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7-Caffeoylsedoheptulose from *Nyssa sylvatica*

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Abstract

7-Caffeoylsedoheptulose has been isolated from the wood of *Nyssa sylvatica*, along with two ellagic acid derivatives and scopoletin. Its structure was established by chemical and spectral evidence.

Keywords: *Nyssa sylvatica*; Nyssaceae; 7-caffeoylsedoheptulose

1. Introduction

Nyssa sylvatica Marsh. (Nyssacae) is a tree widely distributed in the southern parts of the United States of America. Previous phytochemical studies of this plant revealed the presence of flavonoids (Bodalski & Malcher, 1970). In this study, a new compound, 7-caffeoylsedoheptulose (**1**) was isolated, as well as 3′-*O*-methyl-3,4-methylenedioxyellagic acid 4′-*O*-β-D-glucopyranoside (nyssoside) (Chen, Luo & Xiong, 1996), 3,3′, 4-tri-*O*-methylellagic acid 4′-*O*-β-D-glucopyranoside (Khac, Sung, Campos, Lallemand & Fetizon, 1990; Hills & Yazaki, 1973) and scopoletin (Shafizadeh & Melnikoff, 1970).

2. Results and discussion

The *n*-butanol soluble portion of the ethanol extract of the wood of *Nyssa sylvatica* was chromatographed on normal and reversed phase silica gel to yield compound **1** as a white amorphous powder, $[\alpha]_D^{25}$ + 13.9° (MeOH). TLC and HPLC analysis of **1** indicated that it seemed to be a pure compound. However, the ^{1}H- and ^{13}C-NMR spectra displayed, in addition to the predominant signals, some less intense signals with close chemical shifts to the respective predominant ones. In the ^{1}H-NMR spectrum of **1**, the downfield region showed two coupled trans-olefinic proton signals at δ 7.52 (*d*, J = 15.8 Hz) and 6.29 (*d*, J = 15.8 Hz), two coupled aromatic signals at δ 7.01 (*d*, J = 7.9 Hz) and 6.78 (*d*, J = 7.9 Hz) and one broad aromatic singlet at δ 7.07, characteristic of a caffeoyl moiety (Warashina, Miyase & Ueno, 1992). The upfield region from δ 3.0–4.5 displayed complex sugar-like signals. The ^{13}C-NMR spectrum also supported the presence of the caffeoyl moiety (Warashina et al., 1992) and sugar-like carbon signals. The presence of a quaternary anomeric carbon signal at δ 102.7 indicated that the sugar was a ketose (Breitmaier & Voelter, 1987). The high resolution ESI mass spectrometry of **1** gave a quasi-molecular ion peak at m/z 395.0977 $[M + Na]^+$, corresponding to the molecular formula $C_{16}H_{20}O_{10}Na$. The above spectral evidence indicated that **1** might comprise a hepatulose attached to a caffeoyl moiety, with tautomerism of the sugar in solution resulting in the complexity of the NMR spectra.

Acetylation of **1** with acetic anhydride in pyridine yielded compounds **1a** and **1b**. Compound **1a** showed seven additional ester carbonyl carbon signals (δ 168 ~

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170) in the ^{13}C-NMR spectrum, indicating that **1a** was a percetate of **1** with seven hydroxy groups acetylated. This was supported by the high resolution ESIMS, which gave a quasimolecular ion peak at m/z 689.1701 [M ($C_{30}H_{34}O_{17}$) + Na]$^+$. The ^{1}H- and ^{13}C-NMR spectra of **1a** clearly indicated the presence of the caffeoyl moiety (Warashina et al., 1992), so emphasis was put on the analysis of the sugar moiety. HMQC spectroscopy was first employed to correlate the protons with attached carbons. COSY was then used to establish the spin-coupling network of the sugar: δ 5.80 (1H, *d*, J = 3.5 Hz) → 5.34 (1H, *m*) → 4.49 (1H, *dd*, J = 5.7, 6.2 Hz) → 5.34 (1H, *m*) → 4.55 (1H, *dd*, J = 12.2, 3.3 Hz) and 4.27 (1H, *dd*, J = 12.2, 5.8 Hz). The signal at δ 5.34 (2H, *m*) which correlated with two carbons at δ 76.0 and 70.0 in the HMQC spectum was split into two signals when the ^{1}H-NMR spectrum was measured in C_6D_6 (Table 1). Two additional sugar signals at δ 4.66 (1H, *d*, J = 12.0 Hz) and 4.32 (1H, *d*, J = 12.0 Hz) were observed as an isolated coupling system. In the NOESY spectrum of **1a**, the signal at δ 5.80 (H-3) correlated with the signals at δ 4.49 (H-4), 4.66 (H-1a), and 4.32 (H-1b). The coupling pattern of these protons and NOE evidence suggested that the sugar moiety of **1a** is a β-D-fructofuranose-like sugar with an additional CHOH group, i.e. D-*altro*-heptulose or sedoheptulose (Franke, Kapuscinski, Macleod & Williams, 1984). Confirmation was made by alkaline hydrolysis of **1a** with 3% NaOH which yielded sedoheptulose and caffeic acid. The absolute configuration of sedoheptulose as D-form was indicated by its $[\alpha]_D^{25}$ + 6.9° (H_2O). The linkage position of the caffeoyl moeity to the sugar moiety was established by the HMBC spectroscopy in which the signals of H-7 at δ 4.55 and 4.27 correlated with the ester carbonyl carbon signal at 166.0. Therefore, the structure of **1a** was assigned as 7-(3′,4′-di-*O*-acetyl) caffeoyl-1,2,3,4,6-penta-*O*-acetyl-β-D-altro-heptulofuranose.

Compound **1b** showed a carbonyl carbon signal at δ 197.9 and 7 ester carbonyl carbon signals (δ 166 ~ 170) in the ^{13}C-NMR spectrum. The presence of the typical carbonyl carbon signal at δ 197.9 instead of an anomeric carbon signal around δ 108 (compared to **1a**) indicated that **1b** is the straight chain form of the peracetylated **1**. This was supported by the ESIMS and the alkaline hydrolysis result (see Section 3). The assignments of the ^{1}H- and ^{13}C-NMR signals of **1b** were facilitated by 2D NMR experiments including COSY and HMQC spectra. Thus, the structure of **1b** is 7-(3′,4′-di-*O*-acetyl) caffeoyl-1,3,4,5,6-penta-*O*-acetyl-D-altro-heptulose.

The above evidence suggested that compound **1** was 7-caffeoylsedoheptulose. The equilibrium state of sedoheptulose in D_2O has been determined by the ^{13}C-NMR spectrum as 66:18:16 for the β-furanose:α-furanose:α-pyranose (Okuda, Saito, Hayashi, Nagakura & Sugiura, 1976). In the case of **1** whose ^{13}C-NMR spectrum was measured in DMSO-d_6, the predominant β-furanose form (anomeric carbon δ 102.6) and the α-furanose form (anomeric carbon δ 104.8) were observed in a ratio of about 5:2. The ^{13}C-NMR signals of the α-pyranose form were not significant. With the aid of the COSY, HMQC, and HMBC spectra, the ^{1}H- and ^{13}C-NMR signals of the predominant from (β-furanose) of **1** were assigned (Table 1).

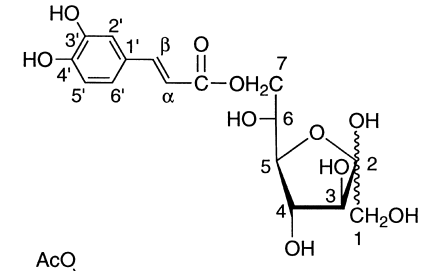
Sedoheptulose, first isolated from *Sedum spectabile* by La Forge and Hudson in 1917 (La Forge & Hudson, 1917), is important in primary and secondary plant metabolism, particularly in the pentose phosphate pathways, the synthesis of sugars during photosynthesis and the biosynthesis of shikimic acid and aromatic amino acids (Dalmas & Demuynck, 1993). Although sedoheptulose is considered to be universally present in plants (Robinson, 1991), glycosides or conjugates of the molecule are rare in nature, to our knowledge consisting only of seduheptulose 1,7-digallate isolated form *Cornus officinalis* (Lee, Tanaka, Nonaka & Nishioka, 1989). From the viewpoint of

Table 1
^{13}C- and ^{1}H-NMR data of the sedoheptulose moiety of compounds **1**, **1a** and **1b** (ppm)[a]

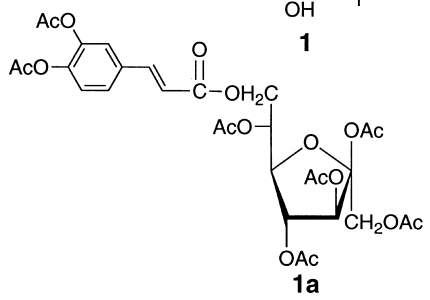
Position	**1**[b] (DMSO-d_6)		**1a** ($CDCl_3$)		**1a**(C_6D_6)	**1b** ($CDCl_3$)	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	62.8	3.28 (*br s*)	61.7	4.66/4.32 (AB*q*, 12.0)	4.95/4.44 (AB*q*, 12.0)	66.7	4.92/4.68 (AB*q*, 17.0)
2	102.6	–	108.2	–	–	197.9	–
3	76.1	3.85	78.4	5.80 (*d*, 3.5)	6.33 (*d*, 4.2)	74.1	5.48 (*d*, 2.1)
4	75.9	4.08	76.0	5.34	5.79 (*dd*, 5.8, 4.2)	68.8	5.69 (*dd*, 8.6, 2.1)
5	81.7	3.58 (*t*, 5.8)	81.4	4.49 (*dd*, 5.7, 6.2)	4.85 (*t*, 6.3)	68.8	5.41 (*dd*, 8.6, 3.2)
6	69.9	3.83	70.0	5.34	5.68 (*m*)	69.9	5.31 (*dt*, 3.5, 7.0)
7	65.6	4.23 (*dd*, 11.2, 3.0) 4.02	62.4	4.55 (*dd*, 12.2, 3.3) 4.27 (*dd*, 12.2, 5.8)	4.76 (*dd*, 12.2, 3.1) 4.39 (*dd*, 12.2, 5.6)	61.7	4.46 (*dd*, 12.1, 3.8) 4.36 (*dd*, 12.1, 7.3)

[a] For ^{1}H-NMR data, coupling patterns well resolved are expressed with multiplicity and coupling constants in Hz in parentheses.
[b] Data for only the β-furanose form.

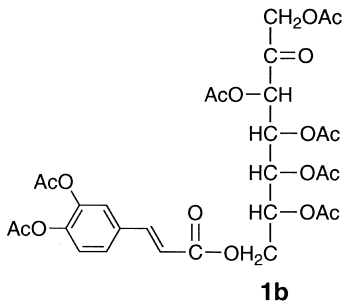
biogenesis, it is postulated that the title compound can be readily synthesized from sedoheptulose-7-phosphate.



1



1a



1b

3. Experimental

3.1. General

Optical rotations were determined on a JASCO DIP-370 digital polarimeter. NMR Spectra were recorded on either a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C or a Bruker Avance DPX-300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts (δ, ppm) are relative to internal TMS. 2D-NMR were measured with standard pulse programs and acquisition parameters. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high resolution HPLC-FT spectrometer by direct injection into an electrospray interface. GC analysis was performed on Hewlett Packard 5890 Series II Gas Chromatograph: DB-1 minibore capillary column (0.18 mm ID, 20 m); detection: FID; column temp.: 100–280° (10°/min → 25 min); carrier gas: He (45 psi). Column chromatography: silica gel (40 μm, J.T. Baker) and reversed-phase silica gel (RP-18, 40 μm, J.T. Baker). TLC: silica gel sheets (Alugram ® Sil G/UV_{254}, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F_{254S}, Merck, Germany). Prep. TLC: J.T. Baker Si500F TLC silica gel plate (500 μm); detection: UV 254 nm.

3.2. Plant material

The wood of *Nyssa sylvatica* Marsh (Nyssaceae) was collected in Mississippi, USA. A voucher specimen (BUR 240693) is deposited at the National Center for Natural Products Research, School of Pharmacy, University of Mississippi.

3.3. Extraction and isolation

The dried powdered wood (1.1 kg) was extracted with 95% EtOH (2.81 × 3) at 37° for 3 h. Removal of the solvent under vacuo at 45° yielded an EtOH extract (52.5 g), of which 50.1 g was suspended in H_2O (1.61), extracted successively with $CHCl_3$ (11 x 3), EtOAc (11 × 3), and *n*-BuOH (satd. with H_2O, 11 × 3). The combined *n*-BuOH layers were evaporated to dryness in vacuo (45°) to give a yellow residue (19.2 g). The residue (18 g) was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (70:10:1 to 30:10:1) as the eluting solvent (7.51), followed by MeOH (1.51). Fractions of 60 ml each were collected. Scopoletin (Shafizadeh & Melnikoff, 1970) was obtained from frs. 15–26 by preparative TLC developed with $CHCl_3$–MeOH (6:1) as colorless needles (3.0 mg). 3,3′,4-Tri-*O*-methylellagic acid 4′-*O*-β-D-glucopyranoside (Khac et al., 1990; Hills & Yazaki, 1973) was crystallized from frs. 26 ~ 30 as granular crystals (7.0 mg). 3′-*O*-Methyl-3,4-methylenedioxyellagic acid 4′-*O*-β-D-glucopyranoside (nyssoside) (Chen et al., 1996) was crystallized from frs 61 ~ 90 as granular crystals (22.3 mg). Identification of the above three compounds was made by comparison of their UV, IR, and NMR spectroscopic data with those reported (Chen et al., 1996; Khac et al., 1990; Hills & Yazaki, 1973; Shafizadeh & Melnikoff, 1970]. Part of frs. 251–290 (200 mg) was chromatographed on a reversed-phase silica gel (RP-18) column using aq. MeOH (0 →100% MeOH) to afford **1** (85 mg).

3.4. 7-Caffeoylsedoheptulose (*1*)

A white powder, $[\alpha]_D^{25} + 13.9°$ (MeOH; *c* 0.42). HPLC (Prodigy 5μ ODS 150 × 4.6 mm column, 10% CH_3CN, 1 ml/min, UV 254 nm): R_t = 21.7 min. ESIMS *m/z*: 395.0977 (calculated for $C_{16}H_{20}O_{10}Na$, 395.0972). ^{1}H-NMR spectral data (400 MHz, DMSO-d_6), β-furanose form: caffeoyl δ 7.52 (1H, *d*, *J* = 15.8 Hz, H-β), 7.07 (1H, *br s*, H-2′), 7.01 (1H, *d*, *J* = 7.9

Hz, H-6′), 6.78 (1H, *d*, *J* = 7.9 Hz, H-5′), 6.29 (1H, *d*, *J* = 15.8 Hz, H-α); sedoheptulose (see Table 1). ^{13}C-NMR spectral data (100 MHz, DMSO-d_6), β-furanose form: caffeoyl δ 166.7 (*s*, C═O), 148.4 (*s*, C-4′), 145.6 (*s*, C-3′), 145.1 (*d*, C-β), 125.6 (*s*, C-1′), 121.4 (*d*, C-6′), 115.8 (*d*, C-5′), 114.7 (*d*, C-2′), 114.1 (*d*, C-α); sedoheptulose (see Table 1).

3.5. Acetylation of *1*

A solution of **1** (75 mg) in Ac_2O–pyridine (1:1, 1 ml) was kept at room temp. overnight. The reaction mixture was evaporated to dryness under a stream of N_2 and then subjected to prep. TLC using $CHCl_3$–EtOAc (5:1) as the developing solvent to yield compounds **1a** (11.2 mg) (R_f = 0.38) and **1b** (5.6 mg) (R_f = 0.29).

3.6. 7-(3′,4′-Di-O-acetyl)caffeoyl-1,2,3,4,6-penta-O-acetyl-β-D-altro-heptulofuranose (*1a*)

A white powder, $[\alpha]_D^{25} + 14.5^\circ$ ($CHCl_3$; *c* 0.88). ESIMS *m*/*z*: 689.1701 (calculated for $C_{30}H_{34}O_{17}$ Na, 689.1688). ^{1}H-NMR spectral data (400 MHz, $CDCl_3$): caffeoyl δ 7.63 (1H, *d*, *J* = 16.0 Hz, H-β), 7.42 (1H, *d*, *J* = 8.3 Hz, H-6′), 7.38 (1H, *br s*, H-2′), 7.23 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.38 (1H, *d*, *J* = 16.0 Hz, H-α); acetyl δ 2.31 (3H, *s*), 2.30 (3H, *s*) 2.17 (6H, *s*), 2.12 (3H, *s*), 2.10 (6H, *s*); sedoheptulose (see Table 1). ^{1}H-NMR spectral data (300 MHz, C_6D_6): caffeoyl δ 7.60 (1H, *d*, *J* = 16.0 Hz, H-β), 6.96 (1H, *d*, *J* = 1.9 Hz, H-2′), 6.89 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.73 (1H, *dd*, *J* = 8.4, 1.9 Hz, H-6′), 6.22 (1H, *d*, *J* = 16.0 Hz, H-α); acetyl δ 1.81, 1.80, 1.78, 1.72, 1.70, 1.63, 1.55 (3H each, *s*); sedoheptulose (see Table 1). ^{13}C-NMR spectral data (100 MHz, $CDCl_3$): caffeoyl δ 166.0 (*s*, C═O), 143.7 (*s*, C-4′), 143.6 (*d*, C-β), 142.5 (*s*, C-3′), 133.0 (*s*, C-1′), 126.6 (*d*, C-6′), 124.0 (*d*, C-5′), 122.8 (*d*, C-2′), 118.4 (*d*, C-α); acetyl δ 170.0, 169.9, 169.5, 169.1, 169.0, 168.1, 168.0 (*s*, C═O × 7), 21.7, 20.9, 20.73, 20.72, 20.66, 20.63 × 2 (*s*, $\underline{CH_3}$C═O × 7); sedoheptulose (see Table 1).

3.7. 7-(3′,4′-Di-O-acetyl)caffeoyl-1,3,4,5,6-penta-O-acetyl-D-altro-heptulose (*1b*)

A white powder, $[\alpha]_D^{25} + 15.6^\circ$ ($CHCl_3$; *c* 0.34). ESIMS *m*/*z*: 667.1854 $[M + H]^+$ (calculated for $C_{30}H_{35}O_{17}$, 667.1869), 689.1637 $[M + Na]^+$ (calculated for $C_{30}H_{34}O_{17}Na$, 689.1688). ^{1}H-NMR (400 MHz, $CDCl_3$): caffeoyl δ 7.64 (1H, *d*, *J* = 16.0 Hz, H-β), 7.42 (1H, *dd*, *J* = 8.4, 1.9 Hz, H-6′), 7.37 (1H, *d*, *J* = 1.9 Hz, H-2′), 7.23 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.36 (1H, *d*, *J* = 16.0 Hz, H-α); acetyl δ 2.31, 2.30, 2.20, 2.16, 2.15, 2.084, 2.079 (3H each, *s*); sedoheptulose (see Table 1). ^{13}C-NMR (100 MHz, $CDCl_3$): caffeoyl δ 166.0 (*s*, C═O), 143.9 (*s*, C-4′), 143.7 (*d*, C-β), 142.5 (*s*, C-3′), 133.1 (*s*, C-1′), 126.6 (*d*, C-6′), 124.0 (*d*, C-5′), 122.9 (*d*, C-2′), 118.3 (*d*, C-α); acetyl δ 170.0, 169.9, 169.72, 169.68, 169.2, 168.0, 167.9 (*s*, C═O × 7), 20.8, 20.65, 20.61 × 2, 20.57, 20.41, 20.34 (*s*, CH_3 C═O × 7); sedoheptulose (see Table 1).

3.8. Alkaline hydrolysis of *1*, *1a* and *1b*

A solution of each compound (1 mg) in 3% NaOH (0.2 ml) was kept at room temp. for 3 h. The reaction mixture was neutralized with Dowex 50W × 8-400 (H^+) washing with H_2O (3 ml) and MeOH (2 ml). The combined washings were evaporated to dryness to afford a residue, which was subjected to TLC analysis using $CHCl_3$–MeOH–HOAc–H_2O (7:3:1:0.5) as the developing solvent. Caffeic acid was detected under UV 254 nm with an R_f value of 0.71 from compounds **1**, **1a** and **1b**. The plate was then sprayed with 10% H_2SO_4 followed by heating. All of the three compounds revealed the presence of sedoheptulose with an R_f value of 0.48 when compared with an authentic sample. For GC analysis, the residue obtained from the hydrolysis product of each of the 3 compounds (**1**, **1a**, **1b**) was converted to the TMS derivative by treatment with BTMSA at 80° for 30 min and the retention time compared with that of the TMS derivative of a reference sample of sedoheptulose. In all cases two peaks were obtained, a minor peak at 14.81 min and a major peak at 14.87 min. Scale-up of the alkaline hydrolysis of compound **1a** (5.6 mg) gave a residue which was purified on a Supelco Discovery DSC-18 column (2 g of C18 silica gel) eluting with H_2O (10 ml) to afford sedoheptulose (1.3 mg). The assignment of the absolute configuration of sedoheptulose as a D-sugar was determined by comparing the value of the optical rotation of the sugar isolated from the hydrolysis product of **1a** with the literature value for D-sedoheptulose {found $[\alpha]_D^{25} + 6.9^\circ$ (H_2O; *c* 0.10), lit. (Dalmas & Demuynck, 1993), $[\alpha]_D^{25} + 8^\circ$}.

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