Isolation and Structure Elucidation of the Major Simmondsin Analogues in Jojoba Meal by Two-Dimensional NMR Spectroscopy

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The major simmondsin analogues have been isolated from jojoba meal; the structures of the compounds could be unambiguously proven by means of two-dimensional NMR spectroscopy and mass spectrometry as 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-glucoside and 2-(cyanomethylene)-3,4,5-trihydroxycyclohexyl β-glucoside.

Keywords: *Mass spectrometry; 2D NMR spectroscopy; liquid chromatography*

INTRODUCTION

Jojoba (*Simmondsia chinensis*) is native to the Sonora desert and is now being cultivated in arid and semiarid countries all over the world. After the oil is removed from the seeds, a protein-rich meal remains. This meal has been described as toxic to rodents and chickens (Elliger et al., 1973, 1974b). Although cyanides have been claimed to be responsible for this toxicity (Williams, 1980), no cyanides were observed as metabolites of simmondsin, when given orally to rats (Cokelaere et al., 1992b). Obviously the "toxicity" is due to the food intake inhibition caused by jojoba constituents of which simmondsin has been identified as the most important inhibitor (Elliger et al., 1973, 1974a; Booth et al., 1974, Cokelaere et al., 1992a). The stereochemistry of simmondsin has been described by Elliger et al. (1974a) and Van Boven et al. (1993). The meal contains two other major simmondsin analogues. Elliger et al. (1974b) described these compounds as two monodemethylsimmondsins, on the basis of the NMR spectra of the acetylated derivatives. Abbott et al. (1988) described the isolation of these compounds by HPLC and characterized them as demethylsimmondsin (DMS) and didemethylsimmondsin (DDMS) without giving any details of structure analysis and stereochemistry. The present paper describes the isolation on a preparative scale and the determination of the exact structure and stereochemistry of the two analogues by two-dimensional (2D) NMR techniques.

MATERIALS AND METHODS

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

Isolation of Simmondsin Analogues. The isolation of these compounds is very analogous with the isolation of simmondsin as described by Van Boven et al. (1993). Jojoba meal (1 kg) was first extracted twice with hexane (5 L) to eliminate any remaining residues of oil. Deoiled meal was 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 methanol (200 mL), and 100 g of silica gel (0.2–0.5 mm) was added. 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 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 parts. The first part contains mainly simmondsin 3′-ferulate and simmondsin 2′-ferulate as described by Van Boven et al. (1994). The second fraction contains mainly simmondsin (Van Boven et al., 1993). The third fraction contains residual simmondsin mixed with more polar compounds. This fraction was filtered over activated carbon and the acetone evaporated. The crude residue (16 g) was further purified on another silica gel column (60 cm length × 6 cm i.d.) consisting of a suspension of 1 kg of silica gel (0.040–0.063 mm) in chloroform. The crude residue was first dissolved in methanol and adsorbed into silica gel (0.2–0.5 mm). After evaporation of the solvent, the silica gel was loaded into the silica gel column. Elution was performed with a mixture of methanol and chloroform (20/80 v/v). The column outlet was conducted through a UV instrument to directly monitor eluting compounds at adequate wavelengths, chosen to keep absorbances in scale. All peaks were collected separately and examined for purity by HPLC and TLC and concentrated. Two major compounds were isolated at 1600–1900 mL (analogue **I**) and at 2600–3100 mL (analogue **II**). The obtained white products were examined by means of mass spectrometry and NMR spectroscopy.

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 (30/70 v/v) as solvent. The spots were localized by short-UV-wavelength radiation (254 nm) and by spraying the plates with 1-naphthol reagent. The naphthol reagent was prepared by adding 10.5 mL of a 15% ethanolic solution of 1-naphtol to a mixture of 40.5 mL of ethanol, 4 mL of water, and 6.5 mL of sulfuric acid. After spraying, the plates were heated at 100 °C in an oven for 5 min.

High-Performance Liquid Chromatography. 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 flow rate of the solvent, a mixture of methanol and water (15/85 v/v), was 1.0 mL/min. The column eluate was monitored at 220 nm with a Hitachi Model L-3000 photodiode array detector. All solvents used were of analytical grade.

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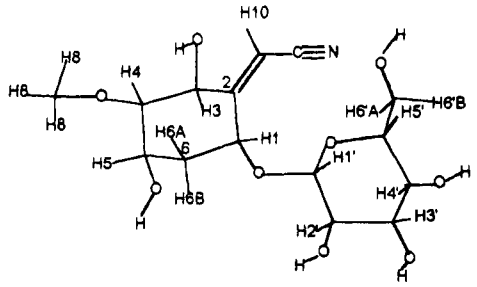
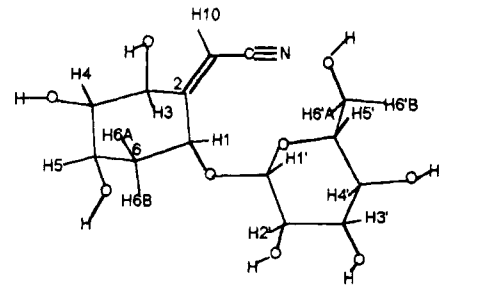


2-(cyanomethylene)-3,5-dihydroxy-4-methoxy-cyclohexyl β-glucoside



2-(cyanomethylene)-3,4,5-trihydroxy-cyclohexyl β-glucoside

Figure 1. 1. Structures of the simmondsin analogues.

Spectroscopy. *Nuclear Magnetic Resonance Spectroscopy.* ^1H and ^{13}C NMR 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 internal reference. The coupling pattern of the ^1H NMR spectra was elucidated by two-dimensional (2D) correlated spectroscopy (COSY) and by homonuclear spin decoupling technique. 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 described method allows the isolation on a preparative scale of two distinct simmondsin analogues. A first eluting compound was identified as 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-glucoside; another major compound was identified as 2-(cyanomethylene)-3,4,5-trihydroxycyclohexyl β-glucoside. The structures of the simmondsin analogues are presented in Figure 1. The purity of the isolated derivatives was checked by TLC and HPLC procedures. TLC showed only a single spot, and the HPLC analysis revealed only one peak for the first compound. The last eluting compound had to be purified a second time by the same chromatographic procedure because of the presence of another compound eluting in the side of didemethylsimmondsin. This impurity was identified by NMR as pinitol or 3-*O*-methylinositol. The R_f values of simmondsin analogue **I** and simmondsin analogue **II** are, respectively, 0.52 and 0.32; the R_f value of simmondsin is 0.69. Simmondsin and the mentioned analogues appeared as violet spots after the plates were sprayed with the 1-naphthol reagent. Both compounds show fluorescence quenching in short-UV-wavelength radiation (254 nm). The retention times of the different products in the mentioned HPLC system are, respectively, 3.7 min for analogue **I**, 5.6 min for analogue **II**, and 7.7 min for simmondsin.

Table 1. Chemical Shifts (Parts per Million) for Simmondsin Analogues in CD_3OD

	simmondsin		demethyl-simmondsin		didemethyl-simmondsin	
H_1	4.88	t	4.90	t	4.92	t
H_3	4.72	dd	4.75	dd	4.69	dd
H_4	3.13	dd	2.97	dd	3.31	dd
H_5	3.9	q	4.28	q	4.08	q
H_{6A}	1.69	dt	1.74	dt	1.77	dt
H_{6B}	2.5	dt	2.42	dt	2.42	dt
$H_8(3)$	3.43	s	3.47	s		
$H_9(3)$	3.47	s				
H_{10}	5.7	d	5.70	d	5.68	d
$H_{1'}$	4.38	d	4.40	d	4.40	d
$H_{2'}$	3.22	dd	3.20	dd	3.20	dd
$H_{3'}$	3.35	t	3.39	t	3.39	t
$H_{4'}$	3.28	t	3.36	t	3.36	t
$H_{5'}$	3.22	m	3.24	m	3.24	m
$H_{6'A}$	3.65	dd	3.69	dd	3.69	dd
$H_{6'B}$	3.82	dd	3.82	dd	3.82	dd

Table 2. $^1H-^1H$ Coupling Constants (Hertz) in Simmondsin Analogues

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

Table 3. ^{13}C Chemical Shifts (Parts per Million) in CD_3OD

carbon no.[a]	S (III)	5-DSIM (I)	DDSIM (II)
1	76.8	77.8	78.0
2	166.4	166.5	166.8
3	70.8	70.1	71.15
4	86.4	87.5	**78.05**
5	76.5	**66.8**	**70.5**
6	32.1	35.1	35.2
10	95.2	94.9	94.6
CN	117.6	117.4	117.4
CH_3O 4	58.2		
CH_3O 5	58.5	58.3	
1′	104.1	104.6	104.5
2′	74.6	74.8	74.8
3′	78.2	77.8	77.8
4′	71.5	71.2	71.2
5′	78.2	78.1	78.1
6′	62.8	62.4	62.4

[a] Carbon numbers correspond to the numbers of the bonded hydrogens in Figure 1.

NMR Spectroscopy. The ^1H NMR chemical shifts from both analogues are mentioned in Table 1 along with the spectral data from simmondsin. The latter were already discussed in detail by Van Boven et al. (1993). Table 2 lists the H–H coupling constants in hertz for the three mentioned compounds. As can be expected, the coupling constants for corresponding protons in the simmondsin analogues and in simmondsin show identical values. The proton spectra clearly demonstrate the presence of one methoxy group in analogue **I** and the absence of a methoxy group in analogue **II**. However, for analogue **I**, the specific position of the methoxy group, either the 4- or 5-position, cannot be definitely proven by the proton spectra, as can be seen in Table 1.

The ^{13}C spectra, on the other hand, are more conclusive for the determination of the exact position of the

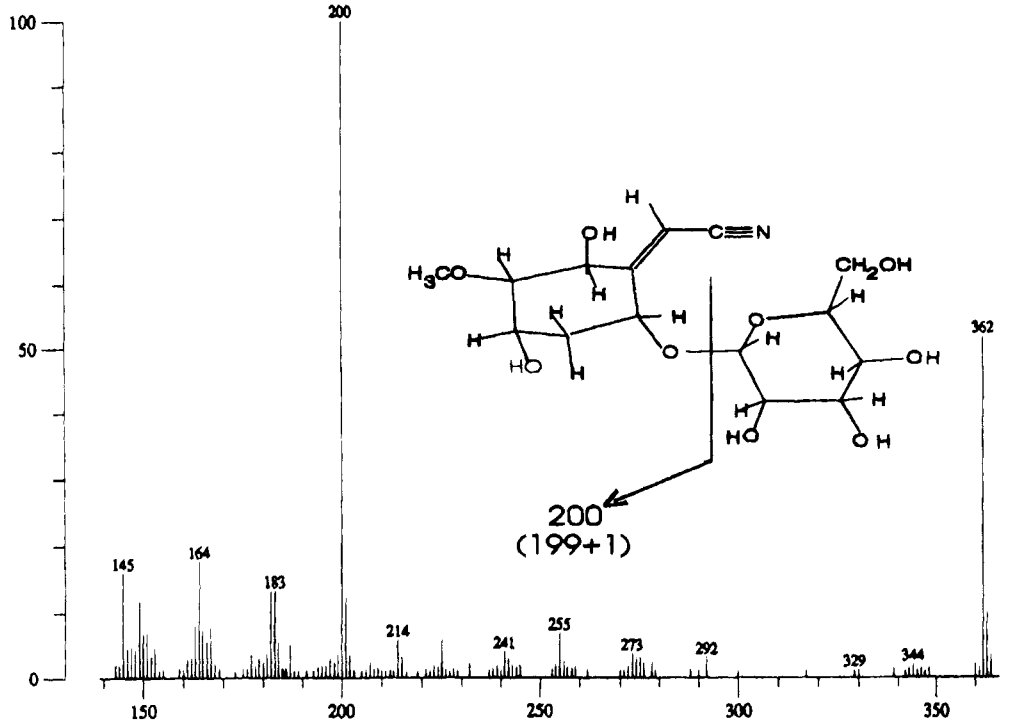


Figure 2. Mass spectrum of 2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl) β-glucoside.

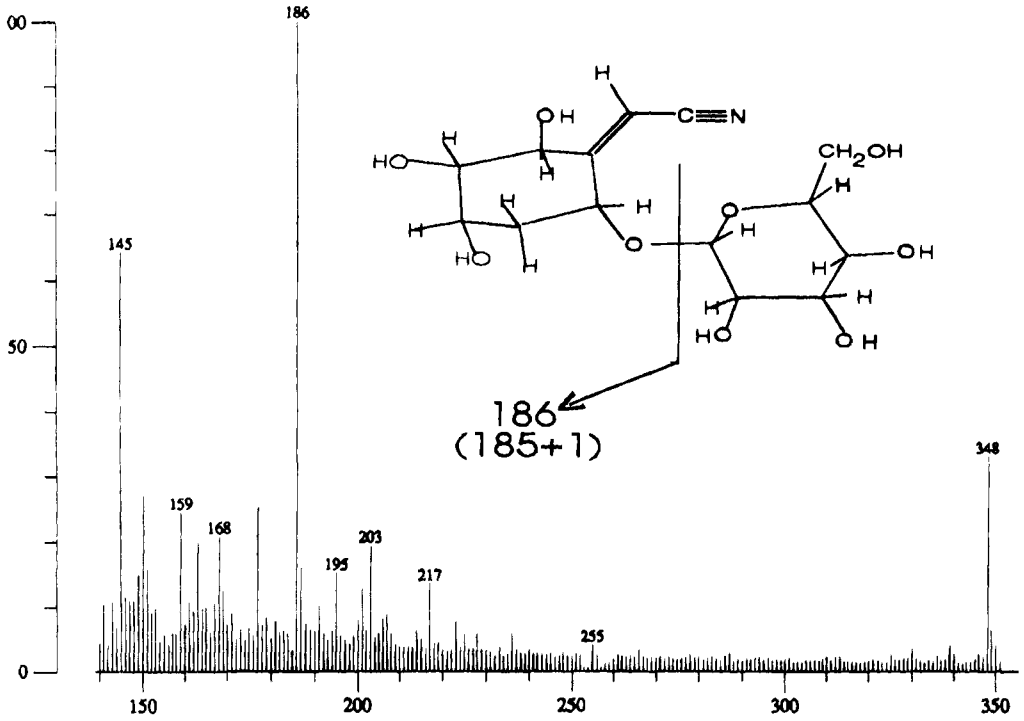


Figure 3. Mass spectrum of 2-(cyanomethylene)-3,4,5-trihydroxycyclohexyl β-glucoside.

methoxy group in the molecule. From the chemical shifts, reported in Table 3 for analogue **I**, in comparison to simmondsin, it can be concluded that the methoxy group is on the 4-position. The signal at 76.5 ppm for carbon 5 in simmondsin is shifted to 66.8 ppm for carbon 5 in analogue **I**, while the resonance signal for carbon 4 at 87.5 ppm remains almost identical to the C_4 signal of simmondsin at 86.4 ppm. This major change in chemical shift for the C_5 signal clearly demonstrates a concomitant chemical change, i.e., replacement of a methoxy group by a hydroxyl group at the actual carbon atom. The same upfield shift is observed for the C_4 signal in analogue **II** from 86.4 ppm in simmondsin to 78.05 ppm in the latter compound. Again this proves the replacement of a methoxy group in simmondsin by a hydroxyl group at carbon 4 in analogue **II**.

The stereochemistry of both compounds is identical to the stereochemistry of simmondsin itself, as is proven by the H−H coupling constants given in Table 2. The axial position of the four glucose ring protons at δ 3.22, 3.28, 3.35, and 3.22 is proven by diaxial coupling constants of 9 Hz. In the cyanomethylenecyclohexyl part of the molecule, the coupling constant of 9 Hz between H_3 and H_4 confirms again the diaxial relationship. H_5 is in the equatorial position, as proven by a coupling constant of 3 Hz with H_4.

Mass Spectrometry. Electron impact ionization mass spectrometry of the underivatized analogues did not result in mass spectra due to intense decomposition of the compounds. On the other hand, the L-SIMS technique, bombarding with cesium ions, results in distinct spectra. Analogue I (Figure 2) shows a M + 1 ion at *m/z* 362 (for simmondsin M + 1 corresponds to *m/z* 376); the major fragment ion from simmondsin at *m/z* 214 is absent in the actual analogue **I** and is replaced by the fragment at *m/z* 200. Fragments at *m/z* 214 and 200 arise by elimination of glucose out of the molecule with a simultaneous rearrangement of hydrogen. The mass spectrum of analogue **II** (Figure 3) shows a M + 1 ion at *m/z* 348. The fragment ion at *m/z* 200 for analogue **I** is absent and replaced by a fragment ion at *m/z* 186, as could be expected. Both analogues show also minor peaks at *m/z* M + Na. As already described by Rinehart (1982) and Barber et al. (1982), the use of glycerol as a solvent in the L-SIMS technique gives rise to both molecular ions, which include M + H and M + Na ions, as well as fragment ions. Glycerol itself gives rise to minor peaks at mass numbers corresponding to $92n + 1$.

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