New Simmondsin 2′-Ferulates from Jojoba Meal

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Two new simmondsin 2′-ferulates have been isolated from jojoba meal by preparative HPLC. The products have been identified as 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-glucoside 2′-*trans*-ferulate and 2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-glucoside 2′-*trans*-ferulate by means of two-dimensional NMR techniques and L-SIMS.

Keywords: *2D NMR; L-SIMS; HPLC*

INTRODUCTION

Jojoba (*Simmondsia chinensis*) is a woody evergreen shrub, native to the Sonora desert (United States), now cultivated as a jojoba wax source in many arid and semiarid countries all over the world. The shrub produces wax-containing nuts. This wax is liquid at room temperature and a highly valued economic product. Specific uses include lubricant and extreme-pressure lubricant, transformer oil, hydraulic fluid, and cosmetics. After the wax is removed from the nuts, either by pressing or by extraction, a protein-rich meal remains, which is a potential animal feed or animal feed ingredient. The meal, however, also contains about 5% simmondsin, a compound with food intake inhibiting activity, as described by Elliger et al. (1973) and Cokelaere et al. (1992). These opposite characteristics of meal components render the meal unsuitable as a livestock feed ingredient and make a pretreatment of the meal necessary before it can be used (Verbiscar and Baningan, 1978; Verbiscar et al., 1980, 1981). The structure and stereochemistry of simmondsin have been described in detail by Elliger et al. (1973, 1974a,b) and Van Boven et al. (1993). Two other simmondsin analogues have been isolated (Elliger et al., 1974b; Abbott et al., 1988; Van Boven et al., 1994b). The meal contains also simmondsin 2′-ferulate as described by Elliger et al. (1973) and Van Boven et al. (1994a) as well as simmondsin 3′-ferulate as described by Van Boven et al. (1994a). Both ferrulates show the same food intake inhibition effect on a molecular basis as simmondsin, these compounds being hydrolyzed to simmondsin in the gastrointestinal system (unpublished results).

The present paper describes the isolation of two demethylsimmondsin 2′-ferulates from jojoba meal by preparative HPLC and identification by two-dimensional (2D) NMR spectroscopy and liquid surface assisted mass spectrometry (L-SIMS).

MATERIALS AND METHODS

Plant Material. Jojoba meal was obtained from EMEC Agro Industries (Antwerp, Belgium).

Isolation of Simmondsin Analogues. The first step in the isolation of the new compounds is very analogous to the isolation of simmondsin, simmondsin 2′-ferulate, and simmondsin 3′-ferulate as described by Van Boven et al. (1993, 1994a,b) and consists in a fractionation in three parts of a crude acetone extract obtained from jojoba meal. For this reason jojoba meal (1 kg) was first extracted twice with hexane (2.5 L) to eliminate any remaining residues of oil; the deoiled meal was then extracted with acetone for 12 h by means of a Soxhlet apparatus. After evaporation of the solvent, a brown residue (40 g) was obtained. This material was taken up in 100 mL of methanol and adsorbed on 100 g of silica gel (0.2–0.5 mm). The solvent was removed under vacuum and the silica gel loaded into a silica gel column (30 cm length, 6 cm i.d.), containing a suspension of 0.5 kg of silica gel (0.040–0.063 mm) in chloroform. The column was first eluted with 1 L of chloroform, which was discarded. The column was further eluted with acetone; 100 mL fractions were collected and aliquot parts analyzed by TLC until simmondsin and analogous compounds were completely eluted. On the basis of the results obtained by TLC the eluates were collected in three fractions. The first fraction (~7 g) contains mainly simmondsin 3′-ferulate and simmondsin 2′-ferulate as described by Van Boven et al. (1994a) along with two other nonidentified compounds. The second fraction contains mainly simmondsin (Van Boven et al., 1993). The third fraction contains residual simmondsin mixed to more polar compounds (Van Boven et al., 1994b). The first fraction was used for the isolation of two new ferulates by preparative HPLC chromatography. For this reason 10 mg quantities were dissolved in 200 μL of a mixture of chloroform and methanol (85/15 v/v) and 100 μL aliquots injected. This procedure was repeated several times to obtain about 100 mg of the new products.

Thin-Layer Chromatography. Thin-layer chromatography was performed on silica gel plates (Polygram Sil G/UV254, Machery-Nagel, Germany) using a mixture of methanol and chloroform (20/80 v/v) as a solvent. The spots were visualized by short-UV-wavelength radiation (254 nm) and long-UV-wavelength radiation (365 nm) and spraying the plates with 1-naftol reagent. The naftol reagent was prepared by adding 10.5 mL of a 15% ethanolic solution of 1-naftol to a mixture of 40.5 mL of ethanol, 4 mL of water, and 6.5 mL of concentrated sulfuric acid. After spraying, the plates were heated at 100 °C in an oven for 5 min.

High-Performance Liquid Chromatography. Analytical high-performance liquid chromatography was performed with a Merck-Hitachi 6200 pump. Samples were injected into a Rheodyne injector (Model 7125) (Berkeley, CA) supplied with a 20 μL injector loop. A stainless steel C_{18} (5 μm particle size) column was used (25 cm × 0.4 cm i.d.) (E. Merck, Darmstadt, Germany). The solvent was a mixture of methanol and water (15/85 v/v); the flow rate was 1.0 mL/min. The column eluate was monitored with a Hitachi Model L-3000 photodiode array detector. All solvents used were of analytical grade.

Preparative HPLC was performed with an L-6250 intelligent pump (Merck-Hitachi, Darmstadt) and a column of 10

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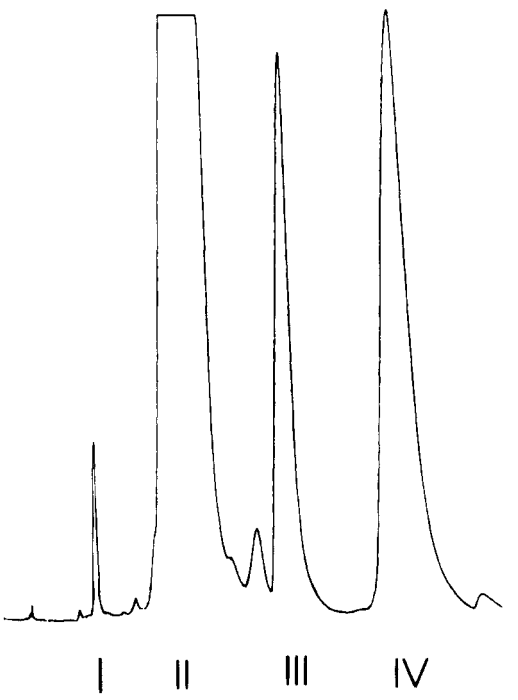


R4 = OCH3 R5 = OCH3 R2′ = fer.ac. R3′ = H simmondsin 2′-ferulate (II)
R4 = OCH3 R5 = OCH3 R3′ = fer.ac. R2′ = H simmondsin 3′-ferulate (I)
R4 = OCH3 R5 = H R2′ = fer.ac. R3′ = H 5-demethyl-simmondsin 2′-ferulate (III)
R4 = H R5 = OCH3 R2′ = fer.ac. R3′ = H 4-demethyl-simmondsin 2′-ferulate (IV)

Figure 1. Typical HPLC chromatogram from a jojoba meal extract (fraction 1).

Table 1

name	R_f value	naftol spray	fluorescence	R_t (min)
simmondsin 3′-ferulate (**I**)	0.80	violet	blue	7.1
simmondsin 2′-ferulate (**II**)	0.64	violet	blue	13.2
5-demethylsimmondsin 2′-ferulate (**III**)	0.55	violet	blue	22.9
4-demethylsimmondsin 2′-ferulate (**IV**)	0.50	violet	blue	31.9

mm × 250 mm, filled with Lichrosorb Si 60 (10 μm). The Rheodyne injector was equipped with a 0.250 mL sample loop. The flow rate of the solvent, a mixture of chloroform and methanol (85/15 v/v), was 3.0 mL/min. The eluate was monitored with the mentioned diode array detector. An analytical silica gel column (0.4 cm × 25 cm) was used to optimize the conditions for the preparative separation.

Spectroscopy. *Nuclear Magnetic Resonance Spectroscopy.* ^1H and ^{13}C NMR spectra and 2D spectra were recorded with a Bruker AMX-400 MHz spectrometer operating at 400 and 100 MHz in CD_3OD as solvent. The ^1H and ^{13}C chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal reference. The coupling patterns of the ^1H NMR spectra were elucidated by two-dimensional (2D) correlated spectroscopy (COSY) and by homonuclear spin decoupling techniques. The ^{13}C assignments were made by selective decoupling of the corresponding protons.

Mass Spectrometry. Electron impact at an ionization potential of 70 eV was performed with an HP 5995A mass spectrometer. The isolated compounds were introduced into the ion source by a direct insert probe (DIP). The ion source temperature was 280 °C, and the probe temperature was raised from room temperature to 280 °C at 60 °C/min. Liquid surface-assisted ionization mass spectrometry (L-SIMS) was performed with a Kratos Concept 1H instrument using a 7-keV Cs beam. The two compounds were dissolved in glycerol on the probe tip.

RESULTS AND DISCUSSION

Isolation Procedure. The crude acetone extract from jojoba meal was first fractionated in three fractions. The first fraction was further separated by preparative HPLC and resulted in the isolation of two new products. A typical chromatogram is shown in Figure 1. The first two peaks correspond to the already described simmondsin 3′-ferulate (peak I) and simmondsin 2′-ferulate (peak II). Peak III is identified as 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl-β-glucoside 2′-*trans*-ferulate and peak IV as 2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-glucoside 2′-*trans*-ferulate. The purity of the isolated products was checked by the mentioned TLC and HPLC procedures. For both isolated compounds TLC showed only one violet spot after the plates were sprayed with the 1-naftol reagent along with an intense blue fluorescence under long-UV-wavelength radiation (365 nm) and fluorescence quenching under short-UV-wavelength radiation (254 nm). The R_f values of the isolated compounds and of simmondsin 2′-*trans*-ferulate and simmondsin 3′-*trans*-ferulate are represented in Table 1 along with the chromogenic behavior with the naftol reagent and the observed fluorescence under long-UV-wavelength radiation. In the mentioned analytical HPLC system the two isolated compounds show only one peak. The retention times of the two new compounds along with the retention times of simmondsin 2′-*trans*-ferulate and simmondsin 3′-*trans*-ferulate are also represented in Table 1.

Mass Spectrometry. Electron impact ionization of the underivatized compounds did not result in spectra,

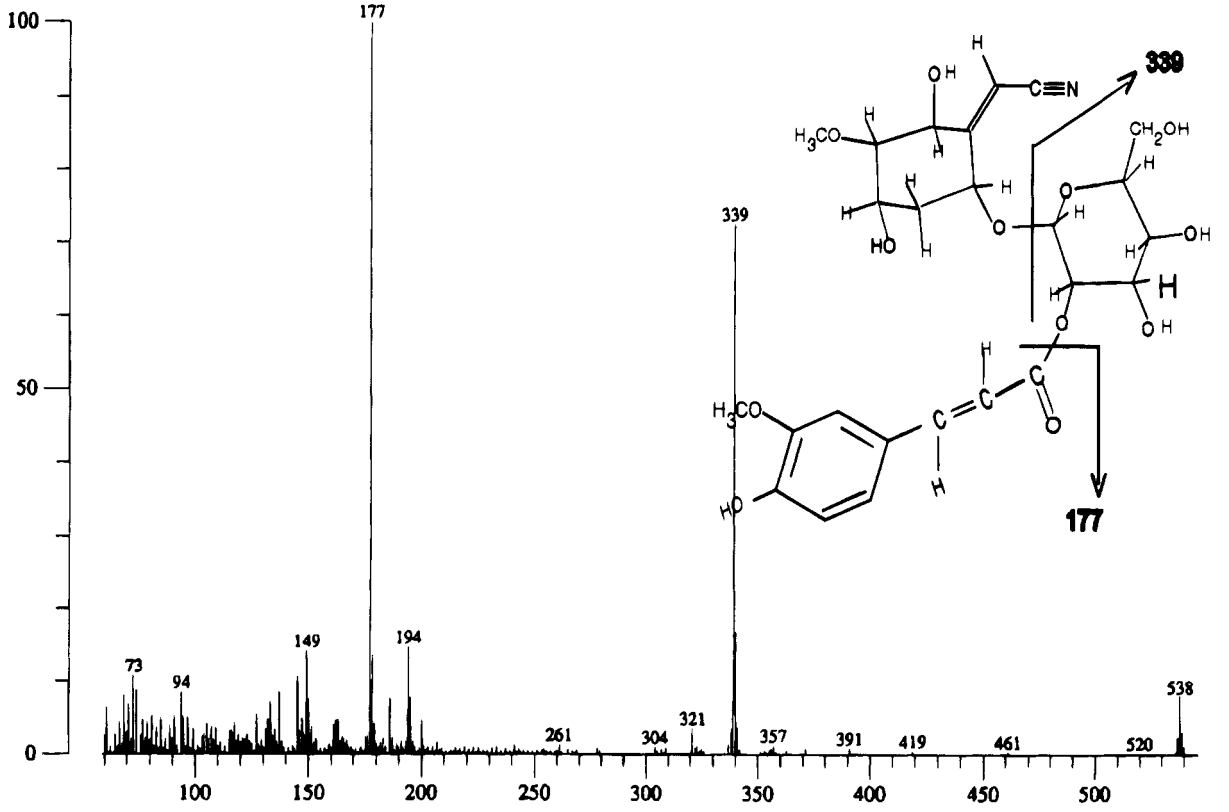


Figure 2. Mass spectrum of 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-glycoside.

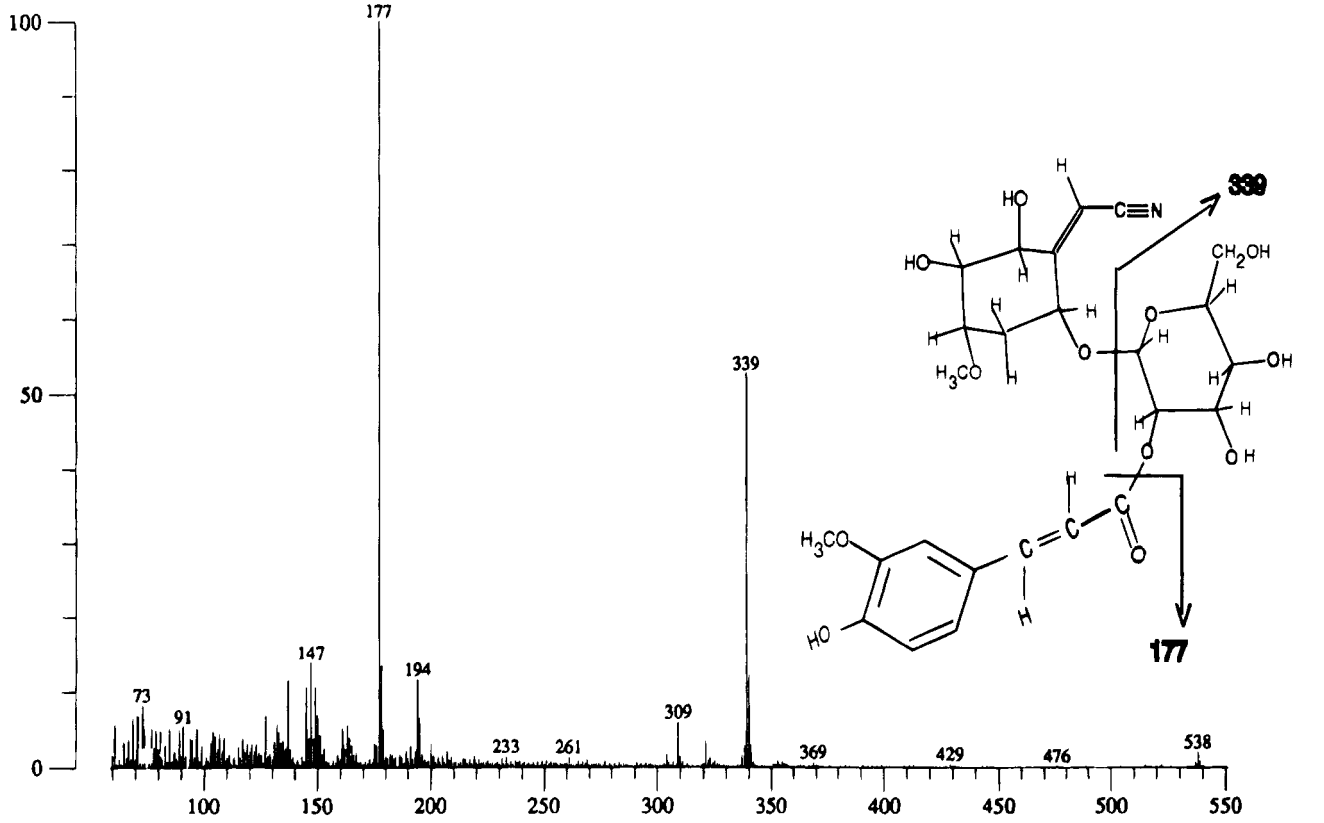


Figure 3. Mass spectrum of 2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-glucoside.

probably due to thermal decomposition of the compounds in the ion source. On the other hand, ionization with cesium ions in the L-SIMS technique (Barber et al., 1982; Rinehart, 1982) results in distinct mass spectra. The spectra from both compounds are very comparable and show the same fragmentation pattern. Compound **III** (Figure2) and compound **IV** (Figure 3) show an M + 1 ion at *m/z* 538. The M + 1 ions of simmondsin 2'-ferulate and simmondsin 3'-ferulate are at *m/z* 552. This means that both new compounds have lost one methyl group compared to the mentioned simmondsin ferulates. The important fragment at *m/z* 177, corresponding to the ferulic acid moiety, is also present in simmondsin 2'-ferulate and simmondsin 3'-

Table 2. ^{1}H Chemical Shifts (Parts per Million) for Simmondsin Ferulates in CD_3OD

	II[a]	I	III	IV
H1	4.81 (dd)	4.9 (dd)	4.89 (dd)	4.79 (dd)
H3	4.74 (dd)	**4.72** (dd)	4.72 (dd)	4.66 (dd)
H4	**3.03** (dd)	**3.14** (dd)	**2.96** (dd)	**3.33** (dd)
H5	**3.81** (ddd)	**3.9** (ddd)	**4.18** (ddd)	**3.60** (ddd)
H6A	**1.52** (dt)	**1.7** (dt)	**1.76** (dt)	**1.53** (dt)
H6B	**2.43** (dt)	2.49 (dt)	**2.22** (dt)	**2.42** (dt)
4-OCH_3	3.4 (s)	3.43 (s)	3.43 (s)	
5-OCH_3	3.28 (s)	3.46 (s)		3.28 (s)
H7	5.72 (d)	5.71 (d)	5.72 (d)	5.69 (d)
H9	6.43 (d)	6.43 (d)	6.43 (d)	6.43 (d)
H10	7.65 (d)	7.65 (d)	7.68 (d)	7.65 (d)
H12	7.19 (d)	7.19 (d)	7.20 (d)	7.20 (d)
H15	6.81 (d)	6.8 (d)	6.81 (d)	6.81 (d)
H16	7.08 (dd)	7.08 (dd)	7.09 (dd)	7.08 (dd)
13-OCH_3	3.88 (s)	3.88 (s)	3.88 (s)	3.88 (s)
H1′	4.62 (d)	4.52 (d)	4.64 (d)	4.62 (d)
H2′	4.85 (dd)	3.46 (dd)	4.83 (dd)	4.85 (dd)
H3′	3.58 (t)	5.05 (t)	3.62 (t)	3.59 (t)
H4′	3.43 (t)	3.55 (t)	3.44 (t)	3.43 (t)
H5′	3.28 (m)	3.35 (m)	3.30 (m)	3.27 (m)
H6′A	3.7 (dd)	3.69 (dd)	3.71 (dd)	3.70 (dd)
H6′B	3.85 (dd)	3.86 (dd)	3.86 (dd)	3.85 (dd)

[a] The Roman numbers correspond to the Roman numbers and the corresponding structures of Figure 4. The numbering of the hydrogens corresponds to the numbering of the carbon atoms to which they are bound.

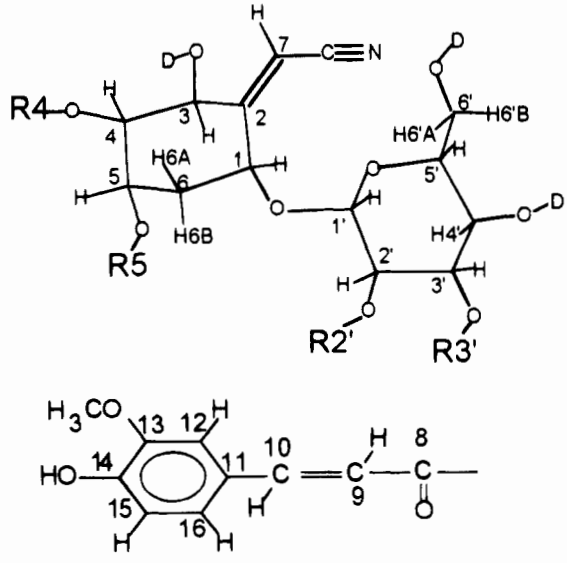
ferulate. This suggests that this part of the molecule remained intact in the new isolated compounds. The intense fragment at *m/z* 339 in both compounds, corresponding to the elimination of the cyanomethylenecyclohexyl part of the molecule, is also seen in simmondsin 2′-ferulate and simmondsin 3′-ferulate, indicating again that this part of the molecule is identical in all four

Table 3. ^{1}H–^{1}H Coupling Constants (Hertz) in Simmondsin Ferulates

$^3J_{H1-H6A} = 3.5$	$^3J_{H1'-H2'} = 7.8$	$^3J_{H9-H10} = 16$
$^3J_{H1-H6B} = 4.0$	$^3J_{H2'-H3'} = 9.0$	$^3J_{H15-H16} = 8$
$^3J_{H3-H4} = 9.0$	$^3J_{H3'-H4'} = 9.0$	$^4J_{H12-H16} = 2$
$^4J_{H3-H7} = 2.0$	$^3J_{H4'-H5'} = 9.0$	
$^3J_{H4-H5} = 3.0$	$^3J_{H5'-H6'A} = 5.2$	
$^3J_{H5-H6A} = 3.5$	$^3J_{H5'-H6'B} = 2.2$	
$^3J_{H5-H6B} = 4.0$	$^2J_{H6'A'-H6'B} = 12.0$	
$^2J_{H6A-H6B} = 15.0$		

compounds. From these observations it can be concluded that the two new compounds differ from simmondsin 2′-ferulate and simmondsin 3′-ferulate by the replacement of a methoxy group by a hydroxyl in the cyanomethylenecyclohexyl part of the molecules. The exact position of the hydroxyl could be determined by NMR spectroscopy.

NMR Spectroscopy. The ^{1}H-NMR chemical shifts from both new products are summarized in Table 2 along with the spectral data of simmondsin 2′-ferulate and simmondsin 3′-ferulate. Table 3 represents the H–H coupling constants in hertz for the four mentioned compounds. Both new products show identical spectral data for the ferulate part of the molecules with the already described ferulates. The coupling constants of 16 Hz between the vinyl protons H_9 and H_{10} (Table 3) again prove that both protons are in a *trans* relationship. The resonances at 6.81 and 7.09 ppm for compound **III** and at 6.81 and 7.08 ppm for compound **IV** correspond to the two vicinal aromatic protons H_{15} and H_{16}. The signal at 7.20 ppm represents the remaining aromatic proton H_{12}. For both compounds the ferulic acid moiety is linked at the 2′-position of glucose. This is proved by an identical shift for the $H_{2'}$ proton in the



ferulic acid moiety

$R_4 = OCH_3$ $R_5 = OCH_3$ $R_2' =$ fer. ac. $R_3' = H$ simmondsin 2′-ferulate (I)

$R_4 = OCH_3$ $R_5 = OCH_3$ $R_3' =$ fer. ac. $R_2' = H$ simmondsin 3′-ferulate (II)

$R_4 = OCH_3$ $R_5 = H$ $R_2' =$ fer. ac $R_3' = H$ 5-demethyl-simmondsin 2′-ferulate (III)

$R_4 = H$ $R_5 = OCH_3$ $R_2' =$ fer. ac. $R_3' = H$ 4-demethyl-simmondsin 2′-ferulate (IV)

Figure 4. Structure of simmondsin ferulate and demethylsimmondsin ferulates isolated from jojoba meal.

Table 4. ^{13}C Chemical Shifts (Parts per Million) in CD_3OD

carbon no.	II	I	III	IV
1	77.8	76.9	78.4	78.1
2	166.5	166.2	166.0	166.9
3	70.4	70.8	70.2	71.6
4	86.3	86.2	87.2	77.2
5	75.6	76.5	67.0	79.0
6	31.3	32.0	35.8	31.4
7	95.4	95.4	95.4	95.0
CN	117.7	117.6	117.4	117.1
4-CH_3O	58.1	58.5	58.1	
5-CH_3O	57.1	58.0		57.1
1′	103.3	104.1	102.3	103.3
2′	75.0	73.2	75.0	75.0
3′	76.4	79.0	76.0	76.4
4′	71.4	69.7	71.3	71.4
5′	78.3	78.0	78.4	78.3
6′	62.4	62.4	62.3	62.4
8	168.0	169.0	168.5	168.0
9	124.1	124.0	124.2	124.1
10	147.0	146.8	147.4	147.0
11	127.7	127.8	127.8	127.7
12	115.6	115.8	115.3	115.6
13-OCH3	56.4	56.4	56.4	56.4
13	149.4	149.4	149.4	149.4
14	150.7	150.5	150.6	150.6
15	116.5	116.5	116.5	116.5
16	111.8	111.8	111.9	111.8

new compounds (respectively at 4.83 and 4.85 ppm) as in simmondsin 2′-ferulate (4.85 ppm). As can be seen from Table 2, the remaining resonances from the glucose part of the two compounds are identical to the resonances of simmondsin 2′-ferulate. For the cyanomethylenecyclohexyl part of the molecule in both compounds there is only one methoxy signal left compared to the simmondsin 2′-ferulate and simmondsin-3′-ferulate, respectively, at 3.43 ppm for compound **III** and at 3.28 ppm for compound **IV**. These values, when compared with the values of simmondsin 2′-ferulate, already suggest that the remaining methoxy group is linked at the 4-position for compound **III** and at the 5-position for compound **IV**.

The ^{13}C chemical shifts reported in Table 4 are even more conclusive about the exact position of the methoxy group in the cyanomethylene part of the molecules. Replacement of a methoxy substitution by a hydroxyl substitution implicates for the aliphatic carbon atom an upfield shift of about 10 ppm as described by Wehrli and Wirthlin (1978). This is clearly demonstrated in the mentioned compounds when the spectra are compared to the spectrum of simmondsin 2′-ferulate. In compound **III** the C5 signal is shifted from 75.6 ppm in simmondsin 2′-ferulate to 67.0 ppm, while the shift at 87.2 ppm for C4 remains nearly identical to the shift at 86.3 ppm in simmondsin 2′-ferulate. This indicates a replacement of the methoxy in simmondsin 2′-ferulate by a hydroxyl in compound **III**. In compound **IV**, on the other hand, the signal at 86.3 ppm in simmondsin 2′-ferulate for C4 is now shifted to 77.2 ppm in this compound, indicating a replacement of the methoxy group by a hydroxyl at C4 in the isolated new product. The structures of the two demethylsimmondsin 2′-ferulates are represented in Figure 4 along with the structures of the previously isolated simmondsin ferulates.

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