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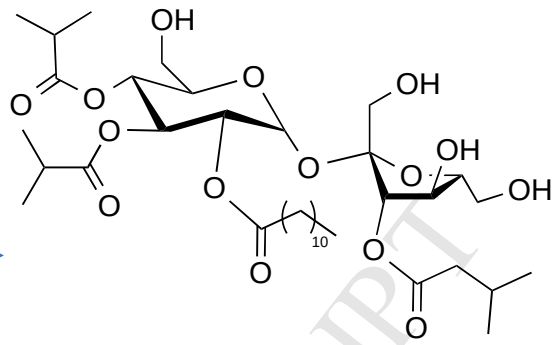

Peruviose D
Inhibition of α-amylase = 84.8%.

Peruvioses A to F, sucrose esters from the exudate of *Physalis peruviana* fruit as *α*-amylase inhibitors

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Abstract

The fruit of *Physalis peruviana* is widely used in traditional Colombian medicine as an antidiabetic treatment. The aim of the study reported here was to identify the compounds responsible for the hypoglycemic activity using the α-amylase inhibition test. Bioguided fractionation of a dichloromethane extract of the sticky exudate that covers the fruit allowed the isolation and identification of three new sucrose esters, named as peruvioses C–E (**1**–**3**), along with the known peruvioses A (**6**), B (**5**) and F (**4**), the structures of which were elucidated by extensive NMR and MS experiments. These compounds proved to be responsible for the hypoglycemic activity observed in the extract. Peruviose D (**2**) showed the highest activity, with an inhibitory activity value of 84.8%. This is the first study to establish the potential of sucrose esters as α-amylase inhibitors and to explain the hypoglycemic effect that has traditionally been attributed to gooseberry fruit.

Keywords: *Physalis peruviana*, Cape gooseberry, Sucrose esters, Peruvioses A–F, hypoglycemic activity, α-amylase inhibitory activity.

1. Introduction

Physalis peruviana is a Solanaceae plant that grows in the Andes Mountains from Bolivia to Venezuela at altitudes between 1700 and 2900 m. Its edible fruit, which is known as cape gooseberry or 'uchuva', is well known for its flavor and for its uses in the prevention and treatment of diabetes and pterygia, along with its use as a diuretic, anti-helminthic, and expectorant, amongst other applications [1]. This wide range of uses has encouraged various studies on the pharmacological properties of different parts of the plant and these have shown antioxidant, anti-inflammatory, diuretic, antispasmodic, anti-asthmatic, anti-lithiasic, immunosuppressive, and antipyretic activities [2,3]. Anti-inflammatory activity, as assessed by ear edema and plantar edema models in mice [1,4–6], along with larvicidal activity [7] were reported for calyces crude ethanolic extract and also for some of its fractions. Studies on the anti-inflammatory activity of extracts and fractions of *P. peruviana* calyces suggest that the compounds responsible for this activity are sucrose esters [5]. These types of sugars bear short-chain fatty acids and they are commonly found in other species of these genera such as *P. angulate* [8], and *P. philadelphica* [8,9], among other species. In addition to this type of compounds, other studies highlighted the presence of withanolides [7,10–13], alkaloids, esters, flavonoids , vitamins, glycosidic flavor precursors and steroids.

As part of our ongoing research on the hypoglycemic activity of functional foods, we decided to study *Physalis peruviana* as a source of compounds that may exert such activity. A worldwide increase in the incidence and prevalence of type 2 diabetes has occurred in recent decades, and a variety of the currently available antidiabetic drugs are known to act by at least one of three different mechanisms: (i) drugs that stimulate insulin secretion such as glibenclamide; (ii) drugs that decrease insulin sensitivity such as metformin; and (iii) drugs that reduce the glucose absorption such as acarbose, which is one of the few drugs that act by this mechanism. Therefore, the discovery of new drugs with this last mechanism has been the focus of recent studies. Several substances isolated from plants show hypoglycemic activity and the main mode of action is to inhibit intestinal carbohydrases such as α-amylase and α-glucosidase [14,15].

Two independent studies in the literature have highlighted the antidiabetic properties of extracts from *P. peruviana* fruits collected in India and Colombia. Sathyadevi et al. reported that the oral administration of ethanolic extracts from *P. peruviana* fruits collected in India improved insulin sensitivity and ameliorated hyperglycemia in high-fat diet/low dose streptozotocin-induced type 2 diabetic rats [16]. A qualitative analysis of this extract was performed by the same authors and this revealed the presence of phenols, flavonoids, glycosides, sterols, saponins, tannins, lactones and alkaloids. Moreover, the fruit extract was also found to contain significant amounts of phenolic compounds and flavonoids, such as rutin, myricetin, quercetin and kaempferol [17]. The same authors reported that the major flavonoids present in the fruit extracts proved to be aldose reductase inhibitors in molecular

docking studies approach against that receptor [16,18]. It was reported that the oral administration of *P. peruviana* extracts from fruits collected in Colombia, reduced blood glucose levels in rats in which diabetes had been induced by a single administration of streptozotocin [19]. In a later study, it was found that the ethanolic extract of *P. peruviana* fruits showed competitive inhibition on α-glucosidase and on the non-competitive type of α-amylase, suggesting that inhibition of intestinal carbohydrases is one of the modes of action by which the fruits of *P. peruviana* exert hypoglycemic and antidiabetic activity [15]. More recently, a dry powder formulation from standardized extracts of the fruit was reported to have hypoglycemic activity [20]. However, the studies outlined above did not address the identities of the compounds responsible for the activity observed.

In order to identify the compounds responsible for the hypoglycemic activity, we studied the organic extracts from the sticky exudate that covers the fruit by carrying out a bio-guided fractionation using an *α*-amylase inhibition test model [15]. These enzymes (carbohydrases) are present in the intestinal villi and they are necessary for polysaccharide digestion because they participate in the cleavage of these compounds to give monosaccharides (glucose, fructose, galactose). Thus, inhibition of the enzymes is one of the different possible mechanisms of antidiabetic drugs because such inhibition slows carbohydrate absorption and decreases postprandial blood glucose levels in both normal and diabetic subjects [14]. We describe here the isolation and structural elucidation of several sugar esters that are responsible for the effects of *P. peruviana* fruit on sugars levels. The biological evaluation of these compounds allowed us to establish some structure-activity relationships.

2. Results and discussion

The α-amylase activity test conducted on the ethanolic extracts from *P. peruviana* fruit showed an inhibitory activity (IA) of 68.3%, which is similar to that determined for acarbose used as a positive control (IA 77.4%). These results, along with the reported antidiabetic properties, indicate that this fruit has great potential as a source of compounds with hypoglycemic activity. The sticky exudate was first extracted by direct immersion of the fruits in CH_2Cl_2 to give the DF extract, followed by MeOH extraction of the fruit (MF), and finally, the remaining fruit were dried, homogenized and extracted with EtOH to afford the ethanolic extract (EF). The *α*-amylase inhibition bioassay on the three extracts allowed us to locate the activity in the DF extract, with an IA value of 77.4% (Figure 1), while the other evaluated extracts where less active.

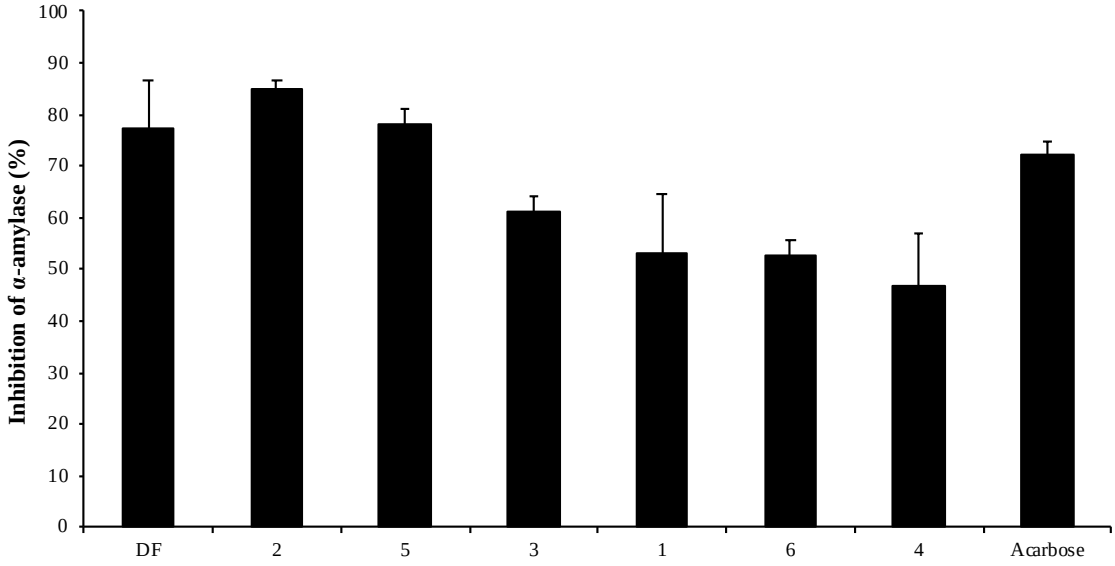

Figure 1. *In vitro* inhibition of *α*-amylase for the DF extract and its major components, peruvioses A–F, displayed from higher to lower inhibitory activity. CH_2Cl_2 extract (DF), peruviose C (**1**), peruviose D (**2**), peruviose E (**3**), peruviose F (**4**), peruviose B (**5**), peruviose A (**6**) and acarbose as a positive control. All compounds were tested at 640 μg/mL. Results are expressed as mean ± S.D.

The bioguided fractionation of the DF extract using several chromatographic techniques, allowed us the isolation of six compounds (**1**–**6**). The structures of these compounds were elucidated by analysis of their 1D and 2D NMR data and mass spectra and by basic hydrolysis and comparison of the products with authentic standards (Figure 2. Supporting information SI 2 – SI 21).

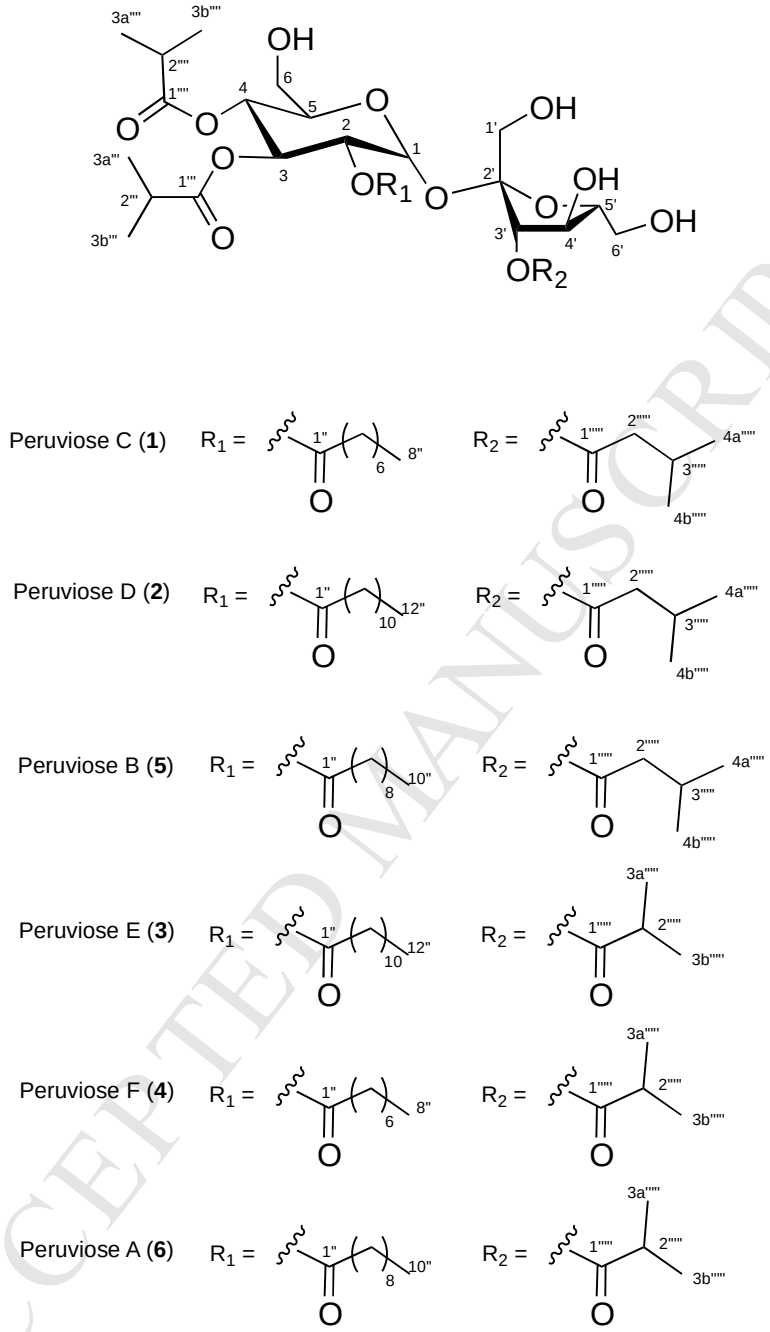

Figure 2. Sucrose esters isolated from *Physalis peruviana* fruits

Compound **1** (12 mg) was isolated as a colorless viscous liquid. The molecular formula of **1** was determined as $C_{33}H_{56}O_{15}$ on the basis of its (+)-HRESIMS, which shows the $[M + Na]^+$ ion peak at *m/z* 715.3511, (calcd for $C_{33}H_{56}O_{15}Na$, 715.3511, Δ 0.0 ppm) and the NMR data. The presence of a disaccharide sucrose structure in **1** was suggested by the ^{1}H-NMR spectrum in $CDCl_3$ (Table 1), which contained eight oxymethine proton signals at δ_H 5.59, 5.46, 5.21, 4.95, 4.91, 4.52, 4.15 and 3.95, along with three

oxymethylene signals at δ_H 3.88 /3.72, 3.55 and 3.59 /3.46. The NMR carbon signals at δ_C 103.84, 89.41, 82.61, 79.11, 72.03, 71.32, 70.10, 69.05, 68.29, 64.62, 61.55 and 59.90 observed in the ^{13}C-NMR spectrum of **1**, which were correlated to their corresponding protons signals by an HSQC experiment and linked by a ^{1}H-^{1}H COSY experiment, confirmed the existence of a sucrose structure. The signals of four ester carbonyl carbons observed in the ^{13}C-NMR spectrum of **1** at δ_C 177.80, 175.96, 175.71 and 172.87 suggested the presence of four acyl substituents. Thus, the presence of two isobutanoyl groups in **1** was deduced from the proton and carbon NMR methine signals at δ_H 2.51 /δ_C 33.92 and δ_H 2.45 /δ_C 33.88, which were connected by a COSY experiment to four methyl protons at δ_H 1.13, 1.11, 1.08 and 1.06 /δ_C 18.67–19.07. The methylene proton signal at δ_H 2.39 /δ_C 43.13, which correlates by COSY to the methine proton signal at δ_H 2.20/δ_C 25.84 and this is in turn to two methyl proton groups at δ_H 1.05 and 1.03, is indicative of a 3-methylbutanoyl moiety. HMBC correlations between the methylene proton signals at δ_H 2.39 and methyl carbon at δ_C 25.84, between the methyl protons at δ_H 1.05 and 1.03 and carbons at δ_C 43.13 and 25.84, and between the methine proton at δ_H 2.20 and methylene carbon at δ_C 43.13 allowed us to confirm the presence of the 3-methylbutanoyl subunit. Finally, methylene NMR signals between δ_H 2.25 and 1.25/δ_C 33.86–22.58 in combination with a terminal methyl group at δ_H 0.87/δ_C 14.03, were indicative of an alkyl chain of a saturated fatty acid in **1**. An HMBC experiment allowed us to connect each acyl group to the sucrose structure by the key long-range correlations shown in Table 1 [5,21].

All of the above data are similar to those of peruviose B (**5**) [5], which was also isolated in this study and is a sucrose ester bearing a C_{10} fatty acid at position C2 in the glucose moiety, two isobutanoyl groups at C3 and C4 and one 3-methylbutanoyl group at C3' in the fructose moiety. The difference of 28 mass units between the molecular weight of compound **1** (*m*/*z* 715 observed for the $[M + H]^+$ ion peak in the ESIMS) and the reported value for peruviose B (**5**, *m*/*z* 743 reported for $[M + Na]^+$ ion peak in the FABMS), are indicative of the lack of two methylene groups in **1**. This finding allowed us to determine the presence of an octanoyl group attached to C2 in **1** rather than the decanoyl subunit in peruviose B (**5**). Thus, the structure of compound **1**, named as peruviose C, was established as is presented in Figure 2.

	Peruviose C (1)			Peruviose D (2)			Peruviose E (3)			Peruviose F (4)		
Position	**xδ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**
					Glucose unit							
1	89.41, CH	5.59, d, (3.6)	5, 2’	89.47, CH	5.47, d, (3.7)	5, 2’	89.40, CH	5.59, d, (3.7)	5, 2’	89.38, CH	5.60, d, (3.3)	5, 2’
2	70.10, CH	4.91, m	3, 1’’	70.16, CH	4.91, m	3, 1’’	70.06, CH	4.92, m	3, 1’’	70.03, CH	4.92, m	3, 1’’
3	69.05, CH	5.46, t, (9.9)	2, 4, 1’’’	69.08, CH	5.46, t, (10.0)	2, 4, 1’’’	69.02, CH	5.47, t, (10.0)	2, 4, 1’’’	68.98, CH	5.47, t, (9.9)	2, 4, 1’’’
4	68.29, CH	4.95, t, (9.9)	3, 5, 6, 1’’’’	68.34, CH	4.95, t, (10.0)	3, 5, 6, 1’’’’	68.25, CH	4.95, t, (10.0)	3, 5, 6, 1’’’’	68.24, CH	4.95, t, (9.9)	3, 5, 6, 1’’’’
5	72.03, CH	4.15, m		72.02, CH	4.14, m		72.18, CH	4.15, m		72.04, CH	4.15, m	
6	61.55, CH_2	3.55, m		61.56, CH_2	3.58, m		61.60, CH_2	3.58, m		61.50, CH_2	3.60, m	
					Fructose unit							
1’a	64.62, CH_2	3.59, m		64.55, CH_2	3.57, m		64.69, CH_2	3.58, d, (12.5)		64.54, CH_2	3.58, d, (13.0)	
1’b		3.46, m			3.47, m			3.46, d, (12.5)			3.46, d, (13.0)	
2’	103.84, C			103.94, C	-		103.81, C	-		103.82, C	-	
3’	79.11, CH	5.21, d, (8.1)	1’, 4’, 1’’’’’	79.21, CH	5.22, d, (8.1)	1’, 4’, 1’’’’’	79.12, CH	5.18, d, (8.2)	1’, 4’, 1’’’’’	79.05, CH	5.20, d, (7.9)	1’, 4’, 1’’’’’
4’	71.32, CH	4.52, t, (8.1)	3’, 5’, 6’	71.28, CH	4.55, t, (8.1)	3’, 5’, 6’	71.19, CH	4.59, t, (8.2)	3’, 5’, 6’	71.30, CH	4.52, t, (7.9)	3’, 5’, 6’
5’	82.61, CH	3.95, m	4’	82.61, CH	3.91, m	4’	82.55, CH	3.93, m	4’	82.54, CH	3.92, m	4’
6’a	59.90, CH_2	3.88, m	4’	59.88, CH_2	3.73, m	4’	59.73, CH_2	3.89, m	4’	59.92, CH_2	3.89, m	4’
6’b		3.72, br d, (13.4)			3.70, m			3.73, br d, (12.3)			3.75, m	
					Substituent at C2							
1’’	172.87, C	-		172.88, C	-		172.78, C	-		172.87, C	-	
2’’	33.86, CH_2	2.25, m	3’’	33.93, CH_2	2.25, m	3’’	34.05, CH_2	2.25, m	3’’	33.83, CH_2	2.25, m	3’’
3’’	24.54, CH_2	1.53, m	2’’	24.57, CH_2	1.56, m	2’’	24.57, CH_2	1.54, m	2’’	24.54, CH_2	1.54, m	2’’
4’’	29.14, CH_2	1.24, br s		29.57, CH_2**	1.24, br s		29.08, CH_2**	1.25, br s		28.85, CH_2	1.25, br s	
5’’	29.05 – 29.01, CH_2**	1.24, br s		29.57, CH_2**	1.24, br s		29.22, CH_2**	1.25, br s		29.00, CH_2	1.25, br s	
6’’	31.75, CH_2	1.24, br s		29.39 , CH_2**	1.24, br s		29.31, CH_2**	1.25, br s		31.56, CH_2	1.25, br s	
7’’	22.58, CH_2	1.24, br s		29.30 , CH_2**	1.24, br s		29.39, CH_2**	1.25, br s		22.56, CH_2	1.25, br s	
8’’	14.03, CH_3	0.86, t, (6.8)	6’’, 7’’	29.21, CH_2**	1.24, br s		29.58, CH_2**	1.25, br s		14.04, CH_3	0.87, t, (6.7)	6’’, 7’’
9’’				29.09, CH_2**	1.24, br s		29.69, CH_2**	1.25, br s				
10’’				31.88, CH_2	1.24, br s		31.89, CH_2	1.25, br s				
11’’				22.66, CH_2	1.24, br s		22.66, CH_2	1.25, br s				
12’’				14.07, CH_3	0.87, t, (7.0)	6’’, 7’’	14.08, CH_3	0.88, t, (6.8)	10’’, 11’’			

Substituent at C3												
1’’’	175.71, C	-			174.05	-	175.73, C	-		175.76, C	-	
2’’’	33.88, CH	2.45, sp, (7.0)			33.87	2.45, sp, (7.0)	33.89, CH	2.45, sp, (7.0)		33.87, CH	2.45, sp, (7.0)	
3’’’a	18.67-19.07, CH_3*	1.08 d, (7.0)	2’’’	2’’’	18.88-18.75, CH_3	1.08 d, (7.0)	18.71-19.06, CH_3*	1.07, d, (7.0)	2’’’	18.66-19.08, CH_3*	1.07, d, (7.0)	2’’’
3’’’b	18.67-19.07, CH_3*	1.06 d, (7.0)	2’’’	2’’’	18.88-18.75, CH_3	1.07 d, (7.0)	18.71-19.06, CH_3*	1.08, d, (7.0)	2’’’	18.66-19.08, CH_3*	1.08, d, (7.0)	2’’’
Substituent at C4												
1’’’’	175.96, C	-		175.63, C	-		175.98, C	-		176.00, C	-	
2’’’’	33.92, CH	2.51, sp (7.0)	3’’’’	33.89, CH	2.52, sp, (7.0)	3’’’’	33.93, CH	2.51, sp, (7.0)	3’’’’	33.90, CH	2.51, sp, (7.0)	3’’’’
3’’’’a	18.67-19.07, CH_3*	1.13, d (7.0)	2’’’’	18.88-18.75, CH_3	1.13, d, (7.0)	2’’’’	18.71-19.06, CH_3*	1.14, d, (7.0)	2’’’’	18.66-19.08, CH_3*	1.14, d, (7.0)	2’’’’
3’’’’b	18.67-19.07, CH_3*	1.11, d, (7.0)	2’’’’	18.88-18.75, CH_3	1.11 d, (7.0)	2’’’’	18.71-19.06, CH_3*	1.05, d, (7.0)	2’’’’	18.66-19.08, CH_3*	1.06 , d, (7.0)	2’’’’
Substituent at C3’												
1’’’’’	177.80, C	-		176.06, C			177.93, C	-		177.81, C	-	
2’’’’’	43.13, CH_2	2.39, dd, (12.8, 6.7)	4´´´a	43.15, CH_2	2.40, dd, (14.0, 7.0)	4´´´a	34.02, CH	2.75, sp, (7.0)	3’’’’’	33.99, CH	2.75, sp, (7.0)	3’’’’’
3’’’’’	25.84, CH	2.20, m	2’’’’’	25.87, CH	2.19, m	2’’’’’						
3’’’’’a							18.71-19.06, CH_3*	1.33, d, (7.0)	2’’’’’	18.66-19.08, CH_3*	1.32, d, (7.0)	2’’’’’
3’’’’’b							18.71-19.06, CH_3*	1.29, d, (7.0)	2’’’’’	18.66-19.08, CH_3*	1.29, d, (7.0)	2’’’’’
4’’’’’a	22.54, CH_3	1.05 , d, (6.5)	2’’’’’, 3’’’’’, 4’’’’’b	22.30, CH_3	1.06, d, (6.4)	2’’’’’, 3’’’’’, 4’’’’’b						
4’’’’’b	24.54, CH_3	1.03, d, (6.5)	2’’’’’, 3’’’’’, 4’’’’’a	22.42, CH_3	1.04, d, (6.4)	2’’’’’, 3’’’’’, 4’’’’’a						

*The multiplicity could not be determined
** Signals may be interchanged

Table 1. NMR-^{1}H data ($CDCl_3$, 400 MHz) for Compound **1** to compound **4** (Peruvioses C-F).

Compound **2** (7 mg) was isolated as a colorless viscous liquid. The molecular formula of **2** was established as $C_{37}H_{64}O_{15}$ by the $[M + Na]^+$ sodium adduct ion observed in the (+)-HRESIMS at *m*/*z* 771.4156 (cald for $C_{37}H_{64}O_{15}Na$, 771.4137, Δ 1.9 ppm) and the NMR data. The ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra along with 2D NMR experiments (COSY, HSQC and HMBC) of **2** are consistent with a sucrose ester structure bearing two isobutanoyl groups at C3 and C4, one 3-methylbutanoyl group at C3' and a fatty acid chain at C2. The NMR data for **2** were very similar to those of peruviose C (**1**) and peruviose B (**5**), only differing in the length of the fatty acid chain attached to C2. The difference of 56 mass units between the molecular weight of compounds **1** and **2**, indicating the presence of four additional methylene groups, allowed us to establish presence of a dodecanoyl group bonded to C2 in **2** rather than the octanoyl subunit in peruviose C (**1**). Thus, the structure of compound **2**, named as peruviose D, was established and is presented in Figure 2.

Compound **3** (4 mg) was isolated as a colorless viscous liquid with molecular formula $C_{36}H_{62}O_{15}$, as evidenced by (+)-HRESIMS at *m*/*z* 757.3982 (calcd for $C_{36}H_{62}O_{15}Na$, 757.3981, Δ 0.1 ppm). Once again, the presence of a sucrose structure in **3** was suggested by its ^{1}H-NMR spectrum (Table 1), with eight oxymethine signals at δ_H 5.59, 5.47, 5.18; 4.95, 4.92, 4.59, 4.15 and 3.93 along with three oxymethylenes at δ_H 3.89 /3.73, 3.58 and 3.58 /3.46. This was confirmed by the presence of two anomeric carbons (δ_C 103.81 and 89.40), seven oxymethines (δ_C 82.55, 79.12, 72.18, 71.19, 70.06, 69.02 and 68.25) and three oxymethylenes (δ_C 64.69, 61.60 and 59.73) in the ^{13}C-NMR spectrum (Table 1). Similarly, the presence of four acyl substituents in **3** was suggested by the four characteristic ester carbonyl carbons at δ_C 177.81, 176.00, 175.76 and 172.87 observed in the ^{13}C-NMR spectrum of **3** (Table 1). However, the proton and carbon NMR data of **3** show the presence of three methine NMR signals at δ_H 2.75/δ_C 34.02, δ_H 2.51/δ_C 33.93 and δ_H 2.45/δ_C 33.89, which correlated by COSY to the corresponding methyl groups at δ_H 1.33, 1.29, 1.14, 1.08, 1.07, 1.05 /δ_C 19.06–18.71, thus indicating the presence of three isobutanoyl groups in **3** instead of two isobutanoyl and one 3-methylbutanoyl subunits as in **1** and **2**. The presence of a fatty acid group was also deduced from the methylene signals between δ_H 2.25 and 1.27 and the terminal methyl at δ_H 0.88. The NMR chemical shifts of **3** are very similar to those of peruviose A (**6**), a sucrose ester previously isolated from *P. peruviana* calyces [5] and also isolated in this study .

This compound is characterized by a long-chain fatty acid (C_{10}) bonded to position C2 and three isobutanoyl groups at positions C3, C4 and C3' of the sucrose moiety (Figure 2). Analysis of the 2D NMR spectra of **3**, including COSY, HSQC and HMBC (Table 1), allowed us to assign unambiguously most of the signals for compound **3**. The difference of 28 mass units between the molecular weight of **3** (its (+)-LRESIMS shows an $[M + H]^+$ ion peak at *m*/*z* 757) in regard to that of peruviose A (reported *m*/*z* 729) indicated the presence of dodecanoic acid bonded at position C2. The 12 carbon signals assigned to carbons C-1" to C-12" (Table 1) of the fatty acid confirmed that proposal. Thus, the structure of **3**, named as peruviose E, was established and is presented in Figure 2.

Compound **4** (9 mg) was isolated as a colorless viscous liquid. The molecular formula of **4** was determined as $C_{32}H_{54}O_{15}$

on the basis of its HRFABMS, which shows the $[M + Na]^+$ ion peak at *m/z* 701.3375 (calculated for $C_{32}H_{54}O_{15}Na$, 701.3355, Δ 2.0 ppm), and its NMR data. The ^1H- and ^{13}C-NMR spectra in combination with COSY, HSQC and HMBC experiments on **4** (Table 1) showed the signals of eight oxymethine and three oxymethylenes that are characteristic of another sucrose ester, along with three septets that are indicative of three isobutanoyl groups at δ_H 2.75/δ_C 33.99, 2.51/δ_C 33.90 and δ_H 2.45/δ_C 33.87 and the typical methylene signals corresponding to a fatty acid subunit. All of these NMR signals were very similar to those of peruviose E (**3**) and only differ in the long-chain fatty acid that forms the ester on the hydroxyl group at C2 of the glucose unit. The presence of a C_8 fatty acid in **4** was proposed from the eight carbon signals at δ_C 172.87, 33.83, 24.54, 28.85, 29.00, 31.56, 22.56 and 14.04 observed in the ^{13}C-NMR spectrum and from the molecular formula $C_{32}H_{54}O_{15}$. Thus, the structure of peruviose F (**4**) is proposed as shown in Figure 2. Although the structure of this compound was recently included in an International Patent related to a *P. peruviana* extract comprising sucrose esters as the active agents for use in a cosmetic application, the NMR and mass spectral data for **4** are here reported for the first time [22].

Peruviose B (**5**, 42 mg) and peruviose A (**6**, 44 mg) were also isolated from the DF extract and identified by comparison of their ^1H- and ^{13}C-NMR data with those reported by Franco et al., from a mixture of these compounds from the same plant [5]. Thus, this is the first time that peruviose A (**6**) and peruviose B (**5**) have been isolated as pure compounds. For the absolute configuration of the sucrose moiety, the basic hydrolysis of **1**–**4** was performed. The work up of the reaction gave a residue that showed an optical rotation value ($[\alpha]_D$ = +52.7) that is similar to the one reported for a standard of sucrose ($[\alpha]_D$ = +66).

Once compounds **1**–**6** had been fully characterized, they were tested in the *α*-amylase inhibition assay. The results obtained (Figure 1), were very similar to those obtained for the DF extract. Peruvioses D (**2**) and B (**5**) gave IA values of 84.8% and 78.2%, respectively, and they were the most active compounds followed by peruvioses E (**3**), C (**1**), A (**6**) and F (**4**). These results highlight the importance of the length of the fatty acid chain at C2 of the glucose and the presence of an isobutanoyl group at C3 of the fructose subunit for *α*-amylase inhibition. Thus, an increase in the length of the fatty acid results in increased inhibition. Indeed, for compounds bearing one 3-methylbutanoyl and two isobutanoyl units, the inhibition activity (IA) values increase from an IA < 50% for peruviose F (**4**), which has a C_8 fatty acid, to an IA of 78% for peruviose B (**5**), which has a C_{10}, and to an IA of 84% for peruviose D (**2**) with a C_{12} fatty acid. A similar increase was found for compounds bearing three isobutanoyl units: peruviose F (**4**, C_8, < 50%) < peruviose A (**6**, C_{10}, 53%) < peruviose E (**3**, C_{12}, 61%). Those compounds bearing the same fatty acid chain but with one 3-methylbutanoyl and two isobutanoyl units, peruvioses D (**2**), B (**5**) and C (**1**), were more active than those having three isobutanoyl units (peruvioses E (**3**), A (**6**) and F (**4**), respectively).

Thus, peruvioses A to F are described here as a family of sucrose esters characterized by the presence of an isobutanoyl group at C4 in the sucrose moiety. This class of compounds has been described in other *Physalis* species such as *P. philadelphica*

Lam. *P. angulate* [8,9], as well as in other exudates commonly observed in Solanaceae such as those from the genera *Lycopersicon*, *Nicotiana*, *Petunia*, and *Solanum* [23,24]. Besides the fruits, these sucrose esters are also present in the trichome and they are believed to protect plants and fruits from insects and fungi. Moreover, this type of compound is responsible for the anti-inflammatory activity found in the extracts of these species [9]. As in other species, these sucrose esters are considered to be the main material in the exudates obtained mainly in the DF fraction.

3. Conclusion

In conclusion, bioguided fractionation of a dichloromethane extract of *P. peruviana* fruit, which showed a considerable inhibitory effect on the α-amylase enzyme, allowed us to obtain a fraction with a high sucrose ester content. Subsequent separation of that mixture afforded three new sucrose esters, named as peruvioses C–E (**1**–**3**), along with three known esters, namely peruvioses A (**6**), B (**5**) and F (**4**). The results of α-amylase enzyme inhibition assays showed that peruviosides D (**2**) and B (**5**) are the most active. The structure-activity relationships of the isolated compounds highlighted the importance of the fatty acid length at C2 and the presence of a 3-methylisobutnoyl at C3' on the sucrose ester skeleton. Although peruvioses A (**6**) and B (**5**) were previously reported as a mixture, this is the first time that they have been isolated as pure compounds. Moreover, the NMR and MS spectroscopic data are reported for the first time for peruviose F (**4**). This work constitutes the first study in which the potential of sucrose esters as α-glucosidase inhibitors has been described and the hypoglycemic effect that has been attributed to gooseberry fruit has been explained. Studies for the evaluation of hypoglycaemic activity in mice are ongoing in our laboratories.

4. Experimental section

4.1. General Experimental Procedures.

^{1}H- and ^{13}C-NMR (1D and 2D) spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using $CDCl_3$ as a solvent and residual solvent signals as internal standards. High-resolution mass data were collected on an Applied Biosystems QSTAR Elite hybrid quadrupole Time-of-Flight (TOF) mass spectrometer, ESI positive mode. Column chromatography (CC) was carried out under vacuum using silica gel 60 G Merck and flash chromatography was carried out on silica gel 60 (230–400 mesh, Macherey-Nagel). Optical rotations were measured on a Polartronic E, Schmidt + Haensch polarimeter. HPLC-PDA was carried out with a Perkin-Elmer LC 480 system equipped with an AD224 controller, a Perkin-Elmer series 410 LC pump and a LC-480 auto scan diode array detector. HPLC-ELSD was performed on a Thermo Dionex ultimate

3000 system, coupled to an ELSD Sedex 85 detector with a gain of 10 for the ELSD detector and a temperature of 40 ºC for the analysis. A TECAN GENIOS spectrophotometer was used to study the α-amylase inhibition activity.

4.2 Plant Material. *Physalis peruviana* fruit (uchuva or cape gooseberry) was purchased in a commercial culture located at Subia in the state of Cundinamarca (Colombia). A voucher specimen of the plant was identified and deposited (COL-574701) at the Herbario Nacional Colombiano of the Universidad Nacional de Colombia at Bogotá (Colombia).

4.3. Extraction and Isolation. The entire mass of fruit (5000 g) of *Physalis peruviana* was immersed in CH_2Cl_2 for 5 minutes to obtain, after filtration and evaporation of solvent under vacuum, the dichloromethane extract (DF, 4.1 g). The fruit containing the CH_2Cl_2 insoluble material was immersed in MeOH for 5 minutes to give, after evaporation under vacuum, the methanolic extract (MF, 6.7 g). After these extractions, the remaining fruit matter was ground, dried in an air oven and then extracted with EtOH to yield, after removal of the solvent, the ethanolic extract (EF, 283.7 g). All of the extracts were evaluated *in vitro* for hypoglycemic activity using the *α*-amylase inhibition test.

The most active extract, DF, was fractionated by silica gel column chromatography and eluted under a gradient using hexane (100%), Hex-EtOAc (80:20, v/v), Hex-EtOAc (50:50, v/v), Hex-EtOAc (20:80, v/v), EtOAc (100%), EtOAc-MeOH (90:10, v/v), EtOAc-MeOH (50:50, v/v), MeOH (100%) and MeOH-H_2O (90:10, v/v). Fractions of 125 mL were collected to obtain 16 fractions (DF1 to DF16). The *in vitro α*-amylase inhibition bioassay showed that DF8 was the most active fraction. Fraction DF8 (676.3 mg) was separated by HPLC-PDA on a semi-preparative Zorbax Eclipse XDB C18 column (250 mm × 9.4 mm, 5 μm) using a mobile phase consisting of a gradient of H_2O-MeOH mixtures: (30:70, v/v) for 5 min, (0:100, v/v) for 20 min, (0:100, v/v) for 10 min and (30:70, v/v) for 5 min at a flow rate of 2 mL/min. Fractions were collected every 5 min to give eight sub-fractions (DF8-1 to DF8-8). Fraction DF8-6 (300 mg), which showed the highest α-amylase inhibition activity, was further fractionated by HPLC-ELSD on a Zorbax Eclipse XDB C18 column (250 mm × 9.4 mm, 5 μm) using a mobile phase consisting of a gradient of H_2O-MeOH mixtures: (10:90, v/v) for 30 min, (0:100, v/v) for 15 min and finally, (10:90, v/v) for 5 min, at a flow of 1.5 mL/min, to yield compounds **1** (12 mg), **2** (7 mg), **3** (4 mg), **4** (9 mg), **5** (42 mg) and **6** (44 mg).

4.4. Basic hydrolysis of compounds 1 to 4. Compounds **1**–**4** (2 mg each) were separately heated under reflux in a 5% methanolic KOH solution (10 mL) for 1 h. The basic solution was cooled, neutralized carefully with 2 N HCl and diluted with water (10 mL). The resulting solution was extracted with Et_2O (3 × 10 mL) to give, in the aqueous fraction after evaporation under vacuum, an amorphous powder. The solid was treated under standard acetylation conditions (Ac_2O/pyridine, overnight) and the peracetylated product was analysed by NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (1H, d, J = 8.3 Hz, anomeric), 5.47 (1H, t, J =

9.9 Hz, –CHOH), 5.36 (1H, d, *J* = 5.7 Hz, –CHOH), 5.24 (1H, dd, *J* = 20.2, 10.7 Hz, CHOH), 4.95 (1H, d, *J* = 5.4 Hz, –CHOH), 4.61 (1H, d, *J* = 11.2 Hz, $–CH_2OH$), 4.21 (1H, m, –CH), 3.87 (1H, s, –CH), 3.84 (1H, d, *J* = 9.7 Hz, –CHOH), 3.72 (1H, d, *J* = 13.3 Hz, –CHOH), 3.64 (2H, m, $–CH_2OH$), 3.55 (1H, d, *J* = 12.0 Hz, –CHOH), 3.49 (1H, d, *J* = 9.0 Hz, –CHOH), 1.9–2.2 (overlapped singlets, AcO). These NMR data matched those of a sucrose standard and the NMR data reported for sucrose in the literature [25,26,23,24]

4.5. α-Amylase inhibition assay. All of the extracts, fractions and compounds **1**–**6** were evaluated for α-amylase inhibition activity according to the methodology described by Hansawasdi-Kawabata [27] and modified by us [15]. Briefly, 200 µL of insoluble corn starch covalently linked with Remazol Brilliant Blue [28] (10 mg/mL) were boiled for 5 min with subsequent addition of 100 µL of Tris-HCl buffer (0.05 M, pH 6.9 with calcium chloride 0.01 M), 100 µL of porcine pancreatic *α*-amylase (PPA, 2.1 IU/mL) and 200 µL of the extract, fraction or pure compounds of *P. peruviana* or acarbose (positive control) at a concentration of 640 µg/mL. After incubation at 37 °C for 1 h, the absorbance was measured at 595 nm using a TECAN GENIOS spectrophotometer.

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	Peruviose C (1)			Peruviose D (2)			Peruviose E (3)			Peruviose F (4)		
Position	**xδ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**	**δ_C, type**	**δ_H, m, (*J* in Hz)**	**HMBC**
					Glucose unit							
1	89.41, CH	5.59, d, (3.6)	5, 2’	89.47, CH	5.47, d, (3.7)	5, 2’	89.40, CH	5.59, d, (3.7)	5, 2’	89.38, CH	5.60, d, (3.3)	5, 2’
2	70.10, CH	4.91, m	3, 1’’	70.16, CH	4.91, m	3, 1’’	70.06, CH	4.92, m	3, 1’’	70.03, CH	4.92, m	3, 1’’
3	69.05, CH	5.46, t, (9.9)	2, 4, 1’’’	69.08, CH	5.46, t, (10.0)	2, 4, 1’’’	69.02, CH	5.47, t, (10.0)	2, 4, 1’’’	68.98, CH	5.47, t, (9.9)	2, 4, 1’’’
4	68.29, CH	4.95, t, (9.9)	3, 5, 6, 1’’’’	68.34, CH	4.95, t, (10.0)	3, 5, 6, 1’’’’	68.25, CH	4.95, t, (10.0)	3, 5, 6, 1’’’’	68.24, CH	4.95, t, (9.9)	3, 5, 6, 1’’’’
5	72.03, CH	4.15, m		72.02, CH	4.14, m		72.18, CH	4.15, m		72.04, CH	4.15, m	
6	61.55, CH_2	3.55, m		61.56, CH_2	3.58, m		61.60, CH_2	3.58, m		61.50, CH_2	3.60, m	
					Fructose unit							
1’a	64.62, CH_2	3.59, m		64.55, CH_2	3.57, m		64.69, CH_2	3.58, d, (12.5)		64.54, CH_2	3.58, d, (13.0)	
1’b		3.46, m			3.47, m			3.46, d, (12.5)			3.46, d, (13.0)	
2’	103.84, C			103.94, C	-		103.81, C	-		103.82, C	-	
3’	79.11, CH	5.21, d, (8.1)	1’, 4’, 1’’’’’	79.21, CH	5.22, d, (8.1)	1’, 4’, 1’’’’’	79.12, CH	5.18, d, (8.2)	1’, 4’, 1’’’’’	79.05, CH	5.20, d, (7.9)	1’, 4’, 1’’’’’
4’	71.32, CH	4.52, t, (8.1)	3’, 5’, 6’	71.28, CH	4.55, t, (8.1)	3’, 5’, 6’	71.19, CH	4.59, t, (8.2)	3’, 5’, 6’	71.30, CH	4.52, t, (7.9)	3’, 5’, 6’
5’	82.61, CH	3.95, m	4’	82.61, CH	3.91, m	4’	82.55, CH	3.93, m	4’	82.54, CH	3.92, m	4’
6’a	59.90, CH_2	3.88, m	4’	59.88, CH_2	3.73, m	4’	59.73, CH_2	3.89, m	4’	59.92, CH_2	3.89, m	4’
6’b		3.72, br d, (13.4)			3.70, m			3.73, br d, (12.3)			3.75, m	
					Substituent at C2							
1’’	172.87, C	-		172.88, C	-		172.78, C	-		172.87, C	-	
2’’	33.86, CH_2	2.25, m	3’’	33.93, CH_2	2.25, m	3’’	34.05, CH_2	2.25, m	3’’	33.83, CH_2	2.25, m	3’’
3’’	24.54, CH_2	1.53, m	2’’	24.57, CH_2	1.56, m	2’’	24.57, CH_2	1.54, m	2’’	24.54, CH_2	1.54, m	2’’
4’’	29.14, CH_2	1.24, br s		29.57, CH_2**	1.24, br s		29.08, CH_2**	1.25, br s		28.85, CH_2	1.25, br s	
5’’	29.05 – 29.01, CH_2**	1.24, br s		29.57, CH_2**	1.24, br s		29.22, CH_2**	1.25, br s		29.00, CH_2	1.25, br s	
6’’	31.75, CH_2	1.24, br s		29.39 , CH_2**	1.24, br s		29.31, CH_2**	1.25, br s		31.56, CH_2	1.25, br s	
7’’	22.58, CH_2	1.24, br s		29.30 , CH_2**	1.24, br s		29.39, CH_2**	1.25, br s		22.56, CH_2	1.25, br s	
8’’	14.03, CH_3	0.86, t, (6.8)	6’’, 7’’	29.21, CH_2**	1.24, br s		29.58, CH_2**	1.25, br s		14.04, CH_3	0.87, t, (6.7)	6’’, 7’’
9’’				29.09, CH_2**	1.24, br s		29.69, CH_2**	1.25, br s				
10’’				31.88, CH_2	1.24, br s		31.89, CH_2	1.25, br s				
11’’				22.66, CH_2	1.24, br s		22.66, CH_2	1.25, br s				
12’’				14.07, CH_3	0.87, t, (7.0)	6’’, 7’’	14.08, CH_3	0.88, t, (6.8)	10’’, 11’’			
					Substituent at C3							
1’’’	175.71, C	-			174.05	-	175.73, C	-		175.76, C	-	
2’’’	33.88, CH	2.45, sp, (7.0)			33.87	2.45, sp, (7.0)	33.89, CH	2.45, sp, (7.0)		33.87, CH	2.45, sp, (7.0)	
3’’’a	18.67-19.07, CH_3*	1.08 d, (7.0)	2’’’	2’’’	18.88-18.75, CH_3	1.08 d, (7.0)	18.71-19.06, CH_3*	1.07, d, (7.0)	2’’’	18.66-19.08, CH_3*	1.07, d, (7.0)	2’’’
3’’’b	18.67-19.07, CH_3*	1.06 d, (7.0)	2’’’	2’’’	18.88-18.75, CH_3	1.07 d, (7.0)	18.71-19.06, CH_3*	1.08, d, (7.0)	2’’’	18.66-19.08, CH_3*	1.08, d, (7.0)	2’’’

Substituent at C4												
1’’’’	175.96, C	-		175.63, C	-		175.98, C	-		176.00, C	-	
2’’’’	33.92, CH	2.51, sp (7.0)	3’’’’	33.89, CH	2.52, sp, (7.0)	3’’’’	33.93, CH	2.51, sp, (7.0)	3’’’’	33.90, CH	2.51, sp, (7.0)	3’’’’
3’’’’a	18.67-19.07, CH_3*	1.13, d (7.0)	2’’’’	18.88-18.75, CH_3	1.13, d, (7.0)	2’’’’	18.71-19.06, CH_3*	1.14, d, (7.0)	2’’’’	18.66-19.08, CH_3*	1.14, d, (7.0)	2’’’’
3’’’’b	18.67-19.07, CH_3*	1.11, d, (7.0)	2’’’’	18.88-18.75, CH_3	1.11 d, (7.0)	2’’’’	18.71-19.06, CH_3*	1.05, d, (7.0)	2’’’’	18.66-19.08, CH_3*	1.06 , d, (7.0)	2’’’’
Substituent at C3’												
1’’’’’	177.80, C	-		176.06, C			177.93, C	-		177.81, C	-	
2’’’’’	43.13, CH_2	2.39, dd, (12.8, 6.7)	4´´´a	43.15, CH_2	2.40, dd, (14.0, 7.0)	4´´´a	34.02, CH	2.75, sp, (7.0)	3’’’’’	33.99, CH	2.75, sp, (7.0)	3’’’’’
3’’’’’	25.84, CH	2.20, m	2’’’’’	25.87, CH	2.19, m	2’’’’’						
3’’’’’a							18.71-19.06, CH_3*	1.33, d, (7.0)	2’’’’’	18.66-19.08, CH_3*	1.32, d, (7.0)	2’’’’’
3’’’’’b							18.71-19.06, CH_3*	1.29, d, (7.0)	2’’’’’	18.66-19.08, CH_3*	1.29, d, (7.0)	2’’’’’
4’’’’’a	22.54, CH_3	1.05 , d, (6.5)	2’’’’’, 3’’’’’, 4’’’’’b	22.30, CH_3	1.06, d, (6.4)	2’’’’’, 3’’’’’, 4’’’’’b						
4’’’’’b	24.54, CH_3	1.03, d, (6.5)	2’’’’’, 3’’’’’, 4’’’’’a	22.42, CH_3	1.04, d, (6.4)	2’’’’’, 3’’’’’, 4’’’’’a						

*The multiplicity could not be determined
** Signals may be interchanged

Table 1. NMR-^{1}H data ($CDCl_3$, 400 MHz) for Compound **1** to compound **4** (Peruvioses C-F).

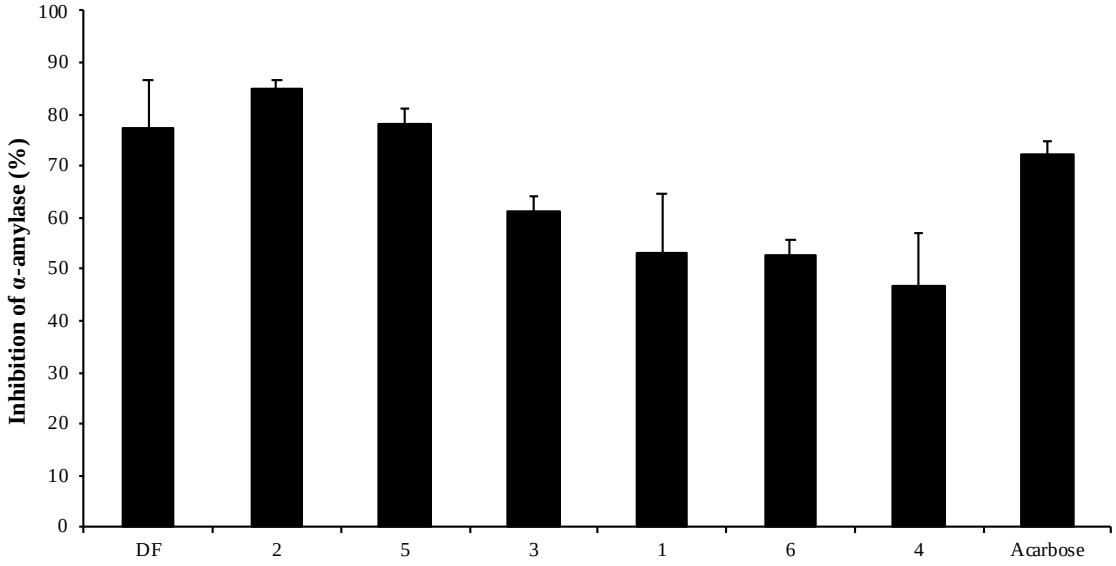
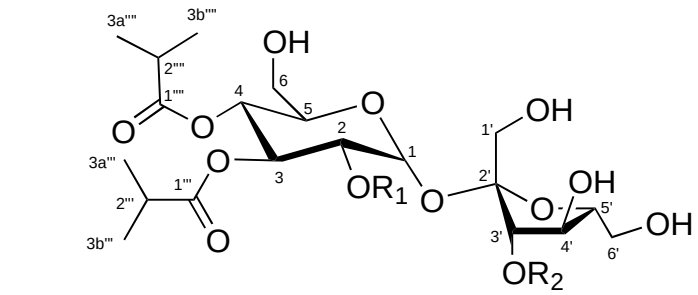
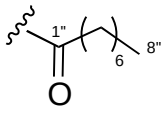
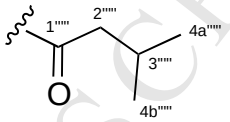
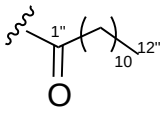
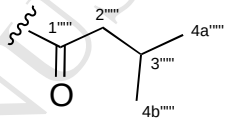
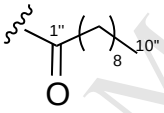
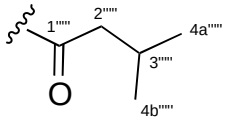


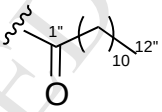
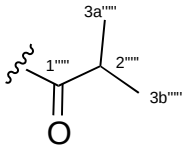
Figure 1. *In vitro* inhibition of α-amylase for the DF extract and its major components, peruvioses A–F, displayed from higher to lower inhibitory activity. CH_2Cl_2 extract (DF), peruviose C (**1**), peruviose D (**2**), peruviose E (**3**), peruviose F (**4**), peruviose B (**5**), peruviose A (**6**) and acarbose as a positive control. All compounds were tested at 640 µg/mL. Results are expressed as mean ± S.D.

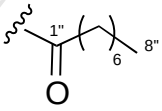
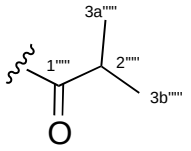


Peruviose C (**1**) R_1 =  R_2 = 

Peruviose D (**2**) R_1 =  R_2 = 

Peruviose B (**5**) R_1 =  R_2 = 

Peruviose E (**3**) R_1 =  R_2 = 

Peruviose F (**4**) R_1 =  R_2 = 

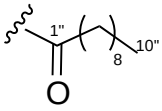
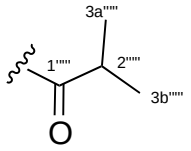
Peruviose A (**6**) R_1 =  R_2 = 

Figure 2. Sucrose esters isolated from *Physalis peruviana* fruits

Highlights

- The dichloromethane extract of *P. peruviana* fruit showed a considerable inhibitory effect on the α-amylase enzyme.
- Sucrose esters were responsible for α-glucosidase inhibition and this explains the hypoglycemic effect that has been attributed to gooseberry fruits.
- Three new sucrose esters were isolated, peruvioses C–E (**1**–**3**), along with three known sucrose esters, peruvioses A (**6**), B (**5**) and F (**4**).
- Peruviosides D (**2**) and B (**5**) showed the highest inhibitory effect on the α-amylase enzyme.