-2}$ µg/ml [25].

*Cis*-annoreticuin (3) was first isolated from the seeds of *A. montana* and reported to exhibit cytotoxic activity against human hepatoma carcinoma cell line, Hep G2 with ED$_{50}$ = 2.4 × 10$^{-3}$ µg/mL [26].

β-Sitosterol (5) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [27]. It was shown to be effective for the treatment of benign prostatic hyperplasia [28]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench radical in-vitro, making it a potential anticancer drug for colon carcinogenesis [29]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [30]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [31].

Stigmasterol (6) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [32]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [33]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells [34], markedly inhibited tumour promotion in two stage carcinogenesis experiments [35], exhibited antimitogenic [36], topical anti-inflammatory [37], anti-osteoarthritic [38] and antioxidant [39] activities.

Triglycerides (7) were reported to exhibit antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes* [40]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [41]. Linoleic acid belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces risk of colon and breast cancer [42] and lowers cardiovascular disease risk and inflammations [43]. Oleic acid has been reported to be responsible for the reduction of blood pressure induced by olive oil [44]. It may hinder the progression of adenoleukodystrophy, a fatal disease that affects the brain and adrenal glands [45]. Oleic acid inhibited cancer cell growth and survival in low metastatic carcinoma cells, such as gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines [46].

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REFERENCES


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