Isolation and Structural Identification of a New Simmondsin Ferulate from Jojoba Meal

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A new simmondsin ferulate was isolated from jojoba meal and identified as simmondsin 3′-*trans*-ferulate by 2D NMR and L-SIMS.

INTRODUCTION

Jojoba [*Simmondsia chinensis* (Link) Schneider, from the family of Buxaceae or Simmondsiaceae] grew originally in the Sonora desert and is now being cultivated in Arizona, California, and Mexico. 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, 1974). Although this toxicity has been attributed to cyanides (Williams, 1980), no cyanides could be detected following metabolism of simmondsin, a cyano-containing glycoside, in the rat (Cokelaere et al., 1992a). Instead, the symptoms described as toxic are probably due to the food intake inhibition caused by jojoba constituents. Simmondsin has been identified as the most important inhibitor (Elliger et al., 1973, 1974; Booth et al., 1974; Cokelaere et al., 1992b). Ellinger et al. (1974) have also isolated and characterized simmondsin 2′-ferulate in the meal. The present study concerns the isolation and structure determination of a new simmondsin ferrulate in jojoba meal, simmondsin 3′-*trans*-ferulate.

MATERIALS AND METHODS

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

Isolation of Simmondsin 2′-Ferulate and Simmondsin 3′-Ferulate. Jojoba meal was extracted twice with hexane to eliminate any remaining residues of oil. The deoiled meal (1 kg) was extracted with acetone for 12 h by means of a Soxhlet apparatus. After evaporation of the solvent, a brown residue was obtained. This material was taken up in methanol, and silica gel (0.2–0.5 mm, 100 g) was added. The solvent was removed under vacuum, and the silica gel was loaded into a silica gel column (30-cm length, 6-cm i.d.), containing a suspension 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. Fractions (100 mL) were collected and analyzed by TLC until simmondsin and analogous compounds were eluted completely. The fraction eluting prior to simmondsin was collected separately and filtered over activated carbon, resulting in a yellow solution. After evaporation of the acetone, the crude residue was further purified on another silica gel column (60 cm × 6-cm i.d.), containing a suspension of 0.5 kg of silica gel (0.040–0.063 mm) in chloroform. The crude residue was first dissolved in methanol and absorbed into silica gel (0.2–0.5 mm, 100 g) which was loaded into the silica gel column. Elution was performed with a mixture of methanol and chloroform (5/95 v/v). The column output was passed through a UV instrument to directly monitor eluting compounds at adequate wavelengths, chosen to keep absorbances in scale. All peaks were collected separately, examined for purity by HPLC and TLC, and concentrated. Three major compounds were isolated at 2800–3200 mL (compound **I**, approximately 1 g), at 3600–4200 mL (compound **II**, 8 g), and at 5800–7200 mL (simmondsin, 40 g).

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 the solvent. The spots were visualized by long-UV-wavelength radiation (365 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-naphthol 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.

HPLC. High-performance liquid chromatography was performed with a Merck Hitachi-6200 apparatus. Samples were injected into a Rheodyne injector (Model 7125) (Berkeley, CA) supplied with a 20-μL injector loop. A stainless steel C_{18} (7-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 (50/50 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.

Spectroscopy. *Nuclear Magnetic Resonance Spectroscopy.* ^1H and ^{13}C NMR spectra were recorded in CD_3OD as solvent on a Bruker AMX-400 MHz spectrometer operating at 400 and 100 MHz. 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) for compound **III** and by homonuclear spin decoupling technique for compounds **I** and **II**. 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

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Simmondsin	R2' = H	R3' = H
Simmondsin-2'-ferulate	R2' = ferulic acid moiety	R3' = H
Simmondsin-3'-ferulate	R3' = ferulic acic moiety	R2' = H

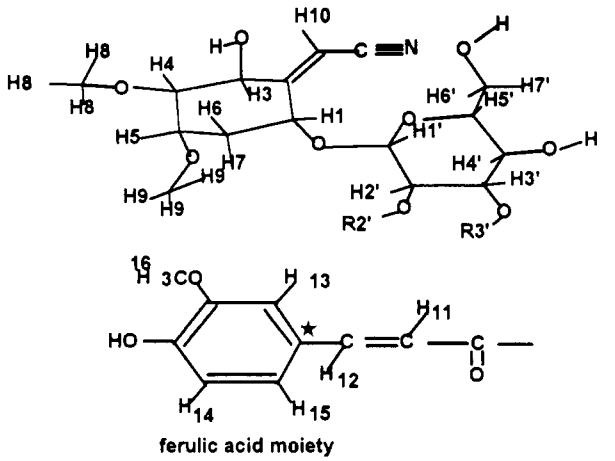

Figure 1. Structure of simmondsin and simmondsin ferulates.

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

sim-mondsin			sim-mondsin	2'-ferulate	sim-mondsin	3'-ferulate
H_1	4.88	t	4.81	t	4.9	t
H_3	4.72	dd	4.74	dd	4.72	dd
H_4	3.13	dd	3.03	dd	3.14	dd
H_5	3.9	q	3.81	q	3.9	q
H_6	1.69	ddd	1.52	ddd	1.7	ddd
H_7	2.5	dt	2.43	dt	2.49	dt
H_8(3)	3.43	s	3.4	s	3.43	s
H_9(3)	3.47	s	3.28	s	3.46	s
H_{10}	5.7	d	5.72	d	5.71	d
H_{11}			6.43	d	6.43	d
H_{12}			7.65	d	7.65	d
H_{13}			7.19	d	7.19	d
H_{14}			6.81	d	6.8	d
H_{15}			7.08	dd	7.08	dd
H_{16}(3)			3.88	s	3.88	s
$H_{1'}$	4.38	d	4.62	d	4.52	d
$H_{2'}$	3.22	dd	4.85	dd	3.46	dd
$H_{3'}$	3.35	t	3.58	t	5.05	t
$H_{4'}$	3.28	t	3.43	t	3.55	t
$H_{5'}$	3.22	m	3.28	m	3.35	m
$H_{6'}$	3.65	dd	3.7	dd	3.69	dd
$H_{7'}$	3.82	dd	3.85	dd	3.86	dd

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. Simmondsin was dissolved in glycerol on the probe tip.

RESULTS AND DISCUSSION

Isolation Procedure. The above-described method led to the isolation of two distinct simmondsin ferulates. The faster eluting compound was identified as simmondsin 3'-*trans*-ferulate (compound **I**). The second major compound (compound **II**), which eluted just before simmondsin, was identified as simmondsin 2'-*trans*-ferulate. The structures of simmondsin, simmondsin 2'-*trans*-ferulate, and simmondsin 3'-*trans*-ferulate are shown in Figure 1. The purity of the isolated ferulates was confirmed by TLC and HPLC; each method showed only one compound. The R_f values of simmondsin 3'-ferulate, simmondsin 2'-ferulate, and simmondsin in the described TLC system were 0.79, 0.64, and 0.42, respectively. Both

Table 2. ^{1}H–^{1}H Coupling Constants (Hertz) in Simmondsin Derivatives

$^3J_{H1-H6}$ = 3.5	$^3J_{H1'-H2'}$ = 7.8	$^3J_{H11-H12}$ = 16
$^3J_{H1-H7}$ = 4.0	$^3J_{H2'-H3'}$ = 9.0	$^3J_{H14-H15}$ = 8
$^3J_{H3-H4}$ = 9.0	$^3J_{H3'-H4'}$ = 9.0	$^4J_{H13-H15}$ = 2
$^4J_{H3-H10}$ = 2.0	$^3J_{H4'-H5'}$ = 9.0	
$^3J_{H4-H5}$ = 3.0	$^3J_{H5'-H6'}$ = 5.2	
$^3J_{H5-H6}$ = 3.5	$^3J_{H5'-H7'}$ = 2.2	
$^3J_{H5-H7}$ = 4.0	$^2J_{H6'-H7'}$ = 12.0	
$^2J_{H6-H7}$ = 15.0		

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

carbon no.[a]	S (III)	SF1 (II)	SF2 (I)
1	76.8	77.8	76.9
2	166.4	166.5	166.2
3	70.8	70.4	70.8
4	86.4	86.3	86.2
5	76.5	75.6	76.5
6	32.1	31.3	32.0
10	95.2	95.4	95.4
CN	117.6	117.7	117.6
CH_3O	58.2	58.1	58.5
	58.5	57.1	58.0
1'	104.1	103.3	104.1
2'	74.6	75.0	73.2
3'	78.2	76.4	79.0
4'	71.5	71.4	69.7
5'	78.2	78.3	78.0
6'	62.8	62.4	62.4
CO		168	169
C_{11}		124.1	124.0
C_{12}		147.0	146.8
C★		127.7	127.8
C_{13}		115.6	115.8
C_{15}		111.8	111.8
OCH_3		65.4	56.4
COD		116.5	116.5
$COCH_3$		149.4	149.4
C_{14}		150.7	150.5

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

simmondsin 2'-ferulate and simmondsin 3'-ferulate show as blue fluorescent spots under long-wavelength UV radiation (365 nm). Simmondsin and the two ferulates appeared as violet spots after the plates were sprayed with the 1-naphthol reagent. The retention times of simmondsin 2'-ferulate, simmondsin 3'-ferulate, and simmondsin in the mentioned system were 6.1, 3.6, and 1.2 min, respectively.

NMR Spectroscopy. The ^{1}H NMR chemical shifts of simmondsin 2'-ferulate and simmondsin 3'-ferulate are given in Table 1 along with the spectral data from simmondsin. Table 2 lists the H–H coupling constants in hertz for simmondsin, simmondsin 2'-ferulate, and simmondsin 3'-ferulate. The coupling constants for analogous protons in the isolated ferulates and in simmondsin showed identical values.

Compound **II** has been described by Elliger et al. (1973) as a mixture of cis- and trans isomers of simmondsin 2'-ferulate. However, the coupling constant of 16 Hz between the vinyl protons H_{11} and H_{12} (Table 2) suggests that this compound is the pure trans isomer. The resonances at 6.81 and 7.08 ppm correspond to the two vicinal aromatic protons H_{14} and H_{15} in the ferulate ester. The signal at 7.19 ppm represents the remaining aromatic proton H_{13}. The ferulic acid moiety is linked to simmondsin at the 2'-hydroxyl group of the β-glucose. This is confirmed by the shift of the $H_{2'}$ proton from 3.22 ppm in simmondsin to 4.80 ppm in the isolated compound. All remaining ^{1}H resonances of the molecule are comparable to the resonances of simmondsin.

Compound **I** was identified as simmondsin 3'-*trans*-

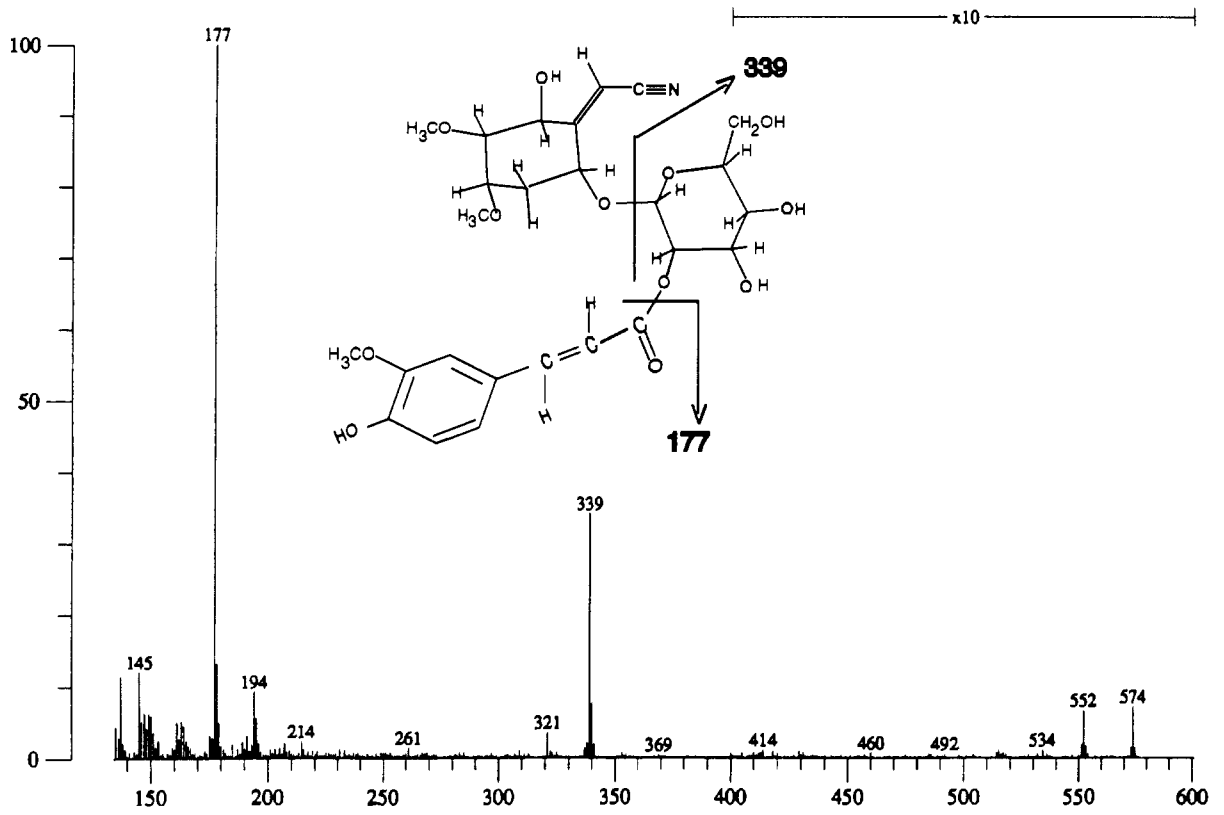

Figure 2. Mass spectrum of simmondsin 2′-ferulate.

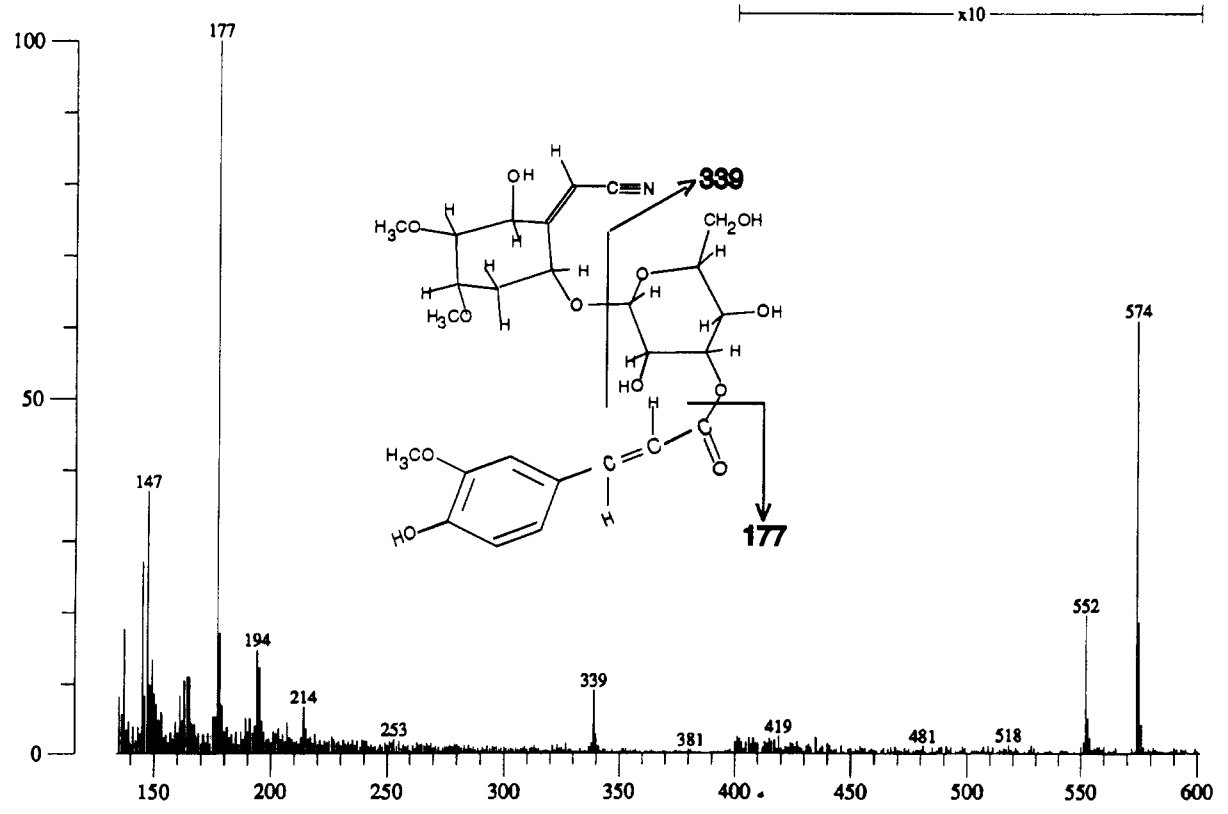

Figure 3. Mass spectrum of simmondsin 3′-ferulate.

ferulate. The aromatic region of its ^1H spectrum is identical to the spectrum of simmondsin 2′-ferulate. Compound **I** also consists of a pure trans isomer as demonstrated by the coupling constant of 16 Hz between the vinyl protons H_{11} and H_{12} (Table 2). The resonance of the 3′ proton is shifted from 3.22 ppm in simmondsin to 5.05 ppm in compound **I**, showing that the ferulic acid moiety is linked to simmondsin at the 3′ position of β-glucose. As seen in Table 1, the other proton resonances in compound **I** are similar to the resonances of simmondsin itself. The spectral data of simmondsin have been discussed in detail by Van Boven et al. (1993).

From the ^{13}C chemical shifts reported in Table 3 for compounds **I**–**III**, linking of the ferulic acid moiety at $O_{2'}$

(II) and $O_{3'}$ (I) results in a small downfield shift (less than 1 ppm) of the substituted carbons $C_{2'}$ (II) and $C_{3'}$ (I) and a somewhat bigger upfield shift (1–2 ppm) of the neighboring carbons $C_{1'}C_{3'}$ (II) and $C_{2'}C_{4'}$ (I). The ^{13}C shifts are less diagnostic than those observed in the ^{1}H NMR spectra.

Mass Spectrometry. Due to intense decomposition, electron impact ionization mass spectrometry of compounds I and II did not result in distinct mass spectra. On the other hand, the L-SIMS technique, with bombardment with cesium ions, provided distinct spectra. Figure 2 shows the spectrum of simmondsin 2′-ferulate and Figure 3 the spectrum of simmondsin 3′-ferulate. M + 1 ions (m/z 552) are seen for both isomers as well as M + 23 (Na) ions (m/z 574). As 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 M + H and M + Na ions, as well as fragment ions. In both compounds the most important fragment ion is at m/z 177, corresponding to the ferulic acid moiety ($C_{10}H_9O_3$). Both compounds also show fragments at m/z 194, corresponding to ferulic acid itself ($C_{10}H_{10}O_4$).

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