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Sterol and Lipids from *Fejervarya vittigera* (Wiegmann)

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ABSTRACT

Fejervarya vittigera (Wiegmann, 1834) commonly known as Luzon wart frog is an edible farm frog endemic to the Philippines. Chemical investigation of the dichloromethane extracts of F. vittigera has led to the isolation of cholesterol (1) and triacylglycerols (2) from the skin and the muscle and bones. The structures of 1 and 2 were identified by comparison of their NMR data with those reported in the literature

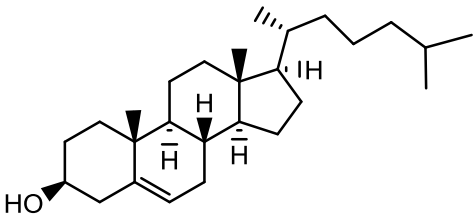
***KEYWORDS**: Fejervarya vittigera, Dicroglossidae, cholesterol, triacylglycerols*

INTRODUCTION

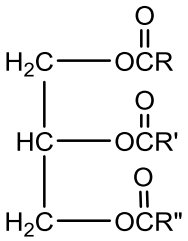
Fejervarya vittigera (Wiegmann, 1834) commonly known as Luzon wart frog is native to the Philippines. It is usually found in anthropogenic habitats such as agricultural areas, ditches, artificial ponds, and lakes [1]. They are abundant during the rainy season when they are found along rice paddies and ponds. In the Philippines, they are used as food and are sold in the local markets during the rainy season. There is no reported study on the chemical

constituents of *F. vittigera.* We report herein the isolation of cholesterol (**1**) and triacylglycerols (**2**) from the skin and the muscle and bones of *F. vittigera.* The structures of **1** and **2** are presented in Figure 1.

Figure-1: Chemical structures of cholesterol (1) and triacylglycerols (2) from *F. vittigera.*



1



2 R, R', R" = long chain saturated fatty acid alkyls

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

The Luzon wart frogs were collected from Florida Blanca, Pampaga, Philippines in December 2015. The samples were authenticated as *Fejervarya vittigera* (Wiegmann, 1834) at the Philippine National Museum.

General Isolation Procedure

The freeze-dried Luzon wart frogs were separated into the skin (9.71 g) and the muscle and bones (80.31 g). A glass column 12 inches in height and 0.5-inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and re-chromatographed in appropriate

solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the chemical constituents from the skin of F. vittigera

The skins (9.71 g) of *F. vittigera* were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.60 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume. The 30% to 40% acetone in CH_2Cl_2 fractions were combined and re-chromatographed using 10% EtOAc in petroleum ether to yield **2** (7 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was re-chromatographed using 20% EtOAc in petroleum ether to yield **1** (5 mg) after washing with petroleum ether.

Isolation of the chemical constituents from the muscle and bones of F. vittigera

The muscle and bones (80.31 g) of *F. vittigera* were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (15.21 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume. The 20% to 40% acetone in CH_2Cl_2 fraction were combined and re-chromatographed using 5% EtOAc in petroleum ether to yield a **2** (15 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was re-chromatographed using 15% EtOAc in petroleum ether to yield **1** (12 mg) after washing with petroleum ether.

Cholesterol (**1**): ^{1}H-NMR (400 MHz, $CDC1_3$): δ 5.35 (brd, *J* = 5.2 Hz, H-6), 3.52 (m, H-3), 1.01 (s, H-19), 0.91 (d, *J* = 6.4 Hz, H-21), 0.87 (d, *J* = 6.8 Hz, H-26), 0.86 (d, *J* = 6.8 Hz, H-27), 0.68 (s, H-18).

Triacylglycerols (**2**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27 (dd, *J* =4, 12 Hz, glyceryl CH_2O), 4.12 (dd, *J* =5.6, 11.6 Hz, glyceryl CH_2O), 5.23 (m, glyceryl CHO); 5.31 (=CH), 2.27 (t, *J* = 7.2 Hz, α-CH_2), 1.80-2.00 (allylic, CH_2), 1.58 (β-CH_2), 1.23-1.25 (CH_2), 0.85 (t, *J* =6.4 Hz, CH_3), 0.86 (t, *J* =6.4 Hz, 2 ×CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the skin and muscle and bones of *F. vittigera* has led to the isolations of cholesterol (**1**) and triacylglycerols (**2**). The NMR spectra of **1** are in accordance with data reported in the literature for cholesterol [2] and **2** for triacylglycerols [3]. Based on integrations and intensities of ^{1}H NMR resonances of **2**, the fatty acids in the triacylglycerols are two saturated and one monounsaturated fatty acids.

According to the American Heart Association, a diet high in cholesterol, saturated fats, and trans fats can raise blood cholesterol levels and can be a risk for heart disease [4].

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