Biologically Active Acylglycerides from the Berries of Saw-Palmetto (*Serenoa repens*)

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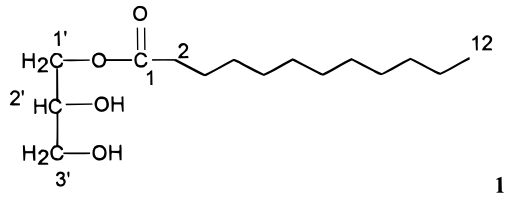
Brine shrimp lethality-directed fractionation of the 95% EtOH extract of the powdered, dried berries of *Serenoa repens* (Bart.) Small (saw-palmetto) (Palmae) led to the isolation of two monoacylglycerides, 1-monolaurin (**1**) and 1-monomyristin (**2**). Compounds **1** and **2** showed moderate biological activities in the brine shrimp lethality test and against renal (A-498) and pancreatic (PACA-2) human tumor cells; borderline cytotoxicity was exhibited against human prostatic (PC-3) cells. The fruits and extracts of saw-palmetto are taken orally as an herbal medicine to prevent prostatic hyperplasias.

Serenoa repens (Bart.) Small (Palmae), commonly known as saw-palmetto, is native to North America in the states of South Carolina, Louisiana, Georgia, and Florida. Extracts of *Serenoa repens*, prepared by supercritical fluid extraction with CO_2 or by extracting with hexane, have been used for the treatment of benign prostatic hyperplasia (BPH) and nonbacterial prostitis.[2,3] Several fatty acids and sterols have been isolated;[4] however, no specific therapeutically useful compounds from this plant have been identified. In the present work, the 95% EtOH extract of the powdered dried berries of *Serenoa repens* was fractionated, directed by the brine shrimp lethality test (BST),[5] to obtain bioactive compounds **1** and **2**, which were then tested for cytotoxic activities against three human tumor cell lines.

Initial screening of the 95% EtOH extract of the berries and subsequent solvent partitions (CH_2Cl_2−H_2O followed by 90% MeOH−hexane partition of the CH_2Cl_2 residue) indicated that the 90% MeOH partition residue was the most lethal to brine shrimp (LC_{50} 79.9 ppm).[5] Hence, this extract was subjected to further bioactivity-directed fractionation. Chromatographic separations over Si gel of the 90% MeOH residue, using open columns and HPLC, led to the isolation of two acylglycerides (**1** and **2**) differing by two methylene groups in the fatty acid side chain. The structures were elucidated by spectroscopic methods including ^{1}H NMR and MS.

The molecular weight of **1** was indicated by the CIMS (isobutane) peak at *m*/*z* 275 [MH^+], and the existence of a glycerol backbone was indicated by a fragment at *m*/*z* 183 [MH − 92 (glycerol part)]. In the same way, the molecular weight of **2** was indicated by the peaks at *m*/*z* 303[MH^+] and 211 [MH − 92(glycerol part)]. Because the ^{1}H-NMR spectra of both appeared very similar, the lengths of the side chains were determined by the molecular weights obtained by low resolution CIMS. The existence of hydroxyl groups in both structures was suggested from the loss of H_2O (*m*/*z* 18) from the [MH^+] in the CIMS spectra. The existence of a glycerol backbone was also suggested by a ^{1}H-NMR resonance at δ 3.61 (2H, H-3′), δ 3.90 (1H, H-2′), and δ 4.12 (2H, H-1′) in **1**, and at δ 3.63 (2H, H-3′), δ 3.90 (1H, H-2′), and δ 4.15 (2H, H-1′) in **2**. Both H-1′ and H-3′ protons were split by H-2′, and, because of the chirality at C-2′, the protons attached to the same carbon were also geminally coupled. The pattern of splitting was agreeable with a standard spectrum found in the Sadtler NMR catalogue.[6] Proton resonances at δ 2.31 (2H, H-2) in **1** and δ 2.32 (2H, H-2) in **2** corresponded to the downfield shift expected for protons of an α-methylene group. Proton resonances at δ 1.60 (2H, H-3) in **1** and δ 1.59 (2H, H-3) in **2** corresponded to the downfield shift expected for protons of a β-methylene group. From the spectral information above, the mono-acylglycerides were elucidated to be the known compounds, 1-monolaurin (**1**) and 1-monomyristin (**2**).



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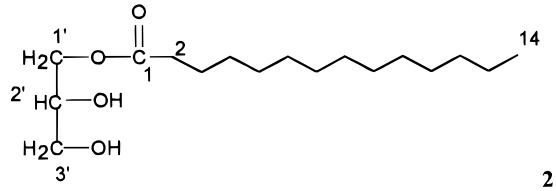

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Both **1** and **2** were moderately bioactive in the BST. Table 1 summarizes their bioactivities and those of the FOO5 fraction. Compounds **1** and **2** were moderately but significantly cytotoxic to the renal (A-498) and pancreatic (PACA-2) cell lines. Significant cytotoxicity is usually considered if the ED_{50} value is ≤ 4 μg/mL. Activity of **2** against the prostatic cell line (PC-3) is borderline. Compound **2** is slightly more active than **1**. This level of cytotoxic efficacy is surprising for such simple plant metabolites and may help to explain the apparent effectiveness of the herbal products against prostatic hyperplasia. Although these compounds could be expected to be rapidly metabolized in vivo, it is

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Table 1. Bioactivities of FOO5, **1** and **2**

	BST[a]	A-498[b]	PC-3[c]	PACA-2[d]
FOO5 (MeOH)	79.9	31.5	35.7	29.9
1	79.2	3.77	23.28	2.33
2	53.3	3.58	8.84	1.87
adriamycin[e]		1.22×10^{-2}	1.19×10^{-2}	3.92×10^{-2}

[a] Brine shrimp lethality test; LC_{50} values are in μg/mL. [b] Kidney carcinoma. [c] Prostate adenocarcinoma. [d] Pancreas carcinoma. [e] Positive control standard for MTT test; all cytotoxicities are ED_{50} values in μg/mL.

noteworthy that mammalian metabolism of triacylglycerides produce 2-acylglycerides and not 1-acylglycerides.[7] Phosphorylation of **1** and **2** could create highly cytotoxic surfactants as are created by the lipase in certain snake venoms.[7] They could exhibit membrane asymmetry or change the membrane order.[8] More studies will be needed to reevaluate the bioactivities of this kind of molecule.

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp apparatus. LRMS were recorded on a Finnigan 4000 quadrupole mass spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ with TMS as internal reference on a Bruker 300s spectrometer at 300.13 MHz. Si gel (60−200 mesh) was used for open columns. Si gel 60 PF-254(EM 5714) was used for TLC analysis, and 5% phosphomolybdic acid in EtOH spray was used for visualization. HPLC was carried out with a Rainin UV-1 detector set at 220 nm.

Plant Material. The Saw Palmetto Berry Powder (lot no. 09651) was purchased from the Indiana Botanical Garden, Inc.

Extraction and Isolation. The powder of berries of saw-palmetto (2.27 kg) was extracted with 95% EtOH at room temperature and evaporated, under rotary vacuum. The residue of the 95% EtOH extract was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer was partitioned between hexane and 90% MeOH to give a MeOH layer and a hexane layer. The MeOH residue (135.0 g oil, FOO5) was the most active fraction in the BST (LC_{50} 79.9 ppm). Thus, FOO5 was repeatedly chromatographed over Si gel columns, directed at each step by BST activity, using gradients of hexane−Me_2CO and CH_2Cl_2−MeOH. The mono-acylglycerides **1** and **2** were purified by HPLC [Si gel, 5% MeOH−THF (9:1) in hexane]. All partitions and chromatographic fractions were monitored for bioactivity using the BST (5).

1-*Monolaurin* (1): 1-*O*-laurylglycerol obtained as white crystals; mp 62 °C; CIMS (isobutane) *m*/*z* 275 (100) $[MH]^+$, 257 (59), 183 (16); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (3H, t, H-12), 1.26 (20H, br s, H-4−11), 1.60 (2H, m, H-3), 2.31 (2H, t, H-2), 3.61 (2H, m, H-3′), 3.90 (1H, m, H-2′), 4.12 (2H, m, H-1′).

1-*Monomyristin* (2): 1-*O*-myristylglycerol obtained as white crystals; mp 69 °C; CIMS (isobutane) *m*/*z* 303 (100) $[MH]^+$, 285 (43), 211 (12); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, H-14), 1.26 (24H, br s, H-4−13), 1.59 (2H, br t, H-3), 2.32 (2H, t, H-2), 3.63 (2H, m, H-3′), 3.90 (1H, m, H-2′), 4.15 (2H, m, H-1′).

Biological Testing. The extracts, fractions, and isolated compounds were routinely evaluated for BST. Seven-day MTT tests for cytotoxicity to the human tumor cell lines A-498 (kidney carcinoma),[9] PC-3 (prostate adenocarcinoma),[10] and PACA-2 (pancreas carcinoma),[11] with Adriamycin as a positive control were carried out at the Purdue Cell Culture Laboratory following established procedures.

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