Physaloside A, an Acylated Sucrose Ester from *Physalis viscosa*

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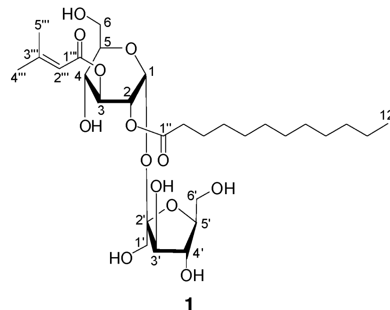
Chemical investigations of the crude MeOH extract of *Physalis viscosa* led to the identification of the novel acylated sucrose ester physaloside A (**1**). The structure of **1** was determined by 2D NMR analysis, and the absolute configuration was determined by chemical degradation and comparison with authentic standards.

One of the major problems in the treatment of bacterial infections is the increased resistance to currently available antibacterial drugs. Currently, about 60% of hospital-acquired bacterial infections are caused by multidrug-resistant microbes.[1] Consequently, the discovery of drug leads with novel chemical structure and unprecedented modes of action is one of the most promising approaches to address the spread of multidrug-resistant microbial strains.

During a high-throughput screening (HTS) campaign trying to identify natural product extracts with antibacterial activity, the crude MeOH extract of the plant *Physalis viscosa* L. (Solanaceae), a native of North and South America, but growing wild in Victoria, Australia, was investigated. It was found to have Gram-positive antibacterial activity versus *Staphylococcus aureus* and *Streptococcus pneumoniae*, but no activity against the Gram-negative bacteria *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and the mammalian cell line THP-1 (human monocytic leukaemia). Chemical investigations into this plant extract led to the identification of the acylated sucrose ester physaloside A (**1**) as the sole compound responsible for the activity of the extract. Discussed below in detail is the structure elucidation, determination of absolute configuration, and the biological profile of physaloside A (**1**).

Physaloside A (**1**) was isolated as a colorless oil with a molecular ion in the HRESIMS at *m/z* 629.3136 ([M + Na]$^+$), suggestive of the formula $C_{29}H_{50}O_{13}$ [Δ +1.3 mmu] and corresponding to five degrees of unsaturation. Analysis of the ^{13}C NMR data revealed resonances consistent with two ester carbonyl moieties (^{13}C: δ 175.1, 168.0 ppm), an observation further supported by characteristic stretches in the IR spectrum at 1733 and 1717 cm^{-1}. Further analysis of the ^{13}C and gHSQC NMR data identified resonances consistent with three hydroxy methines (^{13}C: δ 78.0, 75.8, 69.9 ppm; ^{1}H: δ 4.18, 4.01, 3.61), three hydroxy methylenes (^{13}C: δ 64.1, 63.7, 62.3 ppm; ^{1}H: δ 3.83, 3.73; 3.77; 3.56, 3.39), two oxy methines (^{13}C: δ 84.3, 74.6 ppm; ^{1}H: δ 3.96, 3.77), two ester methines (^{13}C: δ 73.3, 72.8 ppm; ^{1}H: δ 5.41, 4.77), an anomeric methine (^{13}C: δ 91.3 ppm; ^{1}H: δ 5.58), two olefinic methyls (^{13}C: δ 28.1, 21.0 ppm; ^{1}H: δ 2.16, 1.91), an olefinic methine (^{13}C: δ 117.3 ppm; ^{1}H: δ 5.69), a primary methyl (^{13}C: δ 15.0 ppm; ^{1}H: δ 0.89), as well as a quaternary anomeric carbon (^{13}C: δ 106.3 ppm), and 10 methylenes (^{13}C: δ 35.7, 33.6, six between 31.1 and 30.7, 26.4, 24.2 ppm; ^{1}H: δ 2.28, 1.53, 1.32, 1.28). This accounts for three of the five degrees of unsaturation, suggesting that **1** is bicyclic.



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Analysis of the ^{1}H−^{1}H COSY NMR data readily identified a pyranose moiety, while ^{1}H−^{1}H COSY and gHMBC NMR data allowed for the identification of furanose, 2-methyl-2-butenoyl, and dodecanoyl moieties. Diagnostic gHMBC correlations from H-2 to C-1″ and from H-3 to C-1‴ placed the dodecanoyl and 2-methyl-2-butenoyl side chains. Finally, the observation of a gHMBC correlation from H-1 to C-2′ defined the connection of the two sugar moieties and hence the planar structure of **1**.

The relative configuration of the sugars was determined using ^{1}H−^{1}H coupling constants and comparison of ^{1}H and ^{13}C chemical shifts with literature values. For the pyranose sugar, a small coupling constant between H-1 and H-2 established H-1 as equatorial, while large coupling constants between H-2 and H-3, H-3 and H-4, and H-4 and H-5 established H-2, H-3, H-4, and H-5 as axial and therefore identifying the pyranose as α-glucose. Comparison of the ^{1}H and ^{13}C NMR data for the furanose moiety of **1** with that for the known compound arillatose B[2] readily identified the furanose as *β*-fructose.

The absolute configuration of **1** was determined by alkaline hydrolysis and comparison of the product with authentic standards. To this end, alkaline hydrolysis of **1** in 2 M NH_4OH yielded a product identical in respects to that for sucrose ([α]$_D$ product = +52.7, [α]$_D$ standard = +66). This therefore identified the absolute configuration of **1** as shown.

The biological profile of physaloside A (**1**) against two Gram-positive (*S. aureus* and *S. pneumoniae*) and two

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Table 1. NMR Data (CD_3OD) for Physaloside A (**1**)

no.	^{13}C (δ, m)	^{1}H [δ, m, J (Hz)]	COSY	gHMBC ^{1}H to ^{13}C
Glu-1	91.3 (d)	5.58 (d, 3.6)	H-2	C-2′, C-3, C-5
2	72.8 (d)	4.77 (dd, 9.6, 3.6)	H-1, H-3	C-1′, C-3, C-4
3	73.3 (d)	5.41 (t, 9.6)	H-2, H-4	C-1‴, C-2, C-4
4	69.9 (d)	3.61 (t, 9.6)	H-3, H-5	C-2, C-3, C-5, C-6
5	74.6 (d)	3.96 (ddd, 9.6, 4.0, 2.0)	H-4, H_a-6, H_b-6	C-4, C-6
6	62.3 (t)	3.83 (dd, 12.0, 2.0)	H-5	C-4, C-5
		3.73 (dd, 12.0, 4.0)	H-5	C-4
Fru-1′	63.7 (t)	3.56 (d, 12.0)	H_b-1′	C-2′, C-3′
		3.39 (d, 12.0)	H_a-1′	C-2′, C-3′, C-4′
2′	106.3 (s)			
3′	78.0 (d)	4.18 (d, 8.4)	H-4′	C-1′, C-4′
4′	75.8 (d)	4.01 (t, 8.4)	H-3′, H-5′	C-5′, C-6′
5′	84.3 (d)	3.77 (m)	H-4′	C-2′, C-3′, C-4′, C-6′
6′	64.1 (t)	3.77 (m)		C-4′, C-5′
1″	175.1 (s)			
2″	35.7 (t)	2.28 (t, 9.6)	H_2-3″	C-1″, C-3″
3″	26.4 (t)	1.53 (m)	H_2-2″[a]	C-1″, C-2″
4″−9″	31.1−30.7 (d)	1.28 (m)		
10″	24.2 (t)	1.28 (m)		
11″	33.6 (t)	1.32 (m)	H_3-12″	
12″	15.0 (q)	0.89 (t, 6.4)	H_2-11″	C-10″, C-11″
1‴	168.0 (s)			
2‴	117.3 (d)	5.69 (s)	H_3-4‴, H_3-5‴	C-1‴, C-3‴, C-4‴, C-5‴
3‴	159.6 (s)			
4‴	27.2 (q)	1.91 (s)	H-2‴	C-1‴, C-2‴, C-3‴, C-5‴
5‴	20.2 (q)	2.16 (s)	H-2‴	C-1‴, C-2‴, C-3‴, C-4‴

[a] Additional COSY correlations into the methylene envelope at δ 1.28

Table 2. Biological Activities for Physaloside A (**1**) against *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, and THP-1 Cells (IC_{50}'s in μM)

K. pneumoniae	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*	THP-1
>413	>413	52	57	>800

Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*) and its selectivity versus a mammalian tumor cell line (THP-1) are shown in Table 2. As can be seen, there is at least an 8-fold selectivity for Gram-positive versus Gram-negative bacterial strains and more than a 10-fold selectivity for the Gram-positive bacteria versus mammalian cell proliferation. This suggests that **1** is acting selectively against Gram-positive bacteria strains.

There are a number of reports of sucrose compounds acylated with aliphatic side chains.[3−12] Present in these reports are examples where acylation has occurred exclusively on the α-D-glucose moiety of sucrose,[3−6,8−12] although the majority of compounds have acylation on both the α-D-glucose and β-D-fructose moieties.[3−10,12] However physaloside A (**1**) is the only example where acylation has occurred exclusively at carbons 2 and 3. Interestingly, all reports of the isolation of acylated sucrose compounds with aliphatic side chains are from plants belonging to the family Solanaceae. The occurrence of **1** in this specimen of *P. viscosa* adds further support to this observed chemotaxonomic trend.

Experimental Section

General Experimental Procedures. SPE was performed using Varian Megabond Elute C18 SPE cartridges (10 g, 50 μm). HPLC was performed on either a Waters Delta Prep 4000 chromatography system equipped with a Waters 2487 dual wavelength UV detector, a Waters prep LC system controller, and a Waters fraction collector, or a system equipped with a Waters 600 controller, a Waters 996 photodiode array detector, a Waters 717 plus autosampler, and a Waters fraction collector II. All data generated from these chromatographic systems were collected using the Waters Millenium[32] data collection package.

All NMR spectra were collected on either a Varian Unity Inova 400 MHz or a Varian Unity Inova 500 MHz spectrometer in the solvents indicated, with spectra referenced to residual ^{1}H in the deuterated NMR solvents.

Optical rotations were performed on a Jasco Dip-1000 digital polarimeter, while infrared spectra were acquired on a Bio-Rad FTS-165 Fourier transform infrared spectrometer.

Low-resolution mass spectral data were collected on a ThermoFinnigan LCQ ion trap mass spectrometer, with either an ESI or APCI probe. High-resolution mass measurements were collected on a Bruker BioApex FT mass spectrometer.

Plant Material. Flowers and stems of *P. viscosa* L. were collected near Tallygaroopna, Victoria, in January 1999. The plant material was identified by one of the authors (N.G.W.) A voucher specimen (MEL2057826) has been lodged with the National Herbarium of Victoria.

Extraction and Isolation. Dried and ground flowers and stems (10 g) were extracted twice with MeOH (500 mL) over 36 h. The combined MeOH extracts were concentrated in vacuo, then subjected to C18 SPE (10% stepwise gradient elution from 20% MeOH/H_2O to 80% MeOH/H_2O, and a flush with 100% MeOH), generating eight fractions. Activity was localized in the 80% MeOH/H_2O fraction, which was further purified on C18 preparative HPLC [16 mL/min, gradient elution from 3:7 (MeCN/H_2O + 0.1% formic acid) to 9:1 (MeCN/H_2O + 0.1% formic acid) over 25 min through a Varian C18 250 × 50 mm 5 μm preparative HPLC axial compression column] and C8 semipreparative HPLC [6 mL/min, gradient elution from 11:9 (MeCN/H_2O + 0.1% formic acid) to MeCN (+ 0.1% formic acid) over 20 min through a Waters C8 Symmetry 250 × 25 mm 7 μm HPLC column], to generate the acylated sucrose ester physaloside A (**1**) (35 mg, 0.35% yield) as the compound responsible for biological activity.

Physaloside A (1): colorless oil; $[\alpha]_D$ 56.1° (*c* 0.5, MeOH); IR (film) ν_{max} 3383, 2925, 2854, 1733, 1717 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; HRESIMS *m/z* 629.3136 $[M + Na]^+$ (calcd for $C_{29}H_{50}O_{13}Na$, 629.3149).

Alkaline Hydrolysis of 1. A solution of **1** (10 mg) in 2 M aqueous NH_4OH (2 mL) was heated at 50 °C for 4 h. The reaction mixture was adjusted to pH 3 by the addition of 2 M formic acid. The hydrolysate was extracted with EtOAc (3 × 3 mL), and the aqueous phase was repeatedly dried and reconstituted in H_2O in vacuo to remove residual ammonium

formate. Analysis of the hydrolysate showed that it was identical in all aspects to an authentic standard of sucrose.

Antibacterial Assays. Strains of *K. pneumoniae, P. aeruginosa, S. aureus*, and *S. pneumoniae* were initially grown on Mueller-Hinton agar, then subcultured into Mueller-Hinton broth supplemented with $CaCl_2$/$MgCl_2$ and grown at 37 °C for 18 h. Serial dilutions were made (*K. pneumoniae* and *P. aeruginosa* diluted to 10^{-6} cfu/mL, *S. aureus* to 10^{-8} cfu/mL, and *S. pneumoniae* to 10^{-4} cfu/mL) and all broths left to equilibrate for 30 min at 37 °C. Extracts were added to a 96-well microtiter plate, and 200 μL of each culture was dispensed into each well. Negative control wells contained the respective bacterial strain without inhibitors, while positive control wells contained bacterial strains with 50 μg/mL streptomycin. The plates were incubated and shaken at 37 °C and 60% humidity for 18 h, after which optical density at 650 nm was measured. Test wells showing little or no growth/turbidity were indicative of antibacterial activity.

Mammalian Cytotoxicity Assay. Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 5 units/mL penicillin, 5 mg/mL streptomycin, and 10 μM 2-mercaptoethanol at 37 °C, 5% CO_2. The assay was conducted in a 96-well microtiter plate at a cell density of 2.5 $\times$ 10^4 cells per well, in a total volume of 200 μL. Natural product samples in each well were tested at 250 μg/mL final concentration. Positive control wells contained 10 μM camptothecin, while negative control wells contained 0.1% DMSO. Plates were incubated for 72 h at 37 °C, 5% CO_2, prior to the addition of WST-1 reagent (20 μL) and further incubated for 1 to 2 h. Absorbance at 450 nm was measured and activity compared to positive controls.

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