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Cinnamate derivatives of fructo-oligosaccharides from *Lindelofia stylosa*

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Abstract—Phytochemical investigation on the whole plants of *Lindelofia stylosa* (Kar. and Kir.) has led to the isolation of eight fructo-oligosaccharide cinnamate esters **1**–**8**. Six new compounds **1**, **2**, and **5**–**8** were isolated from the butanol extract of the plant. Compounds **1**–**4** belong to sucrose derivatives, while compounds **5**–**6** and **7**–**8** belong to 1-kestose- and nystose-type oligosaccharides, respectively. The fructo-oligosaccharides have been obtained from *L. stylosa* for the first time.

Keywords: *Lindelofia stylosa*; Boragniaceae; Fructo-oligosaccharide cinnamate derivatives; 1-Kestose; Nystose

1. Introduction

Fructo-oligosaccharides are the nonstructural polysaccharides that play an important role in nutrition studies, particularly in lipid metabolism.[1] They are distributed among flowering and higher plants where they serve as carbohydrate storage compounds.[2,3] Fructo-oligosaccharides are recognized as healthy food ingredients. In the course of our phytochemical studies on the medicinal plants of Pakistan, *Lindelofia stylosa* (Kar. and Kir.), a rare species, has been revealed for the first time to contain this important class of fructo-oligosaccharides. During previous investigations on the chemical constituents of *L. stylosa* (Kar. and Kir.), a variety of phenyl propanoids, belonging to rosmarinic and lithospermic acid classes, were isolated.[4] In continuation of this work, we have now obtained sucrose and fructo-oligosaccharide derivatives of phenyl propanoids. These include six new (**1**, **2**, and **5**–**8**) and two known (**3** and **4**) fructo-oligosaccharide cinnamoyl ester derivatives (Fig. 1). Trisaccharides **5** and **6** were found to be 1-kestose-type fructans, while compounds **7** and **8** were nystose-type fructans. To our knowledge, the secondary metabolites, belonging to fructo-oligosaccharide class, have been identified for the first time from the family Boragniaceae. Several plant families are known as rich sources of these classes of compounds.[10] 1-Kestose and nystose-type oligosaccharides are the smaller units of inulin-type fructans.[5] These fructans consist of (2→1)-linked β-D-fructofuranosyl residues terminated with a (1→2)-linked α-D-glucopyranosyl unit.

2. Results and discussion

L. stylosa subsp. *stylosa* (Boraginaceae) was collected from Britswarr Gali, Leepa Valley, Azad Kashmir (Pakistan). The whole plants were extracted with 80% aqueous methanol. The combined crude extracts were concentrated in vacuo and partitioned between hexane, ethyl acetate, and *n*-butanol. The butanol-soluble material was fractionated by Diaion HP-20 and Sephadex LH-20 column chromatography, followed by preparative recycling reversed-phase HPLC to obtain oligosaccharides **1**–**8**. These compounds were structurally

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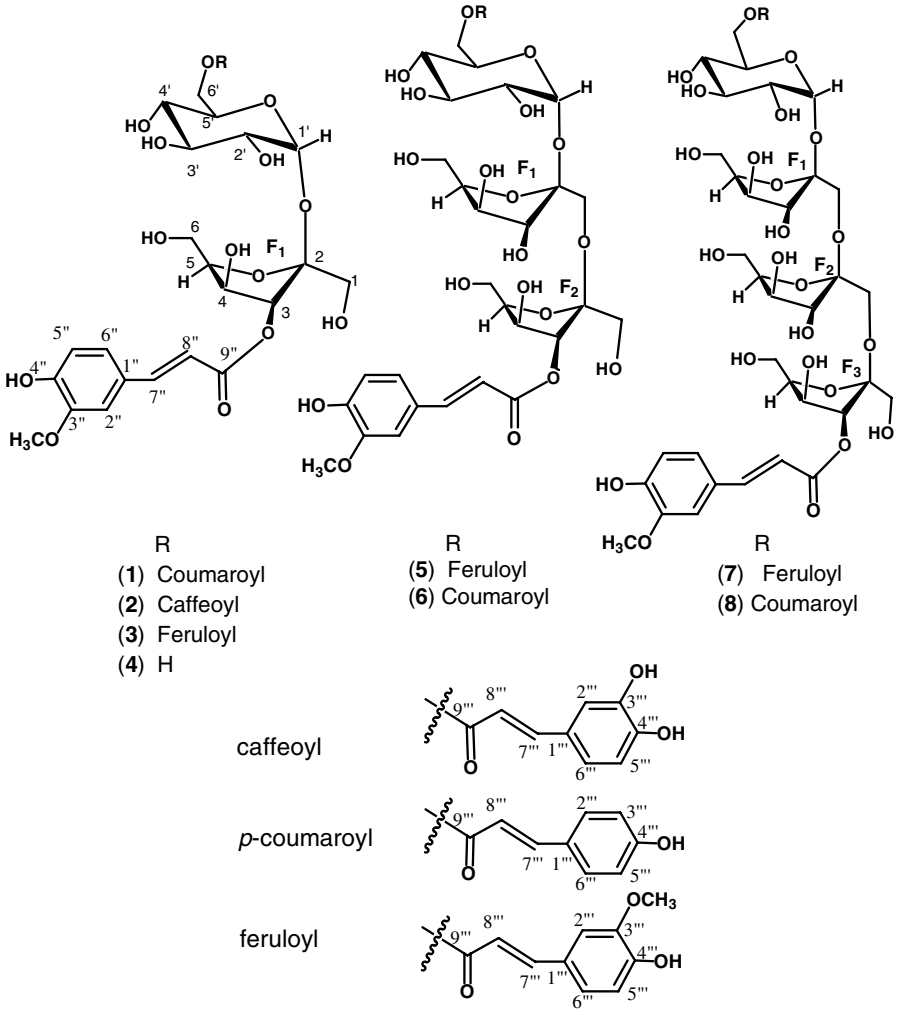

Figure 1. Fructo-oligosaccharides (**1**–**8**) from *Lindelofia stylosa*.

identified as 6′-*O*-coumaroyl-3-*O*-feruloylsucrose (**1**), 6′-*O*-caffeoyl-3-*O*-feruloylsucrose (**2**), 3,6′-*O*-diferuloylsucrose (**3**), 3-*O*-feruloyl fructofuranosyl sucrose (**4**), 3,6′-*O*-diferuloyl-1-kestose (**5**), 6′-*O*-coumaroyl-3-*O*-feruloyl 1-kestose (**6**), 3,6′-*O*-diferuloylnystose (**7**), and 6′-*O*-coumaroyl-3-*O*-feruloylnystose (**8**). The structures of the isolated oligosaccharides were deduced mainly on the basis of 1D and 2D NMR spectroscopic techniques.

The HRFABMS (negative-ion) of compound **1** showed the pseudo-molecular ion $[M-1]^-$ at *m*/*z* 663.1922 ($C_{31}H_{35}O_{16}$, calcd 663.1925). The ^1H and ^{13}C NMR spectra of compound **1** showed the presence of two substituted benzene rings conjugated with two *trans*-disubstituted double bonds. The substitution pattern at the two benzene rings and their conjugated α,β-unsaturated carbonyl system were inferred from the signals [δ 7.24 (d, $J_{1,3}$ 1.7 Hz, H-2″), 7.11 (dd, $J_{1,2}$ 8.1 Hz, $J_{1,3}$ 1.7 Hz, H-6″), 6.79 (d, $J_{1,2}$ 8.1 Hz, H-5″), 7.70 (d, $J_{1,2}$ 15.9 Hz, H-7″), 6.40 (d, $J_{1,2}$ 15.9 Hz, H-8″), and 3.88 (s, OCH_3)], assigned to the 4-hydroxy-3-methoxycinnamoyl moiety (feruloyl), while signals at δ 7.46 (d, $J_{1,2}$ 8.6 Hz, H-2‴, H-6‴), 6.76 (d, $J_{1,2}$ 8.5 Hz, H-3‴, H-5‴), 7.63 (d, $J_{1,2}$ 15.8 Hz, H-7‴), and 6.43 (d, $J_{1,2}$ 15.8 Hz, H-8‴) were assigned to a 4-hydroxycinnamoyl moiety (*p*-coumaroyl group). The ^{13}C NMR spectrum (Table 1) of compound **1** showed the signals for 31 carbons, resolved as 7 aromatic methine, 4 olefinic methine, 3 oxygenated quarternary aromatic, 2 quarternary aromatic, 2 α,β-unsaturated, 8 oxymethines, 3 oxymethylenes, 1 oxygenated methyl, and one oxygenated quaternary carbons. Among them, 12 signals were characteristic of a sucrose unit.[6]

The ^1H–^1H coupling constants between H-1 and H-2 of glucose ($J_{1,2}$ 3.5 Hz) indicated an α-glucosyl bond of the sucrose moiety. The anomeric proton of the glucose moiety, resonated at δ 5.45, showed $^3J_{CH}$ connectivity with C-2–F (δ 104.8) of β-D-fructose in the HMBC spectrum. Therefore, the 2→1 fructosyl linkage in the sucrose moiety was deduced in compound **1**. The ^1H NMR spectrum showed a complex pattern in the region of δ 3.29–4.29 due to overlapping of sugar protons.

Table 1. ^{1}H and ^{13}C NMR data of cinnamate derivatives of fructo-oligosaccharides from *Lindelofia stylosa* [δ ppm (J = Hz)]

C/no.	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Glc								
1′	5.45 d (3.5)	92.8	5.48 overlap	92.9	5.48 overlap	92.8	5.43 d (3.5)	93.3
2′	3.46 dd (9.7, 3.5)	73.0	3.45 dd (9.6, 3.5)	73.1	3.45 dd (9.6,3.6)	73.1	3.44 br d (3.5)	73.1
3′	3.56–3.68 overlap	74.9	3.65 overlap	74.9	3.65 m	75.0	3.65 overlap	75.0
4′	3.56–3.68 overlap	71.8	3.56–3.70 overlap	71.8	3.30 overlap	71.7	3.40 overlap	71.3
5′	4.28 overlap	72.3	4.23 m	72.3	4.23 overlap	72.4	3.93 m	73.9
6′	4.60 br d (11.2), 4.22 dd (11.2, 5.1)	65.3	4.60 br d (11.4)	65.2	4.60 br d (9.6) 4.22 br d (9.4)	65.6	3.78 br d (4.0)	62.4
Fru								
1	3.80 dd (11.9, 2.7)	65.5	3.59 overlap	65.5	3.6 overlap	64.4	3.60 overlap	65.4
2	—	104.8	—	104.9	—	104.9	—	104.9
3	5.48 d (7.8)	79.4	5.48 overlap	79.5	5.48 overlap	79.5	5.45 overlap	79.8
4	4.42 t (7.8)	74.1	4.42 t (7.9)	74.2	4.46 t (7.9)	74.2	4.37 t (7.7)	74.5
5	3.93 overlap	84.2	3.95 overlap	84.3	3.90 overlap	84.3	3.93 m	84.2
6	3.82 overlap	63.7	3.81 overlap	63.7	3.83 overlap	63.8	3.83 overlap	62.9
Ar-C								
1″	—	127.1	—	127.8	—	127.7		127.7
2″	7.24 d (1.5)	112.0	7.23 br s	112.1	7.19 d (1.6)	111.6	7.21 br s	112.2
3″	—	150.7	—	150.7	—	150.7	—	149.4
4″	—	149.3	—	149.3	—	148.9	—	150.8
5″	6.79 d (8.2)	116.4	6.79 d (8.0)	116.4	6.77 d (8.2)	116.4	6.80 d (8.1)	116.5
6″	7.11 dd (8.2,1.5)	124.2	7.12 br d (8.0)	124.2	7.10 dd (8.2, 1.6)	124.2	7.15 br d (8.1)	124.2
7″	7.70 d (15.9)	147.7	7.68 d (15.8)	147.7	7.69 d (15.8)	147.7	7.71 d (15.8)	147.7
8″	6.40 d (15.9)	114.9	6.45 d (15.8)	115.0	6.41 d (15.8)	115.0	6.42 d (15.8)	115.0
9″	—	168.3	—	168.3	—	168.5	—	168.3
1‴	—	127.6	—	127.7	—	127.5		
2‴	7.46 d (8.5)	131.3	7.03 br s	114.9	7.22 d (1.7)	112.1		
3‴	6.76 d (8.5)	116.8	—	146.7	—	150.8		
4‴	—	161.2	—	149.6	—	149.4		
5‴	6.76 d (8.5)	116.8	6.76 d (8.1)	116.8	6.80 d (8.2)	116.5		
6‴	7.46 d (8.5)	131.3	6.95 br d (8.1)	123.1	7.04 dd (8.2, 1.7)	124.3		
7‴	7.63 d (15.8)	146.9	7.55 d (15.8)	147.3	7.61 d (15.8)	147.1		
8‴	6.43 d (15.8)	115.0	6.36 d (15.8)	115.3	6.45 d (15.8)	115.4		
9‴	—	169.3	—	169.2	—	169.0		
OMe″	3.88 s	56.5	3.88 s	56.5	3.89 s	56.5	3.88	56.5
OMe‴					3.86 s	56.4		

The signals of the C-3 proton of fructose and the C-6′ proton of glucose resonated downfield about δ 1–1.5 ppm, as compared to unsubstituted sucrose,[6] indicating the ester linkages at these carbons.

The substitution of cinnamoyl moieties (feruloyl and *p*-coumaroyl groups) at the sucrose unit was deduced from the HMBC spectrum of compound **1**. The $^{3}J_{CH}$ correlation was observed between H-6′ methylene protons (δ 4.60 and 4.22) and the α,β-unsaturated carbonyl carbon (δ_C 169.3, C-9‴) of the *p*-coumaroyl group. The carbonyl carbon at δ_C 169.3 also exhibited HMBC cross peaks with *trans*-olefinic protons, resonating at δ 7.63 and 6.43 (H-7‴ and H-8‴), respectively. These olefinic protons were further correlated with the aromatic carbons δ_C 127.6 (C-1‴) and 131.3 (2 × CH, C-2‴, C-6‴). These interactions indicated that a *p*-coumaroyl residue was attached at the C-6′ position of the α-glucose.

The presence of a feruloyl moiety was further inferred from the downfield shift of C-3–F proton resonated at δ 5.48 (d, $J_{1,2}$ 7.8 Hz). The coupling constant of 7.8 Hz for C-3–F proton signal was attributed to the vicinal coupling with the C-4–F proton, resonating at δ 4.42 (t, $J_{1,2}$ 7.8 Hz). Further evidence for the substitution of the feruloyl moiety at the C-3–F position was obtained by the HMBC correlation. The C-3–F proton (δ 5.48) showed cross peaks with the ester carbonyl carbon (δ_C 168.3, C-9″), anomeric quaternary carbon of β-D-fructosyl residue (δ 104.8, C-2–F), and oxymethylene C-1–F (δ 65.5). These data led to the assignment of structure **1** for this new compound.

Compound **2** displayed an $[M-1]^-$ ion at m/z 679.1868 in the HRFABMS (negative-ion) ($C_{31}H_{35}O_{17}$, calcd 679.1874). Its IR spectrum showed the presence of hydroxyl (3380 cm^{-1}), ester carbonyl (1735 cm^{-1}), and phenyl (1604 and 1516 cm^{-1}) functionalities. The ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **2** attributed the signals to two aromatic ABX systems [δ 7.23 (br s, H-2″), 7.12 (br d, $J_{1,2}$ 8.0 Hz, H-6″), and

6.79 (d, $J_{1,2}$ 8.0 Hz, H-5″)]. *trans*-Olefinic protons of an α,β-unsaturated carbonyl system resonated at δ 7.68 (d, $J_{1,2}$ 15.8 Hz, H-7″), 6.45 (d, $J_{1,2}$ 15.8 Hz, H-8″), and 3.88 (s, OCH_3), and were assigned to a 4-hydroxy-3-methoxycinnamoyl moiety (feruloyl). Other ABX signals [δ 7.03 (br s, H-2‴), 6.76 (d, $J_{1,2}$ 8.1 Hz, H-5‴), and 6.95 (br d, $J_{1,2}$ 8.1 Hz, H-6‴), and signals for α,β-unsaturated carbons at δ 7.55 (d, $J_{1,2}$ 15.8 Hz, H-7‴) and 6.36 (d, $J_{1,2}$ 15.8 Hz, H-8‴)] were due to a 3,4-dihydroxycinnamoyl moiety (caffeoyl). Four oxygenated aromatic quaternary carbons resonated at δ 150.7, 149.6, 149.3, and 146.7. Signals for the ester carbonyls (δ 169.2 and 168.3), and for the α,β-unsaturated carbonyl carbons (δc 147.7, 147.3, 115.3, and 115.0) also appeared in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectra of compound **2** showed one anomeric proton (δ 5.48, overlap H-1′) corresponding to the carbon signal (δ 92.9) of the glucose residue. The sugar assignments were made on the basis of comparison with compound **1**. The overall spectral data of compound **2** were distinctly similar to that of compound **1**, with the only difference being the presence of a caffeoyl group, instead of coumaroyl moiety, at the C-6′ position of the glucose residue.

Compound **3** showed an $[M-1]^-$ ion at *m/z* 693.2010 in the HRFABMS (negative-ion), supporting the formula $C_{32}H_{37}O_{17}$ (calcd 693.2030). The ^{1}H and ^{13}C NMR data indicated two feruloyl units linked to the C-6′ of glucose and the C-3 of the fructose residues (see Table 1). The ^{1}H and ^{13}C NMR data, together with comparison of its physical data with data reported in the literature,[7,8] indicated **3** to be a known 3,6′-*O*-diferuloylsucrose derivative that was previously isolated from the bulbs of *Lilium speciosum*.[7] This is the first report of its isolation from *L. stylosa*.

Compound **4** exhibited an $[M-1]^-$ ion at *m/z* 517.1563 in the HRFABMS (negative-ion) ($C_{22}H_{29}O_{14}$, calcd 517.1557). The ^{1}H NMR spectrum showed the presence of a feruloyl unit attached to C-3–F (δ_C 74.5) of fructose. The overall spectral data of compound **4** were found to be identical to the data reported for 3-*O*-feruloylfructofuranosylsucrose, previously obtained from the roots of *Polygala sibirica*.[9] This is the first report of its isolation from *L. stylosa*.

Compound **5** showed an $[M-1]^-$ ion at *m/z* 855.2561 in the HRFABMS (negative-ion), suggestive of a formula $C_{38}H_{47}O_{22}$ (calcd 855.2559). The presence of α,β-unsaturated carbonyl systems was deduced from the IR absorption at 1694 cm^{-1}. Two sets of trans olefinic protons appeared as AB doublets at δ_H 7.70 ($J_{1,2}$ 15.8 Hz, H-7″)/6.42 ($J_{1,2}$ 15.8 Hz, H-8″) and 7.60 ($J_{1,2}$ 15.8 Hz, H-7‴)/6.42 ($J_{1,2}$ 15.8 Hz, H-8‴) in the ^{1}H NMR spectrum. Signals in ^{1}H and ^{13}C NMR spectra were indicative of two ABX systems of 1,3,4-trisubstituted phenyl rings, finally identified as two feruloyl subunits (see Table 2). In addition to these signals, compound **5** also showed some additional signals in its ^{1}H and ^{13}C NMR spectra that are characteristic of the 1-kestose type trisaccharides.[10] The anomeric proton, resonated as a doublet at δ_H 5.48 ($J_{1,2}$ 3.7 Hz, H-1′), correlated with a carbon signal at δ_C 93.3, suggesting a glucose residue. The presence of fructose units was also inferred from the characteristic quaternary carbon signal (C-2–F_1, δ_C 104.6), which showed a $^{3}J_{CH}$ correlation with an anomeric proton (δ_H 5.48) in the HMBC spectrum. This indicated that a sucrose unit was also present in this compound. In the ^{13}C NMR spectrum, peaks in the sugar region were also characteristic of a fructosyl residue. In the HMBC spectra, $^{3}J_{CH}$ correlations were observed between the C-1–F_1 methylene protons resonated at (δ_H 3.72) with the quaternary C-2–F_2 (δ_C 105.3), and the methine C-3–F_1 (δ_C 79.0) carbons, which suggested that two fructose moieties were linked together as found in 1-kestose type trisaccharides. The anomeric H-1′ (δ_H 5.48) showed 1D-TOCSY correlations with H-2′ to H-5′ methine protons and H-6′ methylene protons of glucose, whereas the H-3–F_2 methine proton (δ_H 5.56) showed 1D-TOCSY connectivity with H-4–F_2, and H-5–F_2 methine protons, and H-1–F_2, and H-6–F_2 methylene protons of fructose, respectively. Sugar linkages were finally confirmed by the HMBC, HMQC, and 1D-TOCSY spectra. The overall spectral data of compound **5** were found to closely resemble that of compound **3**, except for the signals of an additional sugar group. Therefore, compound **5** was deduced to be a 1-kestose-type trisaccharide with two feruloyl substituents.

Compound **6** exhibited an $[M-1]^-$ ion at *m/z* 825.2447 in the HRFABMS (negative-ion) that corresponded to the formula $C_{37}H_{45}O_{21}$ (calcd 825.2453), which is 30 amu less than that for compound **5**. The absence of a methoxy group was thus inferred. The overall spectral data of compound **6** was closely resembled that of compound **5**, indicating a close structural resemblance. The ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **6** showed the presence of an aromatic AB system conjugated with a trans olefinic moiety as inferred from the signals [δ 7.45 (d, $J_{1,2}$ 8.5 Hz, H-2‴, H-6‴), 6.78 (d, $J_{1,2}$ 8.5 Hz, H-3‴, H-5‴), 7.62 (d, $J_{1,2}$ 15.9 Hz, H-7‴), and 6.42 (d, $J_{1,2}$ 15.9 Hz, H-8‴)], assigned to a 4-hydroxycinnamoyl moiety (*p*-coumaroyl group). The characteristic signals [δ 7.23 (d, $J_{1,3}$ 1.4 Hz, H-2″), 6.76 (d, $J_{1,2}$ 8.4 Hz, H-5″), 7.10 (dd, $J_{1,2}$ 8.4 Hz, $J_{1,3}$ 1.4 Hz, H-6″), 3.88 (s, OCH_3), 7.70 (d, $J_{1,2}$ 15.9 Hz, H-7″), and 6.40 (d, $J_{1,2}$ 15.9 Hz, H-8″)] were indicative of a 4-hydroxy-3-methoxycinnamoyl moiety (i.e., a feruloyl group). In addition to these signals, compound **6** also showed ^{1}H and ^{13}C NMR signals characteristic of glycoside moieties, indicating that compound **6** also has a 1-kestose unit. Compound **6** differed from compound **5** only because of the *p*-coumaroyl moiety at C-6′ instead of a feruloyl residue.

Table 2. ^{1}H and ^{13}C NMR data of cinnamate derivatives of fructo-oligosaccharides from *Lindelofia stylosa* [δ ppm (J = Hz)]

C/no.	**5**		**6**		**7**		**8**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Glc								
1′	5.48 d (3.7)	93.3	5.48 d (3.7)	93.4	5.47 d (3.7)	93.3	5.47 d (3.5)	93.4
2′	3.42 dd (9.7, 3.7)	73.0	3.42 dd (9.8, 3.7)	73.0	3.42 dd (9.7, 3.7)	73.0	3.42 dd (9.7, 3.5)	72.9
3′	3.58 m	74.9	3.56–3.63 overlap	74.8	3.57–3.67 overlap	74.8	3.57–3.68 overlap	74.8
4′	3.31 overlap	71.9	3.32 overlap	71.9	3.33 overlap	71.9	3.33 overlap	71.8
5′	4.22 overlap	72.4	4.21 overlap	72.3	4.17–4.22 overlap	72.4	4.11–4.16 overlap	72.4
6′	4.61 d (9.9)	65.3	4.60 br d (10.5)	65.2	4.61 br d (10.2)	65.4	4.61 br d (11.2)	65.2
Fru-1								
1	3.72 overlap	64.5	3.75 overlap	64.5	3.75 overlap	64.5	3.75 overlap	64.2
2	—	104.6	—	104.7	—	104.7	—	104.8
3	4.15 br d (8.1)	79.0	4.15 br d (8.1)	79.0	4.08–4.16 m	76.3	4.08–4.16 m	76.2
4	4.03 br d (7.3)	77.0	4.02 br d (7.4)	77.0	4.02 overlap	78.9	4.01–4.06 m	78.8
5	3.74 br d (4.7)	83.8	3.72 overlap	83.8	3.97 m	84.7	3.76 overlap	83.4
6	3.64 overlap	62.0	3.62 m	62.0	3.60 overlap	62.3	3.65 overlap	62.2
Fru-2								
1	3.72 overlap	64.4	3.74–3.83 overlap	64.4	3.71–3.83 overlap	64.2	3.75 overlap	63.8
2	—	105.3	—	105.3	—	104.5	—	104.4
3	5.56 d (7.6)	79.5	5.56 d (7.5)	79.7	4.02 overlap	76.8	4.02 overlap	76.8
4	4.46 t (7.6)	74.4	4.42 t (7.5)	74.4	4.08–4.16 m	80.1	4.15 br d (8.6)	80.1
5	3.94 overlap	84.6	3.95 m	84.6	3.97 m	83.8	3.96 overlap	83.8
6	3.65 overlap	63.4	3.56–3.65 overlap	63.4	3.60 overlap	62.3	3.65 overlap	62.2
Fru-3								
1					3.82 overlap	63.8	3.82 overlap	64.4
2					—	105.2	—	105.2
3					5.55 d (7.4)	79.7	5.55 d (7.3)	79.6
4					4.47 t (7.4)	74.4	4.44 t (7.3)	74.3
5					3.73 overlap	83.5	3.95 overlap	84.7
6					3.71–3.83 overlap	63.5	3.82 overlap	63.4
Ar-C								
1″	—	127.5	—	127.1	—	127.4	—	127.2
2″	7.23 d (1.0)	112.0	7.23 d (1.4)	112.0	7.19 d (1.3)	111.6	7.23 br s	112.0
3″	—	151.0	—	151.2	—	151.2	—	150.9
4″	—	149.4	—	149.5	—	149.8	—	149.4
5″	6.77 d (8.1)	116.4	6.76 d (8.4)	116.5	6.78 d (8.1)	116.5	6.40 d (8.0)	116.4
6″	7.08 dd (8.1, 1.0)	124.5	7.10 dd (8.4, 1.4)	124.5	7.03 dd (8.1, 1.3)	124.4	7.10 br d (8.0)	124.5
7″	7.70 d (15.8)	148.1	7.70 d (15.9)	148.2	7.60 d (15.8)	147.2	7.70 d (15.8)	148.2
8″	6.42 d (15.8)	115.3	6.40 d (15.9)	114.7	6.44 d (15.8)	114.2	6.45 d (15.8)	114.7
9″	—	168.6	—	168.6	—	168.7	—	168.6
1‴	—	127.6	—	127.4	—	127.6	—	127.6
2‴	7.19 d (1.5)	111.6	7.45 d (8.5)	131.3	7.22 d (1.3)	112.1	7.45 d (8.2)	131.5
3‴	—	150.8	6.78 d (8.5)	116.9		151.5	6.79 d (8.2)	116.8
4‴	—	149.5	—	161.5	—	149.5	—	161.3
5‴	6.80 d (8.0)	116.5	6.78 d (8.5)	116.9	6.76 d (8.1)	116.6	6.79 d (8.2)	116.8
6‴	7.03 dd (8.1, 1.5)	124.4	7.45 d (8.5)	131.3	7.10 dd (8.1, 1.3)	124.5	7.45 d (8.2)	131.5
7‴	7.60 d (15.8)	147.1	7.62 d (15.9)	146.9	7.70 d (15.8)	148.3	7.61 d (15.9)	146.8
8‴	6.42 d (15.8)	114.7	6.42 d (15.9)	114.9	6.44 d (15.8)	115.5	6.41 d (15.9)	115.0
9‴	—	169.2	—	169.3	—	169.3	—	169.2
OMe	3.88 s	56.5	3.88 s	56.5	3.88 s	56.5	3.88 s	56.5
OMe	3.86 s	56.4			3.87 s	56.4		

Compound **7** was isolated as a yellow gum and identified as nystose-type glycoside with two cinnamoyl moieties. An $[M-1]^{-}$ ion at m/z 1017.3076 in HRFABMS (negative-ion) was in agreement with $C_{44}H_{57}O_{27}$ (calcd 1017.3087). The spectral data was found to be distinctly similar to compound **6** with some additional signals of a sugar moiety, indicating it to be a tetrasaccharide. The spectral data suggested that two feruloyl moieties were present in compound **7** (see Table 2). The presence of an anomeric proton signal at δ_H 5.47 was due to a glucose residue. The ^{1}H–^{1}H-COSY and 1D-TOCSY spectra showed all connectivities of the sugar moieties. A signal at δ 104.7 was assigned to the C-2–F_1 of the β-D-fructosyl residue of the sucrose moiety. The methylene protons (H-1–F_1) showed $^{3}J_{CH}$ correlations with C-2–F_2 (δ_C 104.5) of the β-D-fructosyl unit. These interresidual

correlations indicated the presence of a 1-kestose unit. The C-2–F_2 (δ 104.5) showed HMBC correlations with the H-1–F_2 methylene protons (δ 3.71–3.83) while the C-1–F_2 methylene protons also showed HMBC correlations with a quaternary carbon C-2–F_3 (δ_C 105.2), belonging to the third fructose unit. The ^{13}C NMR data for the respective fructosyl units and their connectivities were in good agreement with those present in nystose-type glycosides.[5] Compound **7** was therefore deduced to be a new tetrasaccharide.

Compound **8** exhibited an $[M-1]^-$ ion at m/z 987.2988 in the HRFABMS (negative-ion), which corresponded to the formula $C_{43}H_{55}O_{26}$ (calcd 987.2981), indicating the absence of a methoxy group, as compared to compound **7**. The spectral data of compound **8** was distinctly similar to that for compound **7**. The ^1H NMR spectrum exhibited an ABX system for a 1,3,4-trisubstituted phenyl ring (feruloyl subunits), along with an AB system for cinnamoyl moiety (i.e., *p*-coumaroyl) (see Table 2). In addition to these signals, some additional signals in the ^1H and ^{13}C NMR spectra of **8** were found to be characteristic of fructosyl moieties. The anomeric proton resonated at δ 5.47 ($J_{1,2}$ 3.5 Hz) and showed HMBC correlations with other anomeric carbon C-2–F_1 (δ 104.8). This indicated that compound **8** is part of a sucrose moiety. Additional signals in the ^{13}C NMR spectrum corresponded to a fructofuranosyl moiety. The sugar moieties and their connectivities were identified by the HMBC, 1D-TOCSY, and COSY correlations. The spectroscopic data of compound **8** were characteristic of a tetrasaccharide moiety with feruloyl and coumaroyl units. In compound **8**, the *p*-coumaroyl moiety was found to be connected to the C-6′ position of the glucose residue, while the feruloyl residue was located at the C-3–F_3 position of fructose. Compound **8** was therefore deduced to be a nystose-type fructan.

3. Experimental

3.1. General methods

Unless otherwise stated, the following procedures were adopted. UV spectra were measured on a Shimadzu UV240 spectrophotometer in MeOH solutions and presented as λ_{max} nm ($\log\varepsilon$). IR spectra were recorded as KBr discs on a JASCO A-302 spectrophotometer in cm^{-1}. NMR spectra were recorded in CD_3OD solutions on a Bruker AM-300 spectrometer (^{13}C 75 MHz, 1D spectra of **1**, **6**, and **8**), Bruker AM-400 spectrometer (1H 400 MHz, 1D spectra of **3**, **4**, and **8**; ^{13}C 100 MHz, 1D spectra of **2**, **3**, and **4**) and Bruker AMX 500 NMR spectrometer (1H 500 MHz, 1D spectra of **1**, **2**, **5**, **6**, and **7**; ^{13}C 125 MHz, 1D spectra of **5** and **7**) with tetramethylsilane (TMS) as an internal standard and the data are presented in δ (ppm). 2D NMR spectra were taken on Bruker AMX 500 NMR spectrometer. Electron-impact mass spectra (EIMS) were taken at 70 eV on a Finnigan MAT-112 or MAT-312 instrument and major ions are given by m/z (%). Fast-atom bombardment mass spectra (FABMS) were measured as glycerol matrix on a JEOL HX110 mass spectrometer. TLC was performed on pre-coated silica gel plates (E. Merck), and the spots were observed first under UV (254 nm) and then stained with cerium(IV) sulfate spray reagent and heated until coloration developed. Recycling preparative HPLC (RPHPLC) separation was performed on a JAI LC-908W (Japan Analytical Industry) with columns of YMC ODS H-80 or L-80.

3.2. Plant material

Whole plants of *L. stylosa* (Kar. and Kir.) were collected from Britswarr Gali, Leepa Valley, Azad Kashmir (Pakistan), during September–October 2002 by Professor Shafiq-ur-Rehman, Department of Botany, University of Azad Jammu and Kashmir (voucher specimen No. AJKUH-786165).

3.3. Extraction and isolation

Air-dried powdered forms of *L. stylosa* (3 kg) were extracted three times (each one week) with MeOH (6 L) at room temperature. The resulting MeOH extract (ca. 150 g) was partitioned with hexane, EtOAc, BuOH, and H_2O to give 15, 24, 50, and 40 g extracts, respectively.

A part of BuOH fraction (15 g) was passed through a Diaion HP-20, the column of which was eluted with H_2O, 1:1 H_2O–MeOH, 1:2 H_2O–MeOH, and MeOH. The 1:1 H_2O–MeOH fraction (9.5 g) was passed through a polyamide column and eluted with $CHCl_3$ with increasing proportions of MeOH. The fraction that eluted at 15–20% MeOH–$CHCl_3$ was collected (3 g). This fraction was again chromatographed on a Diaion HP-20 column and eluted with H_2O with an increasing proportion of MeOH. Fractions obtained with 20% H_2O–MeOH were combined (1 g) and subjected to Sephadex LH-20 column chromatography and eluted with gradients of H_2O–MeOH. The resulting fractions were then purified by recycling RPHPLC on an L-80 column using 1:1 H_2O–MeOH as a mobile phase with a flow rate of 4 mL, to give compound **1** (120 mg, 4.0×10^{-3}%, t_R 30 min) and compound **3** (100 mg, 3.33×10^{-3}%, t_R 32 min). The fraction that eluted with 20% MeOH–H_2O was also purified under RPHPLC on an H-80 column using 1:1 H_2O–MeOH as a mobile phase with a flow rate of 3.5 mL/min to obtain compound **2** (20 mg, 6.66×10^{-4}%, t_R 20 min). The fraction obtained with 10% MeOH–H_2O was first fractionated through RHPLC on an H-80 column and separated into two subfractions A and B. For the final purification of

subfraction B, it was again subjected to RHPLC on an H-80 column with a flow rate of 3.5 mL/min, resulting in the isolation of compounds **5** (15 mg, 5.0×10^{-4}%, t_R 40 min) and **6** (45 mg, 1.50×10^{-3}%, t_R 36 min). Compounds **7** (43 mg, 1.40×10^{-3}%) and **8** (20 mg, 6.66×10^{-4}%) were isolated from subfraction A by RPHPLC on an H-80 column with a flow rate of 3.5 mL/min with the retention times of 30 and 34 min, respectively.

3.4. 6′-*O*-Coumaroyl-3-*O*-feruloylsucrose (1)

Yellowish gum; $[\alpha]_D^{23}$ −435 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε): 317 (4.46), 230 (4.21), 212 (4.19) nm; IR (KBr) ν_{max}: 3380, 2939, 1696, 1632, 1516, 1432, 1270 cm^{-1}; FABMS (negative-ion): *m/z* 663 $[M-1]^-$; HRFABMS: *m/z* 663.1922 (calcd *m/z* 663.1925 for $C_{31}H_{35}O_{16}$); EIMS: *m/z* 338 (2.02%), 194 (37.9%), 177 (90.2%), 147 (100%); ^1H NMR (CD_3OD, 500 MHz), and ^{13}C NMR: δ (CD_3OD, 75 MHz) see Table 1.

3.5. 6′-*O*-Caffeoyl-3-*O*-feruloylsucrose (2)

Yellowish gum; $[\alpha]_D^{23}$ −257 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε): 325 (5.32), 234 (5.11), 218 (5.18), 203 (5.19), 198 (5.75) nm; IR (KBr) ν_{max}: 3353, 2943, 1692, 1598, 1515, 1263 cm^{-1}; FABMS (negative-ion): *m/z* 679 $[M-1]^-$; HRFABMS: *m/z* 679.1868 (calcd 679.1874 for $C_{31}H_{35}O_{17}$); EIMS: *m/z* 212 (6.0%), 194 (3.5%), 123 (100%), 77 (14.4%); ^1H NMR (CD_3OD, 500 MHz), and ^{13}C NMR: δ (CD_3OD, 100 MHz) see Table 1.

3.6. 3,6′-Di-*O*-feruloylsucrose (3)

Pale Yellow gum; $[\alpha]_D^{23}$ −252 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε): 218 (3.96), 232 (3.89), 292 (4.72), 326 (5.05) nm; IR (KBr) ν_{max}: 3359, 2985, 1699, 1596, 1515, 1270 cm^{-1}; FABMS (negative-ion): *m/z* 693 $[M-1]^-$; HRFABMS: *m/z* 693.2010 (calcd 693.2030 for $C_{32}H_{37}O_{17}$); EIMS: *m/z* 338 (3.6%), 194 (17.9%), 177 (38.8%), 150 (56.3%), 57 (100%); ^1H NMR (CD_3OD, 400 MHz), and ^{13}C NMR: δ (CD_3OD, 100 MHz) see Table 1.

3.7. 3-*O*-Feruloyl fructofuranosylsucrose (4)

Amorphous powder; $[\alpha]_D^{23}$ −15.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 217 (3.89), 232 (3.79), 297 (3.75), 326 (3.91) nm; IR (KBr) ν_{max}: 3363, 2985, 1688, 1596, 1515, 1066 cm^{-1}; FABMS (negative-ion): *m/z* 517 $[M-1]^-$; HRFABMS: *m/z* 517.1563 (calcd 517.1557 for $C_{22}H_{29}O_{14}$); EIMS: *m/z* 338 (3.62%), 194 (17.9%), 177 (38.8%), 150 (56.3%), 57 (100%); ^1H NMR (CD_3OD, 400 MHz), and ^{13}C NMR: δ (CD_3OD, 100 MHz) see Table 1.

3.8. 3,6′-Di-*O*-feruloyl-1-kestose (5)

Brown yellow gum; $[\alpha]_D^{23}$ −44.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 309 (3.46), 290 (3.47), 254 (3.46), 224 (3.56), 197 (3.84), 194 (5.04) nm; IR (KBr) ν_{max}: 3358, 2943, 1694, 1631, 1515, 1272, 1163 cm^{-1}; FABMS (negative-ion): *m/z* 855 $[M-1]^-$; HRFABMS: *m/z* 855.2561 (calcd 855.2559 for $C_{38}H_{47}O_{22}$); EIMS: *m/z* 321 (6.7%), 280 (27.6%), 123 (100%), 77 (34.7%); ^1H NMR (CD_3OD, 500 MHz), and ^{13}C NMR: δ (CD_3OD, 125 MHz) see Table 2.

3.9. 6′-*O*-Coumaroyl-3-*O*-feruloyl-1-kestose (6)

Yellow amorphous powder; $[\alpha]_D^{23}$ −117.7 (*c* 0.43, MeOH); UV (MeOH) λ_{max} (log ε): 319 (4.29), 231 (4.04), 197 (5.65) nm; IR (KBr) ν_{max}: 3355, 2940, 1692, 1600, 1514, 1271 cm^{-1}; FABMS (negative-ion): *m/z* 825 $[M-1]^-$; HRFABMS: *m/z* 825.2447 (calcd 825.2453 for $C_{37}H_{45}O_{21}$); EIMS: *m/z* 321 (5.18%), 280 (26.0%), 123 (100%), 110 (8.8%), 77 (50.0%); ^1H NMR (CD_3OD, 500 MHz), and ^{13}C NMR: (CD_3OD, 75 MHz) see Table 2.

3.10. 3,6′-Di-*O*-feruloylnystose (7)

Yellowish gum; $[\alpha]_D^{23}$ −61.3 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε): 325 (5.96), 234 (5.76), 219 (5.78), 198 (7.04) nm, IR (KBr) ν_{max}: 3332, 2929, 1696, 1594, 1515, 1272 cm^{-1}; FABMS (negative-ion): *m/z* 1017 $[M-1]^-$; HRFABMS: *m/z* 1017.3076 (calcd 1017.3087 for $C_{44}H_{57}O_{27}$); EIMS: *m/z* 212 (12.5%), 194 (5.6%), 123 (100%), 110 (16.3%), 77 (11.0%); ^1H NMR (CD_3OD, 500 MHz), and ^{13}C NMR: δ (CD_3OD, 125 MHz) see Table 2.

3.11. 6′-*O*-Coumaroyl-3-*O*-feruloylnystose (8)

Brown amorphous powder; $[\alpha]_D^{23}$ −190.9 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 318 (5.05), 230 (4.81), 191 (5.91) nm; IR (KBr) ν_{max}: 3354, 2941, 1693, 1600, 1514, 1268 cm^{-1}; FABMS (negative-ion): *m/z* 987 $[M-1]^-$; HRFABMS: *m/z* 987.2988 (calcd 987.2981 $C_{43}H_{55}O_{26}$); EIMS: *m/z* 321 (12.8%), 280 (38.6%), 123 (100%), 77 (10.6%); ^1H NMR (CD_3OD, 400 MHz), and ^{13}C NMR: δ (CD_3OD, 75 MHz) see Table 2.

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Supplementary data

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