Simple and Hydrolyzable Compounds in Virgin Olive Oil. 3. Spectroscopic Characterizations of the Secoiridoid Derivatives

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Phenolic compounds are important in relation to nutritional and sensory characteristics and the shelflife of virgin olive oil. Four new phenolic compounds have been separated by HPLC. The compounds are two (3,4-dihydroxyphenyl)ethanol derivatives and two (*p*-hydroxyphenyl)ethanol derivatives. This paper reports the NMR, IR, and UV characterization of three of these phenolic compounds. The compounds identified are an isomer of oleuropeine aglycon, the dialdehydic form of elenolic acid linked to (3,4-dihydroxyphenyl)ethanol, and the dialdehydic form of elenolic acid linked to (*p*-hydroxyphenyl)-ethanol.

INTRODUCTION

The pharmacological interest in olive phenolic compounds is well-known (Panizzi et al., 1960). The bioactivity of virgin olive oil phenolic compounds, particularly in relation to autoxidative stability (shelf life) and sensorial characteristics, was studied (Montedoro et al., 1992a; Gutiérrez Rosales et al., 1992).

The HPLC procedure of the quantitative (simple compounds) and semiquantitative (hydrolyzable fraction) evaluation of some phenolic compounds present in virgin olive oil was previously reported (Montedoro et al., 1992a). A preliminary characterization of the unknown phenolic compounds of the hydrolyzable fraction was previously carried out (Montedoro et al., 1992b).

Four new phenolic compounds (Figure 1) have been identified: two (3,4-dihydroxyphenyl)ethanol derivatives (with HPLC RT of 23.50 and 32.50 min) and two (*p*-hydroxyphenyl)ethanol derivatives (with HPLC RT of 27.70 and 28.40 min). These compounds were separated and examined by NMR, IR, and UV spectroscopy. The molecular structures of three of these phenolic compounds have been determined.

EXPERIMENTAL PROCEDURES

Materials. *Virgin Olive Oil.* Olive drupes from the Moraiolo cultivar were crushed with a hammer mill, and the oil was directly extracted using a laboratory hydraulic press (maximum pressure 200 bars).

Reference Compounds. (3,4-Dihydroxyphenyl)ethanol was synthesized in our laboratory according to the procedure of Baraldi et al. (1983). Oleuropeine glycoside was obtained from Extrasynthèse Co. (Genay, France). (*p*-Hydroxyphenyl)ethanol was obtained from Janssen Chemical Co. (Beerse, Belgium). Elenolic acid was obtained by hydrolysis of the pure oleuropeine glycoside following the procedure of Walter et al. (1973).

Phenolic Extraction. The phenolic extract of virgin olive oil was obtained following the procedure described in a previous paper (Montedoro et al., 1992a).

Methods of Analysis. *Preparative HPLC Separation of Unknown Phenolic Compounds and Elenolic Acid in Phenolic Extract.* The HPLC system used was composed of a Varian liquid chromatograph Model 5000 equipped with a 9.4 mm i.d. × 500 mm Whatman Partisil 10 ODS-2 semipreparative column, coupled with a Varian Polychrom 9060 diode array detector, working in the UV region of the electromagnetic spectrum; furthermore, a sample loop of 200-μL capacity was used.

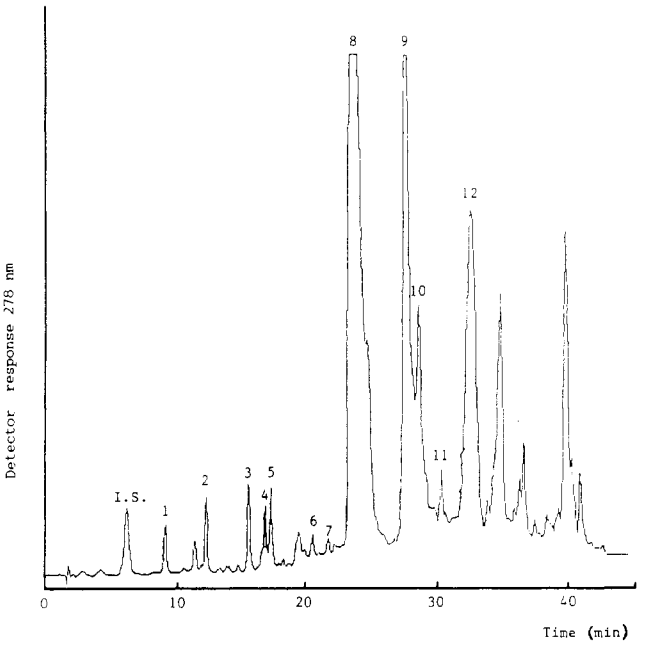

Figure 1. Fractionation of the phenolic extract of virgin olive oil by HPLC at 278 nm. Peak numbers: (I.S.) gallic acid; (1) (3,4-dihydroxyphenyl)ethanol; (2) (*p*-hydroxyphenyl)ethanol; (3) vanillic acid; (4) caffeic acid; (5) syringic acid; (6) *p*-coumaric acid; (7) ferulic acid; (8) RT 23.50; (9) RT 27.70; (10) RT 28.40; (11) cinnamic acid; (12) RT 32.50.

The peaks corresponding to the four unknown phenolic substances under investigation and elenolic acid were recovered using a Gilson Model 201 fraction collector.

The mobile phase utilized in all peak separations was 0.2% acetic acid in water (pH 3.1) (A)/methanol (B) at a flow rate of 6.5 mL/min.

For the unknown phenolic substances the total running time of the analysis was 150 min and the gradient changed in the following manner so as to obtain the maximum separation of the peaks of interest: 95% A/5% B at time 0 min; 74% A/26% B in 2.5 min; 64% A/36% B in 4.5 min, and this percentage was maintained for 33 min; 61% A/39% B in 35 min; 0% A/100% B in 35 min, and this percentage was maintained for 20 min; 95% A/5% B in 20 min.

Each time, a quantity of phenolic extract dissolved in methanol corresponding to about 7 mg of the total phenols and expressed

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Table I. ^{13}C NMR Data (Parts per Million) of Reference and New Phenolic Compounds

	compd										
carbon	1[a]	2[a]	3[b]	3a[a]	4[b]	5[b]	5a[a]	6[b]	6a[a]	7[b]	7a[a]
1			199.4	97.1	95.3	195.7	197.2	195.1	197.1	200.2	99.0
3			156.8	157.7	155.1	200.9	98.2	200.4	98.2	155.8	156.2
4			106.2	108.9	109.4	46.3	40.1	46.2	40.1	106.5	108.0
5			27.9	30.1	31.8	27.3	31.2	27.3	31.3	27.0	28.5
6			38.6	40.1	41.2	37.1	38.5	36.9	38.5	37.2	39.8
7			171.2	177.0	173.2	172.1	174.1	171.9	174.1	171.9	174.0
8			69.6	47.6	124.9	155.0	155.5	155.4	155.4	70.8	47.7
9			50.9	72.2	130.5	143.3	145.0	143.4	145.2	51.3	73.2
10			17.8	18.9	13.5	15.3	15.2	15.2	15.3	19.3	20.3
COOMe			167.2	169.5	168.7					167.6	169.5
COOMe			51.5	51.6	51.9					54.3	51.6
OMe				49.4			49.9		49.7		49.3
1′	64.5	64.4			66.8	65.3	66.4	65.2	66.4	65.3	66.5
2′	39.3	39.4			35.3	34.3	35.3	34.2	35.2	34.3	35.4
3′	131.0	131.8			130.7	130.4	130.7	129.4	130.0	130.2	130.8
4′	116.1	116.3			116.4	115.3	116.4	115.4	116.3	115.3	116.4
5′	130.8	144.4			144.9	142.9	144.8	130.0	130.9	143.0	144.9
6′	156.6	145.9			146.2	143.7	146.1	154.7	157.1	143.8	146.2
7′	130.8	117.1			117.0	116.2	117.0	130.0	130.9	116.1	117.0
8′	116.1	121.3			121.3	121.2	121.2	115.4	116.3	121.1	121.2

[a] In methanol-d_4. [b] In chloroform-d.

as gallic acid equivalent (GAE) as determined by the Folin–Ciocalteu reaction (Montedoro et al., 1992a) was injected in the column, and the eluate was detected at 278 nm. It was necessary to use this amount of total phenols, which is much less than the usual loading capacity of the column, to avoid saturation of the detector. The substances were collected by utilizing a fraction collector with peak detection plus subfractionation within a time windows mode program.

The whole process was repeated many times until we reached a quantity of about 50 mg for each of the four substances.

For the separation of elenolic acid was used a total running time of the analysis of 70 min with the gradient that changed as indicated: 95% A/5% B at time 0 min, and this percentage was maintained for 2 min; 75% A/25% B in 8 min; 60% A/40% B in 10 min, and this percentage was maintained for 7 min; 50% A/50% B in 8 min; 0% A/100% B in 10 min, and this percentage was maintained for 15 min; 95% A/5% B in 10 min.

Repeated injections in the column of an ethyl acetate extract of hydrolyzed oleuropeine were performed, and the eluate was detected at 239 nm.

The collected fractions containing the recovered substances were pooled and concentrated with a Rotovapor under the vacuum of a water pump and under a stream of nitrogen at 25 °C to remove methanol and most of the water; lastly, the aqueous residue was frozen and then freeze-dried.

NMR, IR, and UV Analyses. The NMR spectra were recorded on Bruker AC 200 and Bruker SY WP 80 spectrometers using TMS as internal standard in methanol-d_4 and chloroform-d. For each compound and in each solvent the ^1H, $^{13}C\{^1H\}$ NMR spectra and two multiple-pulse DEPT experiments were performed (Søresen and Ernst, 1983; Sanders and Hunter, 1987). Moreover, homonuclear and heteronuclear selective spin decoupling experiments were used in both solvents to facilitate the assignments of the resonances. In some cases the 2D COSY-45 spectrum was also recorded (Bax and Freeman, 1981). The ^{13}C and ^1H NMR data are reported in Tables I and II, respectively.

The structures of the standards and the compounds are shown in Figures 2 and 3, respectively: the numbering is based on their possible biogenetic origin from oleuropeine aglycon (4).

In Figure 4 the $^{13}C\{^1H\}$ and the two DEPT spectra of compound **5a** in methanol-d_4 are reported.

The 2D COSY-45 spectra of compound **5a** in methanol-d_4 and compound **5** in chloroform-d are reported in Figures 5 and 6, respectively.

The IR spectra, utilized to confirm the assigned structures both in methanol and in chloroform, were recorded on a Perkin-Elmer spectrophotometer Model 1725 X FTIR.

The UV spectra were recorded on a Perkin-Elmer spectrophotometer Model Lambda 16. The wavelengths of maxima and minima of the absorbing bands of the new phenolic compounds are reported in Table III.

RESULTS AND DISCUSSION

Reference compounds were chosen according to the preliminary results obtained in a previous work (Montedoro et al., 1992b).

NMR Characterization of the Reference Compounds. The results of the ^{13}C and ^1H NMR data of (*p*-hydroxyphenyl)ethanol (**1**) and (3,4-dihydroxyphenyl)-ethanol (**2**) are reported in Tables I and II.

(*a*) *Elenolic Acid* (*3*). The ^1H NMR data in chloroform-d are in agreement with the literature data (MacKellar et al., 1973; Gariboldi et al., 1986) and are consistent with structure **3**. The $^{13}C\{^1H\}$ NMR spectrum and the DEPT spectra, to our knowledge never previously recorded, are in agreement with structure **3**, too (see Table I).

On the other hand, both ^1H and ^{13}C spectra in methanol-d_4 show the presence of an equilibrium between structure **3** (30%) and the hemiacetalic form **3a** (70%) deriving from a nucleophilic addition of methanol-d_4 to the aldehydic group. This is well shown from the decrease of the ^1H resonance at 9.61 ppm and the ^{13}C resonance at 199.4 ppm due to aldehydic group 1 and the corresponding increase of the hemiacetalic ^1H resonances at 4.30 ppm and the ^{13}C resonance at 97.1 ppm.

Moreover, adding methanol, evaporating without oil pump, and dissolving again in methanol-d_4, it was possible to see the resonances relative to the OCH_3 group (OCH_3, 3.20 ppm; OCH_3, 49.4 ppm). Due to the equilibrium between the aldehydic and hemiacetalic species, the ^1H NMR spectrum in methanol-d_4 is very complicated, and in some cases, it was not possible to measure exactly all of the coupling constants.

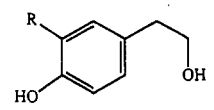
(*b*) *Oleuropeine Glycoside* (**4**). The NMR characterization is in agreement with that reported in the literature (Gariboldi et al., 1986). Only the assignment of the resonances of carbons 4 and 3′ is inverted. Direct comparison with the ^{13}C data of (3,4-dihydroxyphenyl)-ethanol (**2**) (see Table I) suggests that our assignment is very probably right.

NMR Characterization of the Unknown Phenolic Compounds. Peak numbers and retention times (RT) of the unknown phenolic compounds are those quoted in the HPLC chromatogram reported in Figure 1.

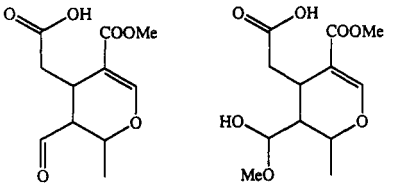
Table II. ^{1}H NMR Data of Reference and New Phenolic Compounds[a]

	compd									
proton	1[b]	2[b]	3[c]	4[c]	5[c]	5a[b]	6[c]	6a[b]	7[c]	7a[b]
1			9.61 s	5.86 s	9.19 d ($^4J_{1,5}$ = 1.9)	9.14 d ($^4J_{1,5}$ = 1.9)	9.24 d ($^4J_{1,5}$ = 1.9)	9.15 d ($^4J_{1,5}$ = 2.0)	9.48 s	4.25 m
3			7.65 s	7.46 s	9.62 s	4.22 m	9.64 s	4.20 m	7.57 s	7.49 s
4					2.79 m ($^2J_{4a,4b}$ = 15.7)	2.00 m	2.78 m ($^2J_{4a,4b}$ = 15.3)	2.00 m		
5			3.36 m ($^3J_{5,6b}$ = 10.4; $^3J_{5,6a}$ = 3.4)	3.86 dd ($^3J_{5,6b}$ = 9.1; $^3J_{5,6a}$ = 4.5)	3.62 m ($^3J_{5,6b}$ = 6.8; $^3J_{5,6a}$ = 6.6)	3.24 m ($^3J_{5,6b}$ = 5.9; $^3J_{5,6a}$ = 4.0)	3.65 m ($^3J_{5,6a}$ = 6.0; $^3J_{5,6b}$ = 5.0)	3.14 m ($^3J_{5,6a}$ = 6.3; $^3J_{5,6b}$ = 5.5)	3.38 m ($^3J_{5,6a}$ = 8.6; $^3J_{5,6a}$ = 3.9; $^3J_{5,9}$ = 5.5)	3.18 m
6a			2.94 d ($^2J_{6a,6b}$ = 15.9)	2.65 dd ($^2J_{6a,6b}$ = 14.1)	2.60 dd ($^2J_{6a,6b}$ = 15.7)	2.62 dd ($^2J_{6a,6b}$ = 15.0)	2.65 m ($^2J_{6a,6b}$ = 18.2)	2.64 dd ($^2J_{6a,6b}$ = 15.0)	2.86 dd ($^2J_{6a,6b}$ = 15.6)	3.20 m
6b			2.28 dd	2.38 dd	2.90 dd	2.46 dd	2.90 m	2.46 dd	2.54 dd	2.50 m
8			4.22 q ($^3J_{8,10}$ = 6.7)	6.03 qd ($^3J_{8,10}$ = 7.1; $^4J_{8,1}$ = 0.85)	6.61 q ($^3J_{8,10}$ = 7.1)	6.61 q ($^3J_{8,10}$ = 6.2)	6.64 q ($^3J_{8,10}$ = 7.1)	6.61 q ($^3J_{8,10}$ = 7.0)	4.41 qd ($^3J_{8,10}$ = 6.6; $^3J_{8,9}$ = 5.6)	4.51 m ($^3J_{8,10}$ = 6.8)
9			2.68 s						2.57 m	
10			1.57 d	1.61 dd ($^5J_{10,1}$ = 1.3)	2.04 d	1.89 d	2.08 d	1.89 d	1.38 d	1.25 d
COOMe			3.74 s	3.66 s						3.60 s
OMe						3.29 s		3.29 s	3.73 s	3.29 s
1′	3.61 t ($^3J_{1',2'}$ = 7.2)	3.33 t ($^3J_{1',2'}$ = 7.2)		4.06 m	4.19 m	4.04 m	4.19 m	3.97 m ($^2J_{1a,1b}$ = 10.8)	4.27 t	4.13 m
2′	2.63 t	2.33 t		2.71 t ($^3J_{2',1'}$ = 6.9)	2.77 t ($^3J_{2',1'}$ = 6.8)	2.65 t ($^3J_{2',1'}$ = 7.0)	2.78 t ($^3J_{2',1'}$ = 7.0)	2.71 t ($^3J_{2',1'}$ = 6.8)	2.80 m ($^3J_{2',1'}$ = 7.1)	2.71 t ($^3J_{2',1'}$ = 7.1)
4′	6.64 d ($^3J_{4',5'}$ = 8.2)	6.34 d ($^4J_{4',8'}$ = 2.0)		6.62 d ($^4J_{4',8'}$ = 2.0)	6.65 d ($^4J_{4',8'}$ = 2.1)	6.57 d ($^4J_{4',8'}$ = 2.0)	7.04 d ($^3J_{4',5'}$ = 8.2)	6.95 d ($^3J_{4',5'}$ = 8.5)	6.78 d ($^4J_{4',8'}$ = 2.0)	6.80 d ($^4J_{4',8'}$ = 2.0)
7′	6.19 d ($^3J_{7',8'}$ = 8.2)	6.36 d ($^3J_{7',8'}$ = 6.9)		6.65 d ($^3J_{7',8'}$ = 8.0)	6.70 d ($^3J_{7',8'}$ = 7.9)	6.63 d ($^3J_{7',8'}$ = 8.0)	6.64 d ($^3J_{7',8'}$ = 8.2)	6.64 d ($^3J_{7',8'}$ = 8.5)	6.74 d ($^3J_{7',8'}$ = 8.0)	6.77 d ($^3J_{7',8'}$ = 8.0)
8′	6.19 d	6.19 dd		6.50 dd	6.60 dd	6.45 dd			6.61 dd	6.63 dd

[a] The values of the chemical shifts and coupling constants are in ppm and Hz, respectively. [b] In methanol-d_4. [c] In chloroform-d.



1 (R=H) and 2 (R=OH)



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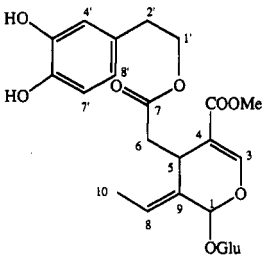

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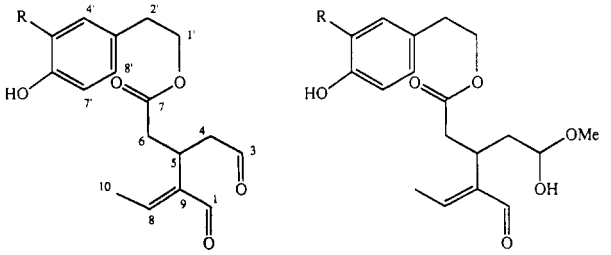
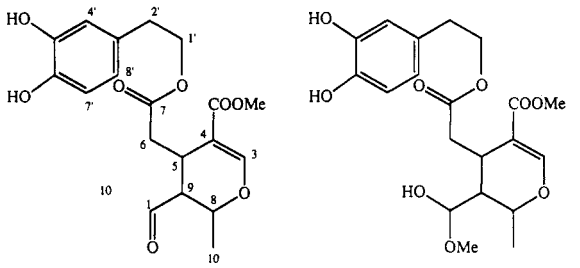
Figure 2. Chemical structures of reference compounds.



5 (R=OH) and 6 (R=H) 5a (R=OH) and 6a (R=H)



7 7a

Figure 3. Chemical structures of new phenolic compounds.

Compound **5** *(Peak 8, RT of 23.50 min).* Compound **5** in chloroform-*d* shows that the resonances of the carbons 1′–8′ relative to the compound **2** are present. The fragment 7–6–5 similar to that of **4** is also observed. On the other hand, there are some differences from **4**: (i) the COOMe group is absent, and two resonances in the aldehydic range are present in the $^{13}C\{^1H\}$ NMR spectrum (195.7 and 200.9 ppm) and in the ^1H spectrum (9.16 and 9.64 ppm), typical, respectively, of α,β-unsaturated and saturated aldehydes; (ii) the ^{13}C data indicate that only two olefinic carbons are present at 155.0 (CH) and 143.3 ppm (quaternary), while a new methylene group at 46.3 ppm is present.

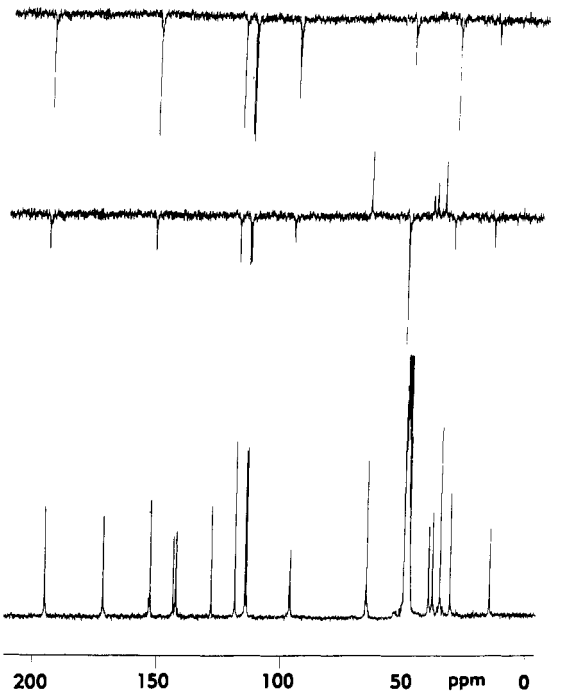


Figure 4. $^{13}C\{^1H\}$ and the two DEPT spectra of compound **5a** in methanol-d_4.

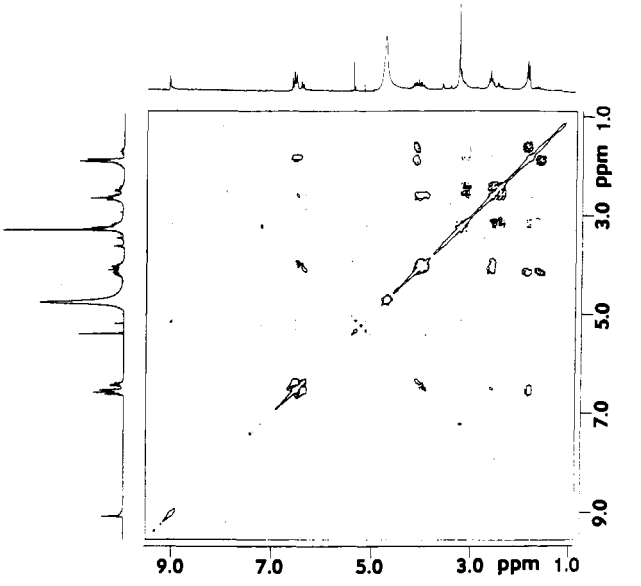


Figure 5. 2D COSY-45 spectrum of compound **5a** in methanol-d_4.

The ^1H NMR spectrum also shows only an olefinic proton that appears like a quartet and, consequently, has to be attributed to a proton that couples to a methyl group: such a proton is that in position 8. This methyl group is downshifted from 6.03 to 6.61 ppm with respect to oleuropeine.

Returning to the ^{13}C NMR spectrum, the two olefinic resonances are relative to carbons 8 and 9, respectively. However, the coincidence in the values of chemical shifts of carbon 8 in **5** and carbon 3 in **4** persuaded us to carry out a heteronuclear selective decoupling experiment. With irradiation of the proton at 6.61 ppm the ^{13}C spectrum shows, besides the quaternary carbons, decoupled resonances at 155.1 ppm and those due to the aromatic CH because their protons are also decoupled with irradiation at 6.61 ppm. This demonstrates that the two olefinic resonances are due to carbons 8 and 9.

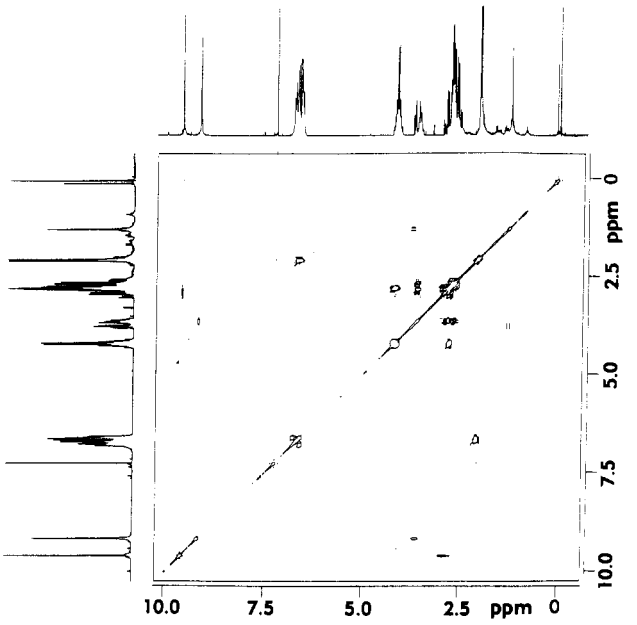


Figure 6. 2D COSY-45 spectrum of compound **5** in chloroform-*d*.

Table III. Wavelengths of the Absorbing Bands in the UV Spectrum of New Phenolic Compounds

compd	solvent	λ_{max} (nm)	λ_{min} (nm)
5	chloroform	281, 287 sh, 320	261
5a	methanol	206, 223, 282, 288 sh, 330	217, 260
6	chloroform	242, 277, 283 sh, 325	265
6a	methanol	225, 277, 284 sh, 330	208, 266
7	chloroform	241, 281, 286 sh, 325	267
7a	methanol	205, 229, 282, 288 sh, 330	216, 266

Another heteronuclear selective decoupling experiment was carried out by irradiating at 4.05 ppm to decouple protons 1′ and 3. The ^{13}C NMR shows two decoupled resonances at 98.2 and 65.3 ppm, confirming the assignment of protons 3 and 1′, respectively. This information is consistent with structure **5** reported in Figure 3.

To confirm structure **5**, homonuclear selective decoupling experiments were performed. Irradiation of proton 10 causes the collapse of quartet 8, and on the contrary when proton 8 is irradiated, the doublet 10 becomes a singlet. Moreover, when 5 is irradiated, mainly 3, 4, and 6 are affected. At the end, alteration of 2′ and 5 is caused by selective irradiation of the multiplet at 4.2 ppm due to 4 and 1′. The downshift of proton 8 can be due to the formation of a hydrogen bond with the oxygen of group 1.

A full confirmation of the assignment derives from the 2D COSY-45 spectrum (Figure 6) that shows cross peaks between 3 and 4, 1 and 5, 8 and 10, 1′ and 2′, 5 and 4 and 6.

The NMR spectra in methanol-d_4 are quite different from those in chloroform-*d*. Both ^1H and ^{13}C NMR data indicate that the alterations involve groups 3 and 4. In fact, the ^{13}C resonance due to saturated aldehydic group 3 at 200.3 ppm disappears and a resonance typical of a carbon directly bonded to two oxygens appears (98.2 ppm).

Also in the ^1H spectrum, the resonance of aldehydic group 3 (9.62 ppm) disappears and a new signal at 4.22 ppm appears. The multiplicity of the latter cannot be well attributed because it is buried under the signal of protons 1′. Moreover, the signals due to 4 go from 2.79 to 2.0 ppm. They were also difficult to assign from the monodimensional spectra because their multiplicity is high and they partially overlap with proton 10. On the other hand, the 2D COSY-45 spectrum, reported in Figure 5, allows us to locate them immediately by their cross peaks.

As in the case of elenolic acid a resonance at 3.2 ppm typical of the OMe group is observed. This information strongly supports the formation of the hemiacetalic structure **5a**.

Homonuclear selective decoupling experiments and the 2D COSY-45 spectrum indicate correlations similar to that of **5**.

It is interesting to note that the resonances observed of at least protons 1, 8, and 10 had an apparent multiplicity higher than that expected from structure **5a**; in fact, they appeared like a triplet, double quartet, and double doublet, respectively.

Both the 2D COSY-45 and the homonuclear selective decoupling experiments emphasized that these further couplings were not real. They were probably due to a slight shift caused by a dynamical process. This was confirmed by recording the ^1H spectrum on an 80-MHz spectrometer, which does not show these splittings owing to the high rate of the dynamical process with respect to the working frequency of the instrument.

The reaction **5**–**5a** is perfectly reversible: on evaporating the solvent from a chloroform solution and by adding methanol, it was possible to observe directly by means of the ^1H NMR the transformation of a mixture of **5** and **5a** to **5a** that occurs in about 20 min.

Compound **6** *(Peak 9, RT of 27.70 min).* All of the measurements carried out for compound **5** were repeated for compound **6**. A comparison of the NMR data for **6** and **5** indicates that the only difference consists of the presence of fragment **1** instead of **2**.

Also, the homonuclear selective decoupling experiments gave the same results as **5**. Compound **6** behaves exactly like **5**, developing in methanol an equilibrium between **6** and **6a**.

Compound **7** *(Peak 12, RT of 32.50 min).* The NMR characterization of compound **7** extracted from the virgin olive oil and the consequent assignment of the structure were immediately obtained. In fact, both ^1H and ^{13}C spectra of **7** almost coincide with the sum of the spectra of **2** and **3** (see Tables I and II). Hence, the structure of **7** was assigned to that reported in Figure 3, a structure previously found by Gariboldi et al. (1986). Our spectroscopic data are totally in agreement with that of Gariboldi et al. (1986) except for the resonances of 4 and 3′, as in oleuropeine.

Compound Corresponding to Peak 10 (RT of 28.40 min). The fourth unknown phenolic compound is still unidentified because the quantity collected was insufficient to interpret the NMR spectra.

IR Characterization. The IR spectra of compounds **3**, **5**, **5a**, **6**, **6a**, **7**, and **7a** completely confirm the assignments obtained from the NMR data.

Compound **5** in chloroform apparently shows only one broad band at 1726 cm^{-1} in the carbonyl range. When the solvent was evaporated and the spectrum was again recorded in methanol where species **5a** is present, two bands at 1736 and 1715 cm^{-1} appeared. They are due to the saturated ester 7 (1736 cm^{-1}) and to the α,β-unsaturated aldehyde 1 (1715 cm^{-1}). Also in **5**, groups 7 and 1 are present, but due to the overlap with the band associated to the stretching of saturated aldehydic carbonyl 3 (1726 cm^{-1}), they cannot be resolved.

The behavior of **6** and **6a** is the same as that of **5** and **5a**.

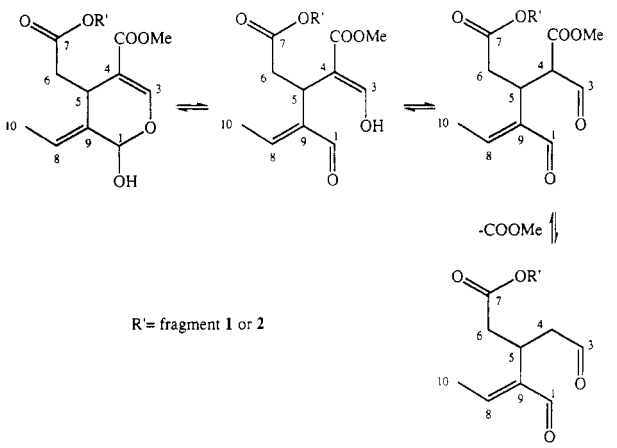


Figure 7. Formation mechanism from oleuropeine aglycon and ligstroside of two new phenolic compounds, **5** and **6**, respectively.

The IR spectrum of **7** in chloroform shows an asymmetric band at 1726 cm^{-1}, assignable to the saturated aldehydic group 1. The other two bands are not resolved but, also in this case, for compound **7a** they are observed at 1734 and 1710 cm^{-1} and are assigned, respectively, to aliphatic ester 7 and to α,β-unsaturated ester COOMe. The same observations were done for elenolic acid (**3**).

The IR spectra of the samples also show the bands typical of the aldehydic C–H stretching (2830–2810 and 2745–2720 cm^{-1}), of the double bonds (1720–1650 cm^{-1}), and of the aromatic ring (1620–1640 cm^{-1}).

UV Characterization. The UV spectra of the new phenolic compounds were recorded both in methanol (in the range 200–400 nm) and in chloroform (in the range 240–400 nm). The UV data of compounds **5**, **5a**, **6**, and **6a**, on the one hand, and of compounds **7** and **7a**, on the other, confirm the presence of the α,β-unsaturated aldehydic group and of the α,β-unsaturated ester group in the structures of these molecules, respectively. In fact, the spectra recorded in methanol show the two typical absorbing bands of these groups near 225 (corresponding to $\pi-\pi^*$ transitions) and 330 nm (corresponding to $n-\pi^*$ transitions), with the latter weak and poorly defined.

The bands at 277 nm for compound **6a** and at 282 nm for compounds **5a** and **7a**, together with their shoulders, are due to hydroxyphenyl and *o*-dihydroxyphenyl groups, respectively. These bands are ascribed mainly to the $n-\pi$ conjugation between electrons from π-orbitals of benzene ring and the nonbonding electrons of the hydroxyl group.

The presence of the band at 205 nm in **5a** and **7a** is due to aromatic $\pi-\pi^*$ transitions.

In chloroform the band near 280 nm is unchanged, while the related shoulder undergoes a little shift. In this solvent bands at 225 and 330 mm are shifted to 242 and 325 nm, respectively.

Formation Mechanism and Occurrence in the Oil. All of the substances analyzed in this paper can be considered as having their origin from oleuropeine (**4**) and ligstroside (whose structure is like **4** but the OH in position 5′ is replaced by a H), compounds isolated from olive fruit (Panizzi et al., 1960; Asaka et al., 1972; Kubo et al., 1984).

For compound **7**, the formation mechanism from oleuropeine was previously hypothesized (Solinas et al., 1975; Gariboldi et al., 1986).

The two new natural samples, **5** and **6**, can form, respectively, from oleuropeine and ligstroside by the opening of the elenolic ring, which involves the formation of an enolic species that successively isomerizes to a dialdehydic structure and is lastly decarboxylated (see Figure 7).

Compounds **5a** and **6a** derive from the reaction of aldehydic group 3 with methanol to give the hemiacetalic species. The nucleophilic addition of methanol does not occur in group 3 because in the carbon of the α,β-unsaturated aldehyde the partial positive charge is less than in the saturated one.

During ripening of the fruit the oleuropeine content decreases. This is probably due to glycosidic activity (β-glucosidase) that is well-known to occur in a variety of fruits (Barz et al., 1985). This same activity has been detected in the ripe olive fruit; nevertheless, oleuropeine aglycon has not been found during some metabolic phases (Amiot, 1986).

When oleuropeine glycoside is transformed to the corresponding aglycon, its lipid solubility is highly increased; thus, it is transferred to the oil droplets (cytoplasmatic inclusions).

Conclusions. Three new natural secoiridoid compounds possessing biological activity (antioxidant, sensorial, oviposition stimulation of *Dacus oleae*) have been isolated from virgin olive oil (*Olea europaea*). The compounds, identified by spectroscopic techniques (NMR, IR, and UV) are (i) an isomer of oleuropeine aglycon, (ii) the dialdehydic form of elenolic acid linked to (3,4-dihydroxyphenyl)ethanol, and (iii) the dialdehydic form of elenolic acid linked to (*p*-hydroxyphenyl)ethanol. Identification of the fourth compound is in progress.

These compounds are derivatives of oleuropeine and ligstroside, and their presence has not been found in the fruit but only in the oil.

The formation mechanism, the transfer process in the oil, and the effect of the agronomic and technological conditions of production on the concentration of these compounds in the oil will be studied.

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