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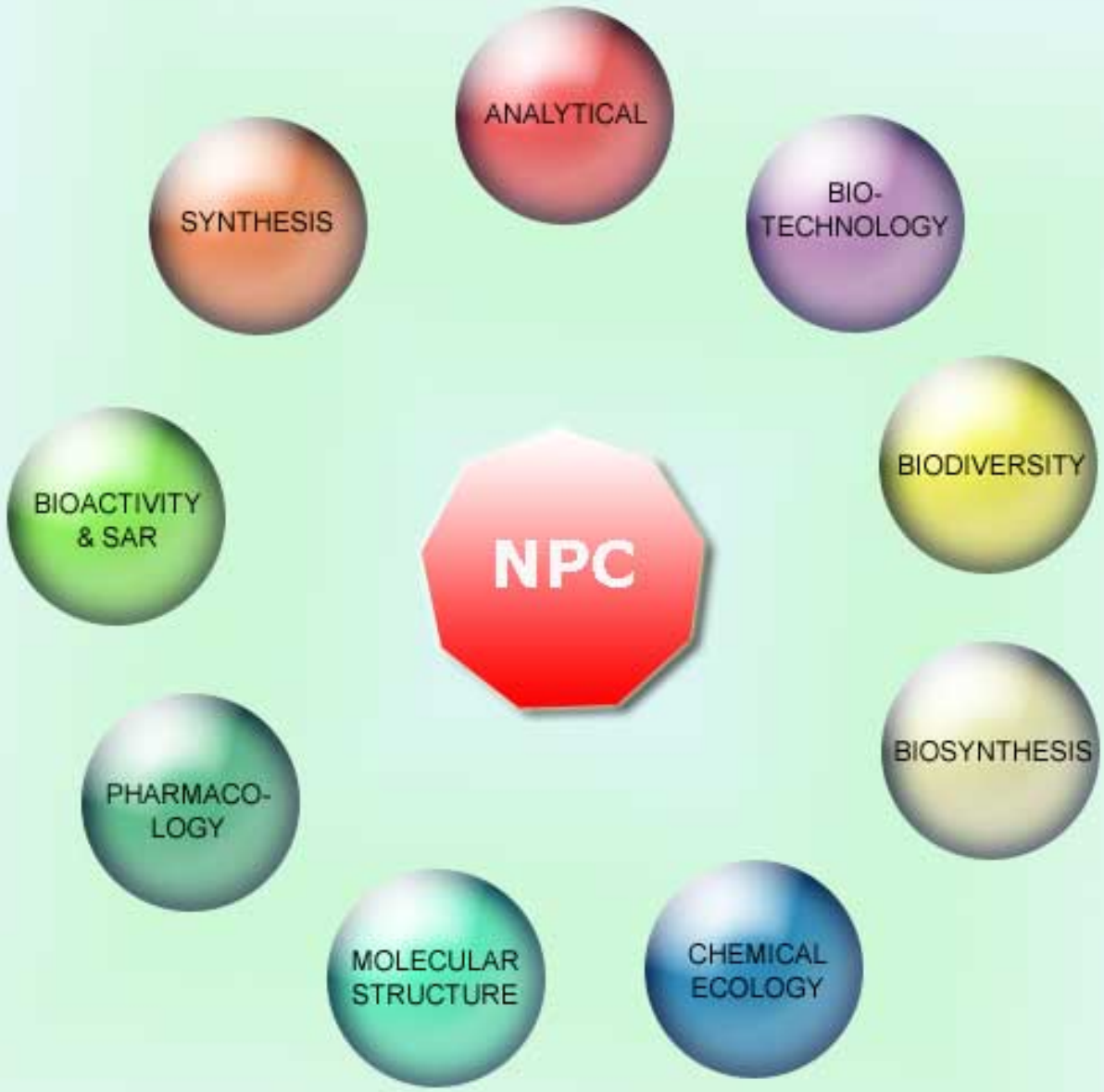

This Issue is Dedicated to
Professor Peter G. Waterman

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Annona muricata (Graviola): Toxic or Therapeutic

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Dedicated to Professor Peter G Waterman, one of the pioneers of phytochemical research.

The medicinal plant *Annona muricata* (Annonaceae), also known as Graviola or Soursop, is reported here to contain imino sugar alkaloids. This is the first report of imino sugars in the Annonaceae. Graviola has very broad medicinal claims and is also widely consumed as a food and in drinks in the tropics. The plant produces a wide range of secondary chemicals, some already known to be toxic, but the discovery here of the imino sugars as a new group of chemicals, including the neurotoxin swainsonine, raises questions about the safety of consumption of this plant.

Keywords: *Annona muricata*, soursop, graviola, imino sugar, swainsonine, toxicity, immune modulator, glycosidase inhibitors.

Of the 60 or more species of the genus *Annona*, family Annonaceae, the soursop or graviola, *A. muricata* L., is the most tropical, the largest-fruited, and the only one lending itself well to preserving and processing. The leaves and bark contain cytotoxic acetogenins and the plant also produces isoquinoline and phenanthrene alkaloids [1]. The bark is high in hydrocyanic acid. The seeds contain 45% of a yellow non-drying oil, which is an irritant poison, causing severe eye inflammation. There are many medicinal claims for *A. muricata*. The juice of the ripe fruit is said to be diuretic and a remedy for haematuria and urethritis. Taken when fasting, it is believed to relieve liver ailments and leprosy. Pulverized immature fruits, which are very astringent, are decocted as a dysentery remedy. To speed wound healing the flesh of the unripe fruit is applied as a poultice and left unchanged for 3 days. Consumption of the plant is also thought to be related to an abnormally high frequency of levodopa-resistant Parkinsonism or progressive supranuclear palsy in the French West Indies [2]. Studies with neurons in culture have indicated that alkaloids from *A. muricata* vegetative tissues can modulate the function and the survival of dopaminergic nerve cells *in vitro*.

We here report, for the first time, the presence of imino sugars, including the neurotoxic alkaloid swainsonine (**1**) in *A. muricata.* Swainsonine is a potent mannosidase inhibitor that causes neurotoxicity through inhibition of lysosomal acidic α-mannosidase [3]. Although the concentration of swainsonine was low in the commercial herbal medicine sample we purchased (0.0004% dry weight), it has been estimated that as little as 0.001% in the diet may cause neurological disorders in livestock [3]. The toxic dose for humans and symptoms are not known. It may well be that the concentrations of swainsonine will vary in *A. muricata* products due to, for example, variety, growth conditions, harvesting time and processing method. Imino sugars are always distributed

throughout plant organs, but are usually concentrated in fruits and seeds [4].

Glycosidase-inhibiting imino sugar alkaloids arouse a lot of interest for potential therapeutic applications including diabetes, viral infections and anti-cancer uses [4]. In view of the range of medicinal claims for *A. muricata* it is interesting, therefore, to note also the immuno-modulatory and anti-cancer activity of swainsonine and of pyrrolizidine imino sugars, such as casuarine (**2**) [5], which was also found in *A. muricata*. The plant material analyzed also contained the glucosidase-inhibiting imino sugars calystegine B_2 (**3**), deoxynojirmycin (DNJ) (**4**) and 2*R*, 3*R*, 4*R*, 5*R*-2,5-dihydroxymethyl-3,4,-dihydroxypyrrolidine (DMDP) (**5**). Another imino sugar was tentatively identified as deoxymannojirimycin (DMJ) (**6**). DMDP (**5**) was the most major imino sugar, being present at 0.01% dry weight. The specificity of glycosidase inhibition is very important for its potential therapeutic activity with DNJ (**4**), for example, having anti-viral activity through inhibition of glycoprotein processing glucosidases [4]. DMJ (**6**) and swainsonine (**1**) inhibit mannosidases very specifically and can have anti-viral activity through inhibition of glycoprotein processing mannosidases. Swainsonine has also been shown to have potent anti-cancer activity [4]. Calystegine B_2 is a strong competitive inhibitor of human liver α-galactosidase and rat liver β-glucosidase [6].

A. muricata highlights the complexity of understanding whether medicinal plants are therapeutically useful. In many cases it is not certain that they have any therapeutically-active components other than perhaps important nutrients. In the case of *A. muricata*, there are many very biologically-active components, including acetogenins and alkaloids. We now add to the known complexity of this plant by reporting glycosidase-inhibiting imino sugars, some of which are known to have direct anti-viral activity (e.g. DNJ, DMJ) and others that stimulate immune responses (e.g. swainsonine and casuarines). However, swainsonine and calystegines have been implicated as neurotoxins in livestock [7] and their presence in *A. muricata* raises the possibility that they might be involved in neurological disorders in man associated with consumption of the plant [2].

The polyhydroxylated alkaloids, as tms derivatives, have good resolution and give characteristic mass spectral fragmentation patterns, as shown for swainsonine (**1**) at 7.9 min (Figure 1). The other imino sugars were identified by comparison with authentic standards and were DMDP (**5**) [retention time 7.6 min and main fragments 217 (50%), 258 (30%), 348 (100%)], DNJ (**4**) [retention time 9.8 min and main fragments 217 (100%), 258 (80%) and 348 (80%)], calystegine B_2 (**3**) [(retention time 10 min with main fragments 217 (100%), 244 (10%), 284 (10%)], and casuarine [(retention time 10.5 min and main fragments 217 (40%), 258 (40%) and 462 (100%)]. In addition there was an unknown imino sugar at 7.8 min, thought to be deoxymannojirimycin (DMJ) (**6**) [(main fragments at 217 (100%), 258 (30%) and 348 (30%)].

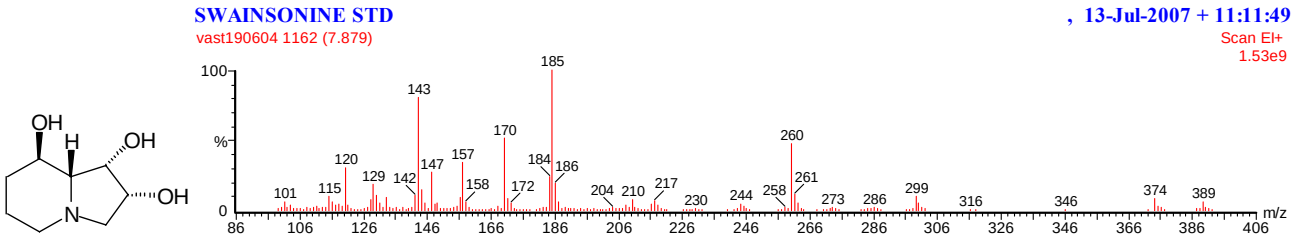
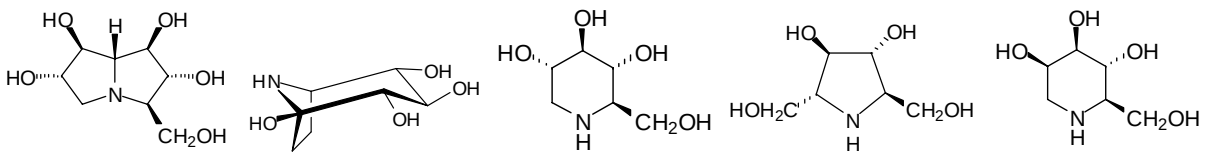


Figure 1: Structure and mass spectrum of swainsonine (**1**) as the *O*-trimethylsilylderivative



(**2**) (**3**) (**4**) (**5**) (**6**)

Figure 2: Structure of casuarine (**2**), calystegine B_2 (**3**), deoxynojirmycin (**4**), DMDP (**5**) and deoxymannonojirmycin (**6**).

Experimental

Plant material: *Annona muricata* material (leaves/stems) from Brazil was purchased from Raintree Nutrition, Inc, Carson City, NV 89701, USA (July 2007 batch 0 12345 10011 6).

Extraction and GC-MS analysis: The plant material powder was extracted at room temperature in 50% aqueous ethanol for 15 h. The extract was filtered and run through strongly acidic cation exchange resin (IR120, Merck, H^+ form) and the alkaloids displaced by 2M ammonia solution, after washing the resin with water. The alkaloid fractions were dried and weighed. GC-MS was conducted on 1 mg samples of each cation exchange bound sample using 200 µL of Sigma Sil A reagent to produce *O*-trimethylsily-derivatives (tms). Samples were heated at 60°C for 15 minutes and then left at room temperature for at least 60 min. Insoluble reaction products were sedimented by centrifugation, and the supernatant was transferred to fresh vials, using a syringe. The GC column was a high polarity, fused-silica column (Varian 'Factor Four' VF-5ms column, 25 m x 0.25 mm i.d., 0.25 µm phase thickness). The carrier gas (helium) flow rate was 1 mL min^{-1}. TMS derivatives were separated using a temperature program that started at 160°C for 5 min, followed by a linear increase to 300°C at a rate of 10°C min^{-1}. The temperature was held at 300°C for an additional 10 min; the total analysis time was 29 min. EI MS of the column eluant was carried out using a Perkin Elmer TurboMass Gold mass spectrometer, with a quadrupole ion filter system, which was run at 250°C constantly during analysis. The detector mass range was set to 100 to 650 amu. The temperature of the transfer line (GC to MS) was held at 250°C. Samples were injected onto the column via a split vent (split ratio 50:1) through a fused silica narrow bore injection liner packed with deactivated quartz wool; the injection port temperature was maintained at 200°C. The injection volume was 1 µL. System control, data collection and mass spectral analysis was carried out using Perkin Elmer TurboMass software (TurboMass v. 4.4). All compounds were identified by GC-MS through comparison with authentic standards. The swainsonine concentration was calculated by comparison with an internal standard of the related alkaloid castanospermine, which was added at a concentration range of 0.0001-0.1mg into 10g of the original plant material prior to extraction. A dose response curve of the two alkaloids together was also used to determine the comparative response factors for the alkaloids. The quantity of DMDP (5) was determined by isolation and the structure confirmed by proton NMR spectroscopy.

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