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BIOSYNTHESIS, PURIFICATION, AND CHARACTERIZATION OF MONK FRUIT SWEETENER, SIAMENOSIDE I

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Siraitia grosvenorii, Siamenoside I, Mogroside V, Bioconversion, Mogrosides, Structure Characterization, Nuclear Magnetic Resonance (NMR). 4

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ABSTRACT: An efficient and high yield synthesis of Siamenoside I (1), from Mogroside V (2) is reported. Siamenoside I (1) was obtained by enzymatic hydrolysis of Mogroside V (2). Bioconversion process involved enzymatic modification followed by separation and purification processes, including reversed-phase chromatography. The final product, a dried white powder, undergoes several stages of concentration, reaction, filtration, and freeze-drying. Siamenoside I (1) was isolated and characterized with NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC-DEPT, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-TOCSY, ^{1}H-^{1}H ROESY and 1D-TOCSY) and mass spectral data. This Siamenoside I (1) possess a 1->2 beta sugar linkage between sugar II and sugar I, making it's structural properties unique. The sensory and spectral properties of Siamenoside I (1) synthesized from mogroside V (2) or isolated from fruit have the same characteristics. Additionally, we emphasize the importance of 1D and 2D NMR techniques when identifying complex mogrosides.

INTRODUCTION: Sweetness is universally regarded as pleasant and it is the preferred taste for beverages, food, pharmaceuticals, and oral hygiene/cosmetic products. To provide sweet taste to consumer products the most commonly used natural caloric sugars are sucrose, fructose, and glucose. Since these natural sugars provide calories, alternative sources must be utilized when the consumer desires a sweet taste with low to no calories. Artificial and natural sweeteners have been developed to fulfill both criteria [1-2]. It is not a simple task to create a non-caloric or low-calorie sweetener because they exhibit a temporal profile, maximal response, flavor profile, mouth feel, and/or adaptation behavior that differ from sugar [3, 4, 13].

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To provide the three criteria of an ideal sweetener (sweet, low to no calorie, and natural) we have looked to mogrosides isolated from *Siraitia grosvenorii* [5-7]. The fruit of *Siraitia grosvenorii* (Swingle) also known as Monk Fruit or Luo Han Guo grows in Guangxi, People's Republic of China, and is used as an expectorant as well as a natural sweetener in that country [8-10]. The fruit has been used for the treatment of dry cough, sore throat, and constipation [4]. Monk Fruit is well known now throughout the world due to its intense sweet taste and has been used as a noncaloric natural sweetener in some countries in beverages as well as juice.

There are more than 50 mogrosides reported in the literature. Saimenoside I is the best tasting mogroside known so far. Several Monk Fruit extract with various levels of Mogroside V (15% – 95%) have received Letter of No Objection concerning its Generally Recognized as Safe (GRAS) status from US FDA [11]. In the present study we report on the novel biosynthesis, isolation, and characterization of one of the best

tasting mogrosides, Siamenoside I (1) [12], from the bioconversion of Mogroside V (2) using beta-galactosidase enzyme **Fig. 1**. The structure of Siamenoside I was determined using a suite of 1D and 2D NMR experiments together with mass spectral data. The sensory properties of Siamenoside I (1) synthesized from Mogroside V (2) or isolated from fruit have the same characteristics.

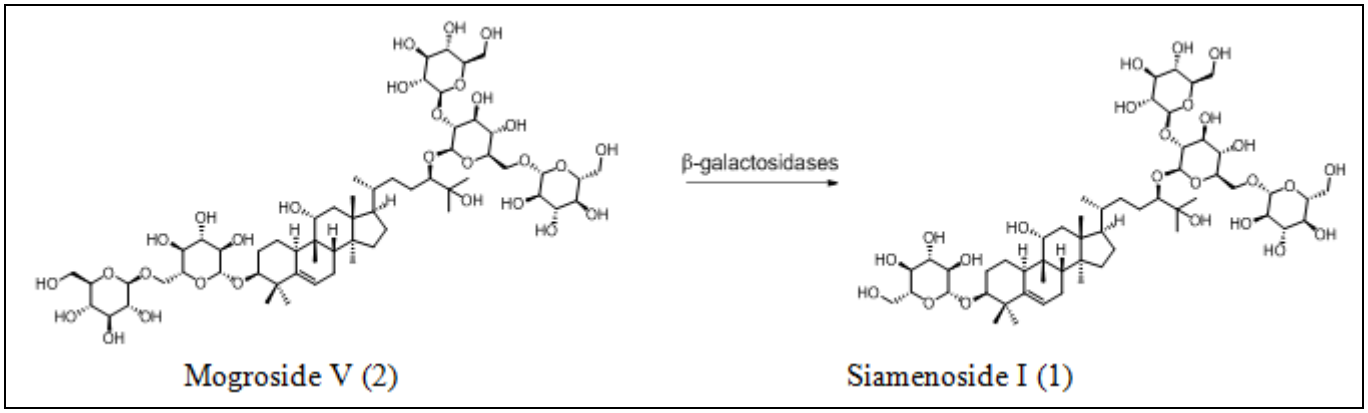


FIG. 1: ENZYMATIC SYNTHESIS OF SIAMENOSIDE I

RESULTS AND DISCUSSION: In our continued efforts to discover natural, non-calorie sweeteners from the bioconversion reaction of Mogroside V, we have isolated and characterized Siamenoside I (1) **Fig. 1**. There are several enzymatic methods known in the literature for the production of Siamenoside I but the yield is low, produces various undesired product and makes it cumbersome to purify. Here we report high yield bioconversion, isolation and complete characterization using NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC-DEPT, ^{1}H-^{13}C HMBC, ^{1}H-^{13}C HSQC-TOCSY, 1D-TOCSY and ^{1}H-^{1}H ROESY) and high-resolution mass spectral data of Siamenoside I (1).

Compound 1 was isolated as a white solid. The analysis of 1D and 2D NMR data indicated that compound 1 is a triterpene mogrol glycoside bearing four sugar units. Presence of a triterpene mogrol core was supported by ^{1}H NMR data which resonated seven methyl singlets between δ_H0.89 – 1.19 (H-18, H-19, H-26, H-27, H-28, H-29, and H-30), a methyl doublet (H-21) at δ_H0.98, eight methylenes between δ_H 1.14 – 2.39 (H-1, H-2, H-7, H-12, H-15, H-16, H-22, and H-23), four methine protons between δ_H 1.46 – 2.50 (H-8, H-10, H-17, and H-20), three additional methines between δ_H 3.40-3.85 (H-3, H-11, and H-24), attached to carbons bearing oxygen groups, and a tertiary hydroxyl carbon at δ_C73.9 (C-25). The triterpenoid aglycone central core for compound 1 was supported by ^{1}H-^{1}H COSY correlations of H-1/H-2; H-2/H-3; H-6/H-7; H-7/H-8; H-10/H-1; H-11/H-12; H-15/H-16; H-16/H-17; H-17/H-20; H-20/H-21; and H-23/H-24 and ^{1}H-^{13}C HMBC correlations of H-3/C-1, C-4, C-5; H-6/C-4, C-7, C-8, C-10; H-7/C-5, C-6, C-14; H-8/C-6, C-7, C-9, C-10, C-14; H-11/C-9, C-12, C-19; H-12/C-13, C-14, C-18; H-18/C-12, C-13, C-14, C-17; H-19/C-10, C-11; H-21/C-17, C-20, C-22; H-24/C-22, C-23, C-27; H-26 and H-27/C-24, C-25; H-28 and H-29/C-3, C-4, C-5 and H-30/C-8, C-13, C-14, C-15. The complete ^{1}H and ^{13}C NMR assignments of the aglycone of 1 was made on the basis of ^{1}H, ^{13}C, COSY, HSQC-DEPT and HMBC data and are given in Table 1. The key ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC correlations used to assign the aglycone unit of 1 are provided in **Fig. 2.**

The relative stereochemistry in the central triterpene core was assigned based on NOE correlations observed in the ROESY experiment. In the ROESY spectrum of 1, NOE correlations were observed between H-10 and H-28, H-10 and H-30 as well as between H-30 and H-17 indicating that H-10, H-17, H-28, and H-30 are on the same face of the rings. Similarly, NOE correlations were observed between H-8 and H-18 as well as between H-18 and H-11 indicating that H-8, H-11 and H-18 are on the same face of the rings. NOE correlations were not observed between H-8/H-11/H-18 and H-10/H-17/H-30 indicating that H-8, H-11 and H-18 were on the opposite face of the rings compared to

H-10, H-17 and H-30. Although, the relative stereochemistry of H-3 and H-19 could not be assigned unambiguously based on the ROESY data, they are expected to be the same as in other mogroside compounds because ^{1}H and ^{13}C chemical shifts of central triterpene core for 1 are consistent with related mogrosides [10].

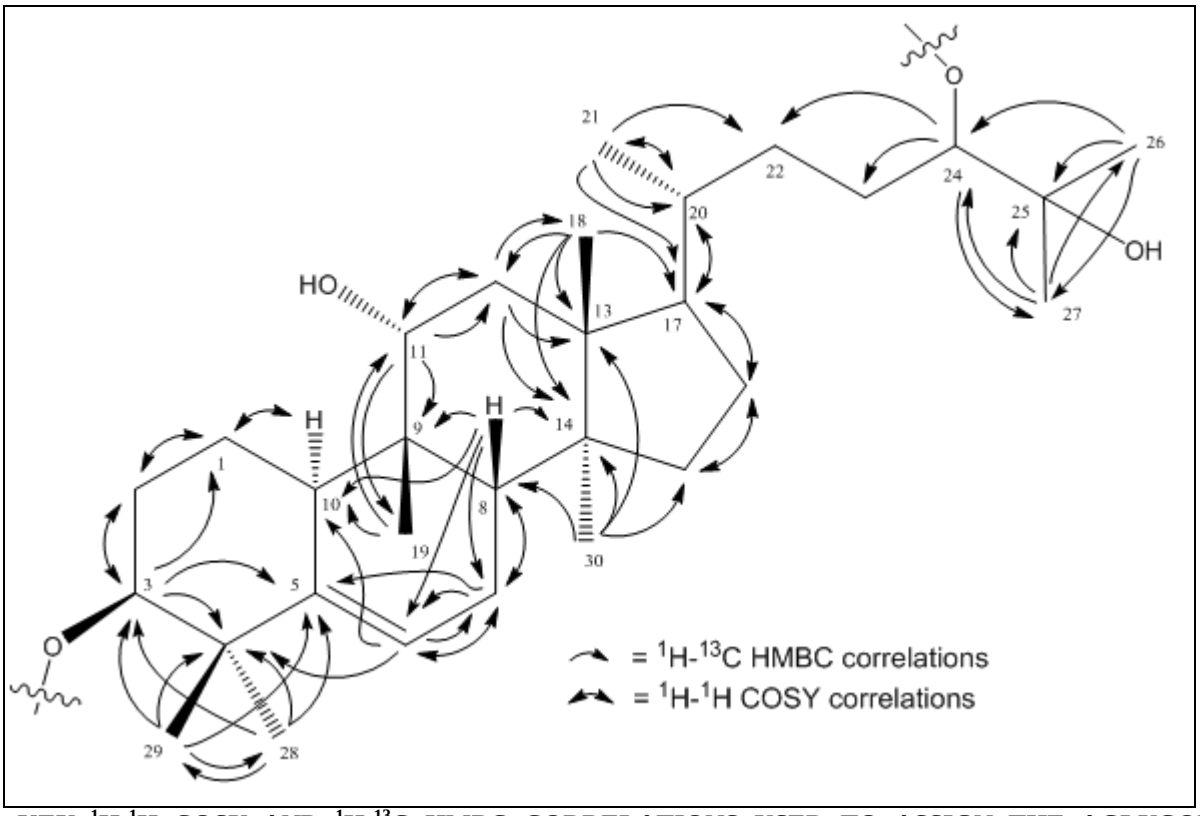


FIG. 2: KEY ^{1}H-^{1}H COSY AND ^{1}H-^{13}C HMBC CORRELATIONS USED TO ASSIGN THE AGLYCONE OF MOGROSIDE V (1)

The ^{1}H and ^{1}H-^{13}C HSQC-DEPT data for compound **1** confirmed the presence of four anomeric protons at δ_H 4.78 (δ_C 104.4 or 104.5), δ_H 4.44 (δ_C 104.2), δ_H 4.29 (δ_C 104.4 or 104.5) and δ_H 4.28 (δ_C 106.6). All four anomeric protons had large couplings (7.4 – 7.8 Hz) indicating β-configurations.

TABLE 1: ^{1}H AND ^{13}C NMR (500 AND 125 MHZ) ASSIGNMENTS OF THE AGLYCONE CORE OF SIAMENOSIDE I IN CD_3OD

Position	^{1}H Chemical Shift	^{13}C Chemical Shift
1	2.22 dd (12.6, 3.0) 1.51 m	27.2
2	1.94 m	29.6
3	3.42 m	88.6
4	---	42.9
5	---	145.0
6	5.49 d (6.0)	119.6
7	2.39 dd (18.5, 7.1) 1.82 m	25.1
8	1.67 m	44.7
9	---	40.9
10	2.50 brd (12.1)	37.3
11	3.85 m	79.4
12	1.87 m, 1.82 m	41.1
13	---	48.3
14	---	50.6
15	1.22 m, 1.14 m	35.3
16	1.99m, 1.33m	29.4
17	1.63 m	51.8

18	0.92 s	17.1
19	1.12 s	26.2
20	1.46 m	37.5
21	0.98d (6.3)	19.3
22	1.57 m, 1.47 m	34.1
23	1.55 m, 1.39 m	29.9
24	3.40 m	93.4
25	---	73.9
26	1.11 s[†]	26.3[†]
27	1.15 s[†]	24.1[†]
28	1.08 s	27.9
29	1.19 s	26.3
30	0.89 s	20.0

[†]Assignments can be interchanged.

The anomeric proton observed at δ_H 4.28 showed an HMBC correlation to C-3 which indicated that it corresponded to the anomeric proton of GlcIV. A reciprocal HMBC correlation from H-3 to the anomeric carbon of GlcIV (δ_C 106.6) was also observed confirming this linkage. Since the anomeric proton of GlcIV at δ_H 4.28 was partially overlapped with the anomeric proton of another sugar unit resonated at δ_H 4.29, analysis of 1D-TOCSY as well as 2D NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC-DEPT, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC-TOCSY) data allowed the complete proton assignments in GlcIV as H-2 (δ_H 3.19), H-3 (δ_H 3.31), H-4 (δ_H 3.27), H-5 (δ_H 3.21), and H-6 (δ_H 3.82 and 3.65) and the HSQC-DEPT data then allowed the assignments of the carbons as C-2 (δ_C 75.6 or 75.7), C-3 (δ_C 77.9-78.2), C-4 (δ_C 71.5-71.7), C-5 (δ_C 77.7) and C-6 (δ_C 62.7 or 62.8). These assignments were further confirmed by HMBC correlations of H-1/C-2 and C-3; H-2/C-1 and C-3; H-3/C-2 and C-4; H-4/C-3, C-5 and C-6 and H-6/C-4. The complete ^{1}H and ^{13}C NMR assignments of GlcIV are provided in **Table 2** while the key COSY and HMBC correlations are provided in **Fig. 3.**

TABLE 2: ^{1}H AND ^{13}C CHEMICAL SHIFTS OF THE SUGAR RESIDUES IN SIAMENOSIDE I

Sugar	Position	^{1}H Chemical Shift	^{13}C Chemical Shift
Glc I	1	4.44 d (7.4)	104.2
	2	3.63 m	81.2
	3	3.59 m	78.7
	4	3.33 m	71.5-71.7[£]
	5	3.51 m	76.4
	6	4.25dd (10.4, 1.8) 3.61 m	70.1
Glc II	1	4.29 d (7.7)	104.4 or 104.5
	2	3.21 m	75.2
	3	3.36 m	77.7[¥]
	4	3.28 m	71.5-71.7
	5	3.28 m	77.9-78.2[€]
	6	3.85 m, 3.65 m	62.7 or 62.8
Glc III	1	4.78 d (7.8)	104.4 or 104.5
	2	3.28 m	75.6 or 75.7
	3	3.37 m	77.9-78.2[€]
	4	3.21 m	72.4
	5	3.28 m	77.9-78.2[€]
	6	3.87 m, 3.64 m	63.5
Glc IV	1	4.28 d (7.8)	106.6
	2	3.19 m	75.6 or 75.7
	3	3.31 m	77.9-78.2[€]
	4	3.27 m	71.5-71.7[£]
	5	3.21 m	77.7[¥]
	6	3.82 m, 3.65 m	62.7 or 62.8

[¥]Two carbon resonances overlapped at 77.65 ppm.[€,£]Chemical shifts could not be unequivocally assigned due to very close chemical shifts.

Further analysis of the 1D and 2D NMR data allowed the assignment of the remaining three sugars in 1. An anomeric proton observed at δ_H 4.44 showed long range HMBC correlations to a carbon at δ_C 93.4 (C-24) and established its connectivity to the central triterpene core at C-24 and allowed its assignment as the anomeric proton of GlcI. HMBC correlation from H-24 (δ_H 3.40) to the anomeric carbon of GlcI (δ_C 104.2) was also observed confirming the attachment of GlcI at C-24. The ^{1}H coupling sequence from GlcI anomeric proton (δ_H 4.44) to H-2 (δ_H 3.63) through H-3 (δ_H 3.59), H-4 (δ_H 3.33), H-5 (δ_H 3.51) and the oxymethylene protons, H-6 (δ_H 4.25 and 3.61), was established using a combination of ^{1}H-^{1}H COSY and 1D-TOCSY data. As done above for GlcIV, the carbon assignments in GlcI were completed based on the HSQC-DEPT and HMBC data and are provided in **Table 2.** The relatively higher ^{13}C NMR chemical shifts of the C-2 and C-6 positions suggested glycosyl substituents at these positions in GlcI which was confirmed by HMBC correlations. Thus long range ^{1}H-^{13}C correlations in the HMBC experiment from GlcI H-2 and GlcI H-6 to the anomeric carbons at δ_C 104.4 or 104.5 (GlcIII, C-1 and GlcII, C-1), as well as from the anomeric protons at δ_H 4.78 (GlcIII, H-1) to the carbon at δ_C81.2 (GlcI, C-2) and δ_H 4.29 (GlcII, H-1) to the carbon at δ_C 70.1 (GlcI, C-6) supported glycoside linkages at positions 2 and 6 and established the 2,6-*O*-branched-D-glucodiosyl substituent in GlcI. The complete ^{1}H and ^{13}C assignments for GlcII and GlcIII were done as described above for GlcIV. The complete ^{1}H and ^{13}C assignments of GlcI, GlcII and GlcIII was done based on extensive analysis of 1D and 2D NMR data and are provided in **Table 2** while the key ^{1}H-^{1}H COSY and HMBC correlations are provided in **Fig. 3.** The structure of 1 was further supported by mass spectrometry data. Accurate mass measurement of 1 provided an exact mass of *m/z* 1123.5938 in the negative ESI mass spectrum. This corresponded to a molecular formula of $C_{54}H_{92}O_{24}$ (calcd. for $C_{54}H_{91}O_{24}$: 1123.5906).

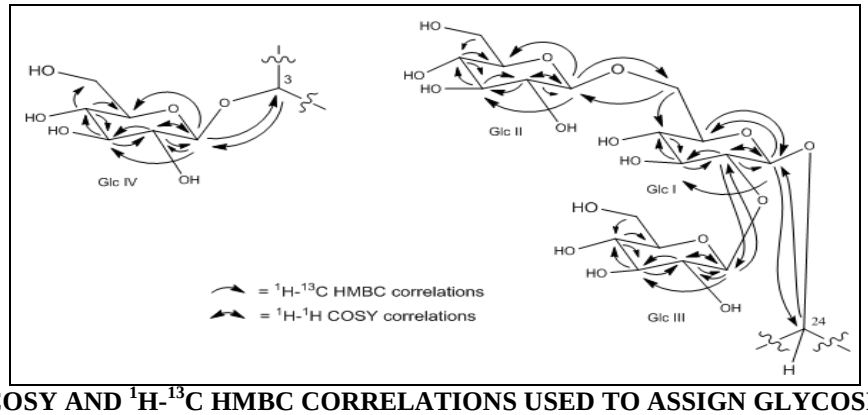
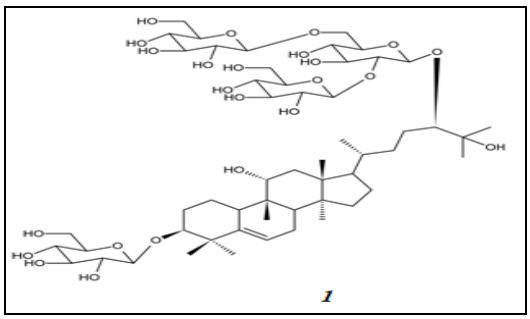


FIG. 3: KEY ^{1}H-^{1}H COSY AND ^{1}H-^{13}C HMBC CORRELATIONS USED TO ASSIGN GLYCOSIDE REGIONS AT C-3 AND C-24 OF COMPOUND 1

Experimental Section:

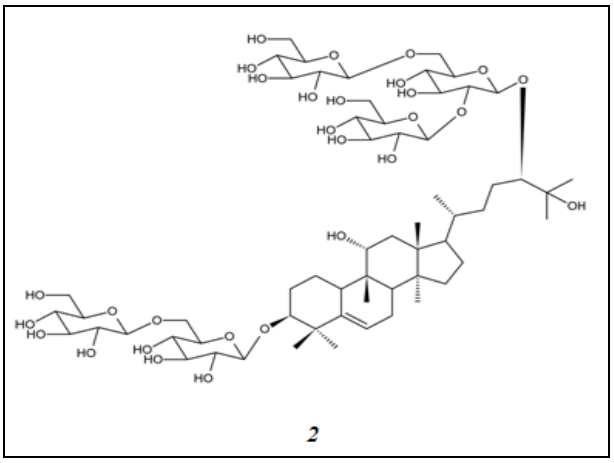
General Experimental Procedure for the purification of Siamenoside I (1): The production of Siamenoside I from Mogroside V involves enzymatic modification followed by separation and purification processes, including reversed-phase chromatography. The final product, a dried powder, is produced after several stages of concentration, filtration, and freeze-drying.



SIAMENOSIDE I STRUCTURE

Overview of Key Steps:

Stage 1: Enzyme Concentration and Microfiltration: Ultrafiltration was used to concentrate the enzyme and.



MOGROSIDE V STRUCTURE

Stage 2: Enzyme Reaction in Bioreactor: Sterilization of the bioreactor vessel and sterile filtration of solutions. Reaction monitoring with regular sampling and pH adjustments to ensure optimal enzyme activity.

Stage 3: Post-Bioreactor and Pre-Column Workup: High pH and low pH ultrafiltration to remove enzymes and concentrate the reaction mixture. Preparation of solutions for chromatography and subsequent filtration processes.

Stage 4: Chromatography: Multiple rounds of column chromatography to separate and collect high purity Siamenoside I. Utilization of ethanol, RO water, and ammonium acetate buffer for effective separation.

Stage 5: Post-Column Workup: Final volume reduction and product concentration by nanofiltration. Final purification steps including freeze-drying and milling.

Enzyme Concentration and Microfiltration: Maxilact A4 (DSM Lot 417111301) was concentrated using an ultrafiltration (UF) system consisting of a stainless steel vessel, a positive displacement pump with flexible blades, and a Koch Romicon PM5 membrane. Initially, the UF system was cleaned with Ultrasil 110 solution followed by UV-treated RO water rinses until the pH was neutral (pH ≤ 7.7). Approximately 2.19 kg of Maxilact A4 enzyme solution was charged into the vessel and concentrated to an estimated volume of 0.46 L, operating over a 13-hour period. The concentrated enzyme was then microfiltered using a Koch Wine filter membrane to remove impurities. Buffer solutions, prepared by dissolving sodium phosphate dibasic dihydrate, magnesium chloride hexahydrate, and sodium acetate in UV-treated RO water, were added to the concentrated enzyme to achieve the desired pH of 6.30 ± 0.02. The microfiltration process involved passing the enzyme solution through the membrane with low turbulence to minimize enzyme denaturation. The final concentrated enzyme solution was covered and stored at ambient temperature overnight before subsequent use in the bioreactor.

Enzyme Reaction in Bioreactor: The enzymatic conversion of Mogroside V to Siamenoside I was carried out in a sterilized 5-L jacketed fermentation vessel equipped with an agitator. The vessel was cleaned with DZolv and UV-RO water before being steam-sterilized at 121°C and 1 barg for 60 minutes, maintaining a post-sterilization pressure of 0.7 barg. Sterile filtration was employed to transfer 33.5 g of Mogroside V (Lot LHGE-17-1228) dissolved in 0. 224 kg of UV-RO water and buffered with 3.34 g sodium phosphate dibasic dihydrate, 0.70 g magnesium chloride hexahydrate, and 1.54 g sodium acetate anhydrous to pH 6.30 ± 0.02 into the bioreactor. Concurrently, the previously prepared concentrated Maxilact A4 enzyme (DSM Lot 417111301) was also sterile-filtered into the reactor. The bioreactor was maintained at 54°C with an operating pressure of 5 psig using sterile air, while agitation was set to 35 rpm. The reaction mixture was periodically sampled and analyzed using HPLC to monitor enzyme activity and reaction progress. Adjustments to the pH were made as needed to optimize the enzymatic conversion. Over approximately 12 days, the enzymatic reaction proceeded, resulting in the conversion of Mogroside V to Siamenoside I. The final reaction mixture was then transferred into a 2 L HDPE container, without further rinsing, and stored at ≤ -15°C for subsequent processing steps.

Post-Bioreactor and Pre-Column Workup: Following the enzymatic reaction, the about 2 L of reaction mixture containing approximately 19.1 g of resultant Siamenoside I was subjected to a series

of purification steps. Initially, the solution was adjusted to pH 12.4 with the addition of 30% sodium hydroxide (NaOH) solution (32.4 g) and mixed with 95% ethanol (82.9 g) to a final concentration of ~16.7% v/v ethanol. Ultrafiltration was performed using a Koch Romicon PM10 6043 membrane system, processing portions of the reaction mixture to remove enzymes and other impurities. Each permeate collected (approximately 0.2 L portions) was subsequently pH-adjusted to 5.5 using 80% acetic acid. The procedure was repeated until the entire reaction mixture was processed.

Following initial ultrafiltration, the permeate underwent secondary ultrafiltration at pH 5.5 to further purify the solution. Additionally, RO UV water and XNS ethanol were charged into the feed tank in specific amounts (e.g., 0.28 kg RO UV water and 48.0 g XNS ethanol) to rinse and facilitate further purification through nanofiltration. The final permeate was adjusted to pH 5.5 and subjected to C18 guard column treatment, which involved passing the solution through a Biotage SNAP KP-C18-HS 40g cartridge at <5 barg to remove remaining byproducts. The purified solution was then portioned into 0.3 kg aliquots and stored in HDPE containers at freezer temperatures pending chromatography.

Chromatography: The purification of Siamenoside I was performed using reversed-phase chromatography with a Biotage Ultra C18, cartridge. Load solutions were prepared by dissolving the post-bioreactor mixture containing approximately 7g of Siamenoside I into 0.3 kg aliquots. For each chromatography run, a 5-L stainless steel container was used as the feed vessel, and a pump with pressure control set at 6 barg was employed. The chromatography process began with column equilibration using Eluent 1, consisting of 3.5 kg UV-RO water, 91.5 g XNS ethanol, and 9.3 g 2 M ammonium acetate buffer (pH 5.0). The mixture was thoroughly stirred before being passed through the column.

Following equilibration, the load solution was applied to the column and eluted with Eluent 3, prepared by mixing 3.88 kg UV-RO water, 1.23 kg XNS ethanol, and 14 g 2 M ammonium acetate buffer (pH 5.0). The elution process continued until collected fractions showed a Siamenoside I purity of over 96%, verified by HPLC. At this point, Eluent 6, containing 3.09 kg UV-RO water, 1.15 kg XNS ethanol, and 11 g 2 M ammonium acetate buffer (pH 5.0), was introduced to elute the remaining product. Fractions were collected in 2 L or 0.5 L HDPE containers, depending on the eluent progress, and analyzed for purity. Fractions with purity greater than 80% Siamenoside I were stored in a food-grade freezer. Each chromatography session ensured careful monitoring and buffer adjustments to maintain optimal conditions for Siamenoside I separation and purification.

Post-Column Workup: After chromatography, the collected fractions of >96% purity Siamenoside I were pooled for further processing. The fractions were first thawed to ambient temperature overnight. Nanofiltration (NF) was used to concentrate and purify the solution further. The NF system comprised a 15.5-L stainless steel vessel, a CS32-260 pump, and a Koch SR3D 200 Da membrane, with a heat exchanger inline to maintain ambient temperature. The pooled fractions were diluted with an equal volume of UV-RO water to reduce ethanol content before starting the NF. The NF process began with a recirculation phase for 20 minutes, followed by directing the permeate to waste until the volume was reduced to ~1.5 L. Three 1 kg portions of UV-RO water were added sequentially to the feed tank, maintaining the volume at 1.5 L. Each addition was followed by sufficient processing time to ensure thorough dilution and concentration reduction. After NF, the solution was further purified through diafiltration using UV-RO water.

To finalize purification, the diafiltered solution underwent freeze-drying in Lyoguard trays. Prior to freeze-drying, any remaining solids were processed through mixed-bed ion exchange and filtration using a 0.2 µm filter to ensure high purity. The final step involved milling the freeze-dried product to achieve a consistent powder form, which was then packed into food-grade HDPE containers for storage.

Isolation and Purification: Small Scale Purification of crude reaction mixtures was accomplished using Waters XBridge Phenyl column systems with HPLC analysis at stages

during the purification process. HPLC analyses were performed on a Waters 2695 Alliance System coupled to a Waters 996 Photo Diode Array (PDA, Waters Corp) detector. In addition, sample purities were assessed using an ESA Corona plus Charged Aerosol Detector (CAD Plus, Dionex, Sunnyvale, CA, USA). The details of the experimental procedure can be found in the reference outlined by Prakash *et al.* [13].

The primary and secondary processing of the samples were performed using a Waters X Bridge Phenyl column (19 x 250 mm, 5 µm). The purification process was performed using a Waters Delta Prep 2000/4000 system coupled to a Waters 2487 UV–Vis detector). The purification process was performed using a Waters 2545 Quaternary Gradient Module system coupled to a Waters 2489 UV–Vis detector.

Primary Purification: Approximately 1 mL of the sample was processed using the primary preparative HPLC method. Sample received in approximately 0.3 mL of Glycerol, and, was syringe filtered in order to minimize Glycerol contamination, mixed with organic solvent (Acetonitrile) and diluted up to 10 mL with Water. Glycerol contamination was noted as an issue in primary processing, in this case it resulted in fractions of interest eluting in the flush. Collected fraction Lot# KTC-B-115(Flush) was selected for reprocessing.

Secondary Purification: Fraction Lot# KTC-B-115(Flush) was reprocessed. Collected fraction KTC-B-123(1), with the retention time approximately 13.947 min on the respective preparative HPLC trace, was deemed sufficiently pure for structural elucidation *via* NMR.

Final Batch Preparation: Fraction Lot# KTC-B-123(1)) was concentrated by rotary evaporation and further dried *via* lyophilization for 24 hours. The final yield of the batch KTC-B-123(1) was 1.4 mg. The final purity was determined using the analytical method summarized in **Table 2** and found to be 97.09% (AUC, CAD) with a retention time of 13.632 min; the analysis is provided in Figure 6. Reference Siamenoside I was run in sequence with KTC-B-123(1), retention time was 13.561 min.

HPLC Analysis: Samples were analyzed on a Phenomenex Synergi-Hydro RP, 250 mm x 4.6 mm, 4µm (part # 00G-4375-E0) ran at 55°C at a flow rate of 0.5 mL. min using a gradient of two solvents. Solvent A was composed of 0.569 g of ammonium acetate and 0.231 g of acetic acid in 2 L of 18 MΩ·cm Water. Solvent B was composed of acetonitrile. Elution of compounds was monitored by a UV dectector set to 215 nm, 4 nm bandwidth, reference at 265 nm with 100 nm bandwidth. The gradient profile was 25% solvent B at 0 minutes, 58% solvent B at 25 minutes, 90% solvent B at 35 minutes, 90 % solvent B at 38 minutes, 25% solvent B at 38.1 minutes, and 25 % solvent B at 43 minutes. With this gradient, all major peaks were well resolved and compared to mogroside standards ran with this method. 11-oxomogroside V eluted at about 8.6 minutes, Mogroside V eluted at about 9.4 minutes, 11-oxosiamenoside I eluted at about 10.1 minutes, Siamenoside I eluted at about 10.6 minutes, Mogroside IV eluted at about 11.3 minutes, and Mogroside IIIE eluted at about 12.7 min. Samples were ultrafiltered with a 10 kDa spin filter and 5 × dilution in 20% acetonitrile in water before injection. The injection volume was 50 microliters.

Mass Spectrometry: The ESI-TOF mass spectra and MS/MS data were generated with a Waters Q-Tof Premier mass spectrometer (Waters Corp., Manchester, UK) equipped with an electrospray ionization source. Samples were analyzed by negative ESI, prior to that samples were diluted with H_2O: MeCN (1:1) by 50 fold and introduced *via* infusion using the onboard syringe pump.

Nuclear Magnetic Resonance: To determine the complete structure of Siamenoside I (1), we carried out a series of NMR experiments including ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC-DEPT, ^{1}H-^{13}C HSQC-TOCSY, ^{1}H-^{13}C HMBC, 1D-TOCSY and ^{1}H-^{1}H ROESY. The sample of Siamenoside I (1) (~3.0 mg in 600 µL of CD_3OD) was prepared and NMR data was acquired on a Bruker Avance III HD 500 MHz instrument (BrukerBioSpin Corp., Billerica, MA,USA) with a 5.0 mm Prodigy inverse detection probe at 25º C. The ^{1}H NMR and ^{13}C NMR spectra were referenced to the residual solvent CD_3OD signal (δ_H 3.31 ppm) and (δ_C 49.00 ppm), respectively.

Material Sources: 90% Mogroside V (2) was sourced from Huachengbio, China.

CONCLUSIONS: To the best of our knowledge this is the first report full isolation and spectral characterization of Siamenoside (I), from an efficient high yield bioconversion reaction of Mogroside V (2). Siamenoside I possesses a 1→2 beta sugar linkage between sugar III and sugar I, making its structural properties unique. Continued search in the area of mogrosides provides great opportunity to find great tasting sweeteners and sweetener systems. In addition to the efficient and high yield process of producing Siamenoside (1), we have re-iterated the importance of multiple 1D and 2D NMR techniques when identifying complex mogrosides.

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