Pinoresinol and 1-Acetoxypinoresinol, Two New Phenolic Compounds Identified in Olive Oil

Manuel Brenes, Francisco J. Hidalgo, Aranzazu García, José J. Rios, Pedro García, Rosario Zamora, and Antonio Garrido*

Food Biotechnology Department, Instituto de la Grasa, CSIC, 41012 Sevilla, Spain

ABSTRACT: Polyphenols of olive oil show autoprotective, sensory, and nutritional-therapeutic effects. Two new phenolic compounds have been isolated from virgin olive oils by preparative high-performance liquid chromatography and their structures established on the basis of their mass spectra and nuclear magnetic resonance spectral data. The compounds identified are the lignans pinoresinol and 1-acetoxypinoresinol. Both have been found in all the commercial virgin olive oils analyzed. Pinoresinol concentration was rather similar in all the oils. In contrast, 1-acetoxypinoresinol concentration was higher in oils of the Arbequina and Empeltre cultivars than in Picual or Picudo cultivars. Pinoresinol and 1-acetoxypinoresinol may represent the major phenolic compounds in some Arbequina and Empeltre oils. Lignans possess biological and pharmacological properties and, therefore, the two new compounds identified in olive oils may contribute to the reported beneficial effects which are attributed to polyphenols on human health of a diet rich in olive oil.

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KEY WORDS: 1-Acetoxypinoresinol, MS, NMR, olive oil, phenols, pinoresinol.

The beneficial effects that a diet rich in olive oil has on human health are well known (1,2). These benefits are mainly due to the elevated oleic acid content and the antioxidant properties of its polyphenols (3,4).

Some of the most representative phenolic compounds in olive oils are the oleuropein and ligstroside aglycons and the dialdehydic form of elenolic acid linked to hydroxytyrosol and tyrosol (5–8), and both hydroxytyrosol and tyrosol have been suggested to play a role in the prevention of oxidative damage in cells (9,10).

In addition, other phenolic compounds recently have been identified in olive oils including flavonoids (11), glucosides of hydroxytyrosol (12) and 4-(acetoxyethyl)-1,2-dihydroxybenzene (13). However, some other peaks in the high-performance liquid chromatography (HPLC) chromatograms of phenolic compounds remain unidentified. In particular, in a recent study carried out in this laboratory (13) two peaks appeared that were located between those of the dialdehydic form of elenolic acid linked to tyrosol and the oleuropein aglycon and that showed large peak areas in some olive oils. Previous studies had suggested that these peaks could correspond to tyrosol derivatives (6,14), however, their structures had not been elucidated.

As a continuation of those studies, the present investigation was undertaken to characterize the molecular structure of these compounds and to quantify them in commercial olive oils obtained from different cultivars.

MATERIALS AND METHODS

Oil. Extra virgin olive oils packed in glass containers were purchased in local markets. These oils corresponded to different regions of Spain and were obtained from Picual, Picudo, Hojiblanca, Arbequina, and Empeltre cultivars. Two different oils from each cultivar were analyzed by using duplicates of each sample. Data are mean values from the duplicates of each oil.

Phenolic extraction. The phenolic extract of virgin olive oils was obtained by following the procedure of Montedoro *et al*. (15). Briefly, 14 g of virgin olive oil was extracted by using 4 × 14 mL of methanol/water (80:20 vol/vol). After removing the methanol, the residue was taken up to 15 mL with acetonitrile. Washings with hexane (3 × 20 mL) were performed, and the resulting acetonitrile solution was evaporated under vacuum, giving a residue that was dissolved in 1 mL of methanol.

HPLC analysis of phenolic compounds. The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600E pump, a Waters column heater module, and a Waters 996 photodiode array detector operated with Millenium 2010 software (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 µm, 25 cm × 4.6 mm i.d., Technokroma, Barcelona, Spain) column was used. Separation was achieved with an elution gradient by using an initial composition of 90% water (pH adjusted to 3.1 with 0.2% acetic acid) and 10% methanol. The concentration of the latter solvent was increased to 30% in 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% in 10 min, maintained for 5 min, increased to 50% in 5 min, and maintained for another 5 min. Finally, the methanol percentage was increased to 60, 70, and 100% in 5-min periods. Initial conditions were reached in 15 min. A flow of 1 mL/min and a temperature of

*To whom correspondence should be addressed at Food Biotechnology Department, Instituto de la Grasa, CSIC, Avda. Padre García Tejero 4, 41012 Sevilla, Spain. E-mail: garfer@cica.es

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35°C were used in all the experiments. Quantification of phenolic compounds was made by using the reference compounds obtained from commercial suppliers or preparative HPLC as described elsewhere (13).

Preparative HPLC purification. The HPLC system was composed of a Waters 600E pump, a Rheodyne 7125 injector with a 1 mL loop, and a Waters 994 photodiode array detector. A phenolic extract, obtained as reported above from Arbequina olive oils, was used. A mixture of different peaks corresponding to retention times between 42 and 46 min was obtained by using a Spherisorb ODS-2 (5 µm, 25 cm × 20 mm i.d., Waters, Inc.) column and a flow of 16 mL/min. The mobile phases were water (A) and methanol (B), and 0.6 mL of the phenolic extract was injected into the chromatograph. Separation was achieved with an elution gradient by using an initial concentration of 90% A, then decreased linearly to 60% in 20 min, with a final decrease to 50% over 15 min. This final concentration was maintained for 5 min, and then a 0% concentration of A was over 10 min to clean the column. The initial conditions were achieved in 5 min and then equilibrated for other 10 min before a new injection. The fractions containing the recovered substances were pooled and evaporated under vacuum, and the residue was dissolved in the same amount of methanol as the original phenolic extract injected into the chromatograph. Subsequently, the methanol extract from which peaks had been isolated was again injected into the chromatograph but using a Spherisorb ODS 2 (5 µm, 25 cm × 10 mm i.d.; Technokroma) column and a 4 mL/min flow under isocratic conditions. The mobile phase was water/methanol (55:45, vol/vol). The new collected fractions were pooled, the methanol was eliminated using vacuum conditions, and the aqueous residue was frozen and freeze-dried (model Alpha 1-4; Martin Christ, Osterode am Harz, Germany).

Characterization of isolated compounds. Pure compounds isolated by preparative HPLC were characterized by high-resolution nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). ^{1}H and ^{13}C NMR spectra were acquired at 300 and 75.4 MHz, respectively, on a Bruker AC-300P spectrometer (Karlsruhe, Germany) using $(CD_3)_2SO$ as solvent and tetramethylsilane as internal standard. The spectrum of the more complex of the two compounds isolated was also acquired at 500 MHz by using a Bruker AMX-500. Both compounds were studied by two-dimensional NMR to assist in their structural determination. Mass spectra were obtained in a Finnigan MAT95s (Bremen, Germany) at 70 eV. The accelerating voltage was 4 kV, and the trap current was 100 µA. Two different derivatives were employed in the MS analysis. They were the trimethylsilyl derivatives obtained by reaction with bis(trimethylsilyl)trifluoroacetamide and the products of the hydrogenation with PtO_2 (16).

RESULTS AND DISCUSSION

The HPLC chromatograms of the phenolic fraction of three virgin olive oils are presented in Figure 1. Peaks were identified according to a previous paper (13), and unidentified peaks numbered 10 (retention time 45.1 min) and 11 (retention time 45.8 min) were studied in more detail.

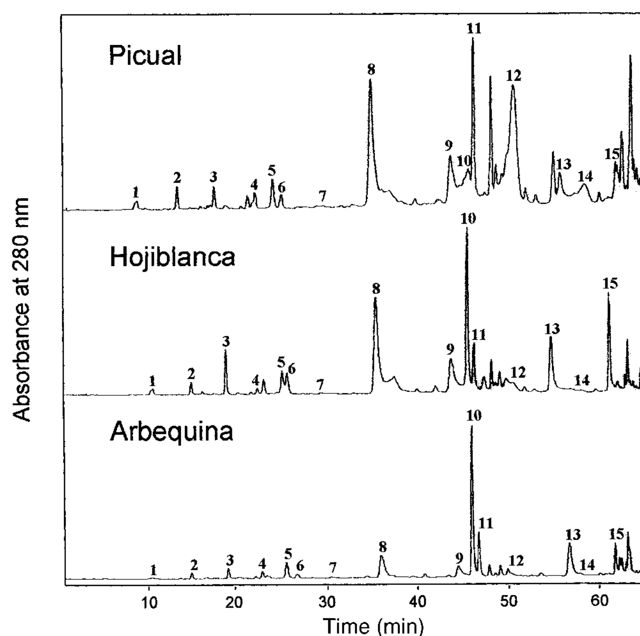


FIG. 1. High-performance liquid chromatograms of the phenolic fraction of virgin olive oils at 280 nm. Peaks corresponded to (1) hydroxytyrosol, (2) tyrosol, (3) vanillic acid, (4) vanillin, (5) 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC), (6) *p*-coumaric acid, (7) ferulic acid, (8) dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), (9) dialdehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EDA), (10) 1-acetoxypinoresinol (identified in this work), (11) pinoresinol (identified in this work), (12) oleuropein aglycon (3,4-DHPEA-EA), (13) luteolin, (14) ligstroside aglycon (*p*-HPEA-EA), (15) apigenin.

The isolated compounds corresponding to peaks 10 and 11 reacted with the Folin-Ciocalteu reagents, were not easily oxidized under air, and were more soluble in organic solvents than in water. It has been tentatively suggested that these peaks could correspond to tyrosol derivatives (6,14,17). However, this possibility was rejected since no signals for tyrosol were found in the NMR spectra.

Peak 10 could be isolated in a higher yield and its characterization was first undertaken. The ^{1}H and ^{13}C NMR and MS data of this compound are shown in Tables 1 and 2, respectively. It had a molecular weight of 416, and the presence of two hydroxyl groups in the molecule could be easily determined by MS after silylation. The reduction with PtO_2 was not easy, but with time the introduction of three molecules of hydrogen was observed. In addition, this reduced compound maintained the presence of the two hydroxyl groups as could be determined by MS. The ^{1}H NMR spectrum also seemed to exhibit signals corresponding to hydroxyl groups, which might be hidden under the water signal that contained the solvent. However, the presence of the three carbon-carbon double bonds was not clear either in the ^{1}H or in the ^{13}C spectra, and these MS data interfered with the structural determina-

TABLE 1
^{1}H and ^{13}C NMR Data of Compound 10 Obtained in $(CD_3)_2SO$

^{1}H NMR		^{13}C NMR		
Assignment	NMR data	Assignment	Chemical shift	Chemical shift[a]
H2	4.89 *s*	C1	96.9	96.9
$H4_e$	4.32 *dd* (*J* = 7.9; *J* = 9.2)	C2	86.3	86.3
$H4_a$	3.64 *dd* (*J* = 4.9; *J* = 9.2)	C4	69.2	69.3
H5	3.27 *dt* (*J* = 4.9; *J* = 7.9)	C5	58.2	58.2
H6	4.97 *d* (*J* = 4.9)	C6	84.6	84.6
$H8_e$	4.20 *d* (*J* = 10.5)	C8	73.9	73.9
$H8_a$	4.12 *d* (*J* = 10.5)	C1′	127.5	127.6
H2′	6.85 *d* (*J* = 1.8)	C2′	112.9	113.0
H5′	6.69 *d* (*J* = 8.1)	C3′	146.9	146.9
H6′	6.74 *dd* (*J* = 1.8; *J* = 8.1)	C2′	146.1[b]	146.4
H2″	6.85 *d* (*J* = 1.8)	C5′	114.8	114.8
H5″	6.75 *d* (*J* = 8.1)	C6′	121.3	121.3
H6″	6.80 *dd* (*J* = 1.8; *J* = 8.1)	C1″	131.2	131.2
OCH_3	3.73 *s*	C2″	110.7	110.7
OCH_3	3.76 *s*	C3″	147.5	147.6
CH_3CO	1.64 *s*	C4″	146.3[b]	146.4
		C5″	115.2	115.3
		C6″	118.9	118.9
		OCH_3	55.6	55.7
		OCH_3	55.7	55.7
		$\underline{C}H_3CO$	20.6	20.6
		$CH_3\underline{C}O$	168.7	168.7

[a]Data from Tsukamoto *et al.* (19).
[b]These assignments are only tentative. NMR, nuclear magnetic resonance.

tion. On the other hand, the ^{1}H and ^{13}C NMR spectra exhibited some signals that were clearly assigned. Thus, the presence of an acetoxy group could be easily detected by the presence of a singlet at δ 1.64 in the ^{1}H NMR spectrum and the corresponding signals at δ 20.5 and 168.7 in the ^{13}C NMR spectrum. In addition, the presence of two methoxy groups was also easily detected by the signals at δ 3.73 and 3.76 in the ^{1}H NMR spectrum and the corresponding signals at δ 55.6 and 55.7 in the ^{13}C NMR spectrum. Finally, the existence of signals corresponding to aromatic rings was also detected. In fact, the ^{1}H NMR spectrum exhibited the presence of six aromatic protons at δ 6.6–6.9. This implied the presence of at least two aromatic rings, data which correlated with the presence of 12 aromatic carbon signals (six CH and six C carbons) at δ 110–150 ppm in the ^{13}C NMR spectrum. Both aromatic rings were trisubstituted at positions 1,2,4 according to the coupling constants of the ^{1}H NMR spectrum (18), and both were substituted similarly. Because of the absence of sig-

TABLE 2
Mass Spectral Data of Compounds 10 and 11[a]

Compound 10		Compound 11	
m/z (intensity)	Assignment	*m/z* (intensity)	Assignment
417 (26)	$[M + H]^+$	358 (66)	$[M^+]$
416 (77)	$[M^+]$	327 (6)	$[M - OCH_3]^+$
358 (5)	$[M - CH_3COO + H]^+$	205 (17)	$[M - ArCHO - H]^+$
221 (24)		163 (33)	$[ArCHCH{=}CH_2]^+$
207 (68)		152 (34)	$[ArCHO]^+$
205 (67)	$[M - ArCHO - CH_3COOH]^+$	151 (100)	$[ArCO]^+$
204 (100)	$[M - ArCHO - CH_3COO]^+$	137 (49)	$[ArCH_2]^+$
189 (29)		124 (13)	$[ArH]^+$
173 (20)			
163 (36)	$[ArCHCH{=}CH_2]^+$		
152 (55)	$[ArCHO]^+$		
151 (80)	$[ArCO]^+$		
137 (73)	$[ArCH_2]^+$		
131 (77)			
103 (25)			

[a]Compound 10, 1-acetoxypinoresinol; compound 11, pinoresinol. For structures see Scheme 1.

nals corresponding to other substituents, the presence of one hydroxy group and one methoxy group in each one of the aromatic rings was postulated, and this hypothesis was supported by the good correlation obtained between the experimental and the calculated chemical shifts.

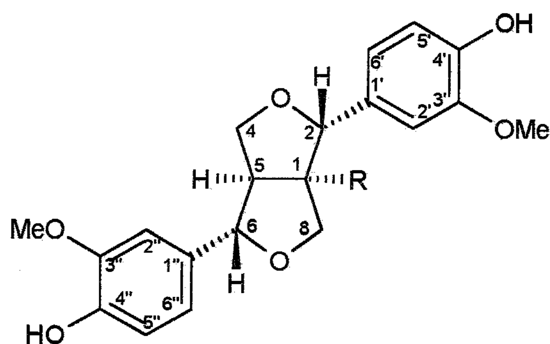
According to all these assignments, only the core of the molecule remained to be determined. This part contained the six carbon signals not previously assigned in the ^{13}C NMR spectrum, and the seven protons not assigned in the ^{1}H NMR spectrum. In addition, it should also contain two oxygen atoms to obtain the molecular weight determined by MS. According to the ^{1}H–^{13}C correlation spectra, one of the carbons was quaternary, three had one proton attached, and the other two had two protons attached. According to these results and the coupling constants observed in the ^{1}H NMR spectrum, the structure proposed for the central core of the molecule was a 3,7-dioxabicyclo[3.3.0]octane, and the final structure for peak 10 was 1-acetoxy-2,6-bis(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane. This structure does not readily explain the hydrogenation undergone by the molecule and detected by MS, but it is likely to be a consequence of the bicyclo opening. The proposed structure is shown in Scheme 1. This compound, better known as 1-acetoxypinoresinol, previously had been found among the lignans obtained from the bark of *Olea* plants (19), but this is the first time that it has been found in olive oils. Table 1 shows the ^{13}C NMR chemical shifts described by Tsukamoto *et al.* (19) for 1-acetoxypinoresinol. They are almost identical to the chemical shifts found in the present study, confirming the identity of the characterized compound found in peak 10.

MS and NMR spectral data of the compound from peak 11 are shown in Tables 2 and 3, respectively, and they were similar to the data for the compound from peak 10. However, compound 10 exhibited much simpler ^{1}H and ^{13}C NMR spectra. In addition, the molecular weight found by MS was 358, suggesting that the molecule had more carbon atoms than the signal observed in the ^{13}C NMR spectrum. These results implied a symmetric molecule that, according to the absence of the acetoxy group in the spectra, was identified as pinoresinol; its structure is shown in Scheme 1. This compound previously had been found in the resin of *Pinus* spp. and of *Picea jezoensis*, the splint wood of *Tsuga heterophylla* (all Pinaceae), the wood of *Araucaria angustifolia* (Araucariaceae), the bark of *Fraxinus mandshurica* var. Japonica (Oleaceae), and *Wikstroemia* spp. (Thymelaeaceae) (20), but it is the first time that it has been found in olive oils. Table 3 shows the ^{13}C NMR chemical shifts described by Lin-gen *et al.* (21) for pinoresinol. For contrast to the data shown above for 1-acetoxypinoresinol, chemical shifts obtained in the present study and the data described previously showed some slight differences, but they are likely a consequence of the different solvents employed for obtaining the spectra. In addition, MS data obtained in the present study were analogous to the pinoresinol described previously, confirming the identity of compound 11 (22).

The ultraviolet spectra of 1-acetoxypinoresinol and pinoresinol are shown in Figure 2. As described previously (19,21), the spectra exhibited two shoulders around 230 and 280 nm. The latter one is a consequence of the hydroxyphenyl groups. The shapes of the absorbance spectra of pinoresinol and 1-acetoxypinoresinol are similar to those of hydroxytyrosol and tyrosol, respectively, the shoulder at 230 nm being higher for 1-acetoxypinoresinol and tyrosol than for the other two.

Pinoresinol and its derivatives are lignans that are widely distributed in the plant kingdom and chemically related to the polymeric lignins of the plant cell wall. It is assumed that pinoresinol is formed biosynthetically by stereospecific reductive coupling of two molecules of coniferyl alcohol (22). Lignans are of considerable pharmacological interest because they are known to have antitumor, antimitotic, and antiviral activities and to specifically inhibit certain enzymes (23–25). Pinoresinol glucosides have also been proposed as hypotensives (26) and antioxidants for lipids in food and living organisms (27).

Pinoresinol and derivatives have been isolated in roots, leaf, flowers, and particularly bark of the trees. They have



SCHEME 1

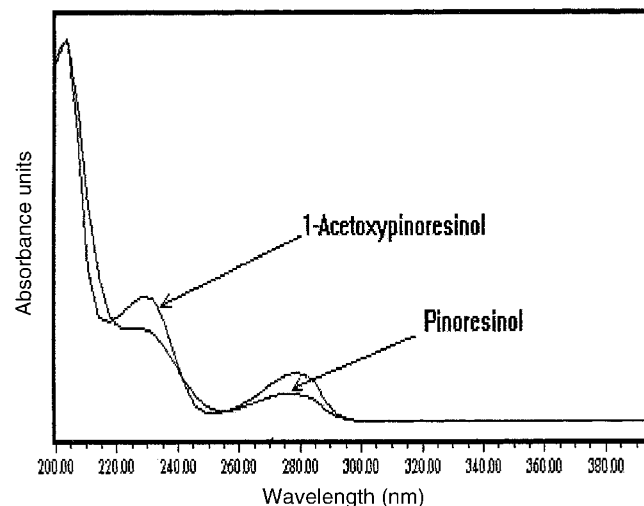


FIG. 2. Ultraviolet spectra of 1-acetoxypinoresinol and pinoresinol obtained in 0.2% acetic acid in water/methanol during the high-performance liquid chromatographic analysis.

TABLE 3
^{1}H and ^{13}C NMR Data of Compound 11 Obtained in $(CD_3)_2SO$

^{1}H NMR		^{13}C NMR		
Assignment	NMR data	Assignment	Chemical shift	Chemical shift[a]
H1/H5	3.03 *m*	C1/C5	53.5	54.2
H2/H6	4.06 *d* (*J* = 4)	C2/C6	85.0	85.9
$H4_e/H8_e$	4.11 *m*	C4/C8	70.8	71.7
$H4_a/H8_a$	3.74 *m*	C1′/C1″	132.1	133.0
Aromatic (6H)	6.73 *m*	C2′/C2″	110.3	108.8
OCH_3	3.76 *s*	C3′/C3″	147.4	146.7
OCH_3	3.75 *s*	C4′/C4″	145.8	145.3
		C5′/C5″	115.0	114.3
		C6′/C6″	118.5	118.9
		OCH_3	55.5	56.0
		OCH_3	55.9	56.0

[a]Data obtained from Lin-gen *et al.* (21) in $CDCl_3$. For abbreviation see Table 1.

also been detected in some foods such as sesame seeds (27) and flaxeed meal (28). This is the first time that lignans have been detected in olive oil and, presumably, also in fruits of *Olea europaea*. However, 1-acetoxypinoresinol and its glucosides and 1-hydroxypinoresinol glucosides have been isolated from bark of olive trees (19,29). Moreover, pinoresinol glucoside linked to oleoside 11-methyl ester, which is a secoiridoid as oleuropein, has been isolated from oleaceous plants (30). Thus, the lipid-soluble lignans detected in olive oils could originate from hydrolysis of compounds similar to lignan linked to secoiridoid glucoside.

The concentrations of phenolic compounds in 10 extra virgin olive oils purchased in local markets are given in Table 4. Aglycons and aglycon derivatives of oleuropein and ligstroside were found in higher concentrations in the oils. However, the two new phenolic compounds identified in the present study, 1-acetoxypinoresinol and pinoresinol, were also found in marked amounts in oils of Arbequina and Empeltre cultivars. Pinoresinol concentration was rather similar in all the analyzed oils, but 1-acetoxypinoresinol was higher in oils of Arbequina and Empeltre cultivars than in Picual or Picudo cultivars. The latter compound was also found in great amount in oils of Razzola and Frantoio Italian cultivars (data not shown). Thus, the sum of these two compounds may represent the major phenolic fraction in the olive oils obtained from some cultivars.

It must also be stressed that differences in concentration were found for all the phenolics between oil samples of the same cultivar. This is a consequence of the variables that affect the concentration of phenolic compounds in oils such as maturation of fruits, extraction, and storage conditions.

It has been recently reported that the concentrations of 1-acetoxypinoresinol (peak 10) and pinoresinol (peak 11) in oils slightly diminished and increased, respectively, with maturation of olives (13). Likewise, Montedoro *et al.* (15) indicated that a peak named 10, which must correspond to the lignans identified in this paper, was very stable during oil oxidation as tyrosol and derivatives were. Thus, tyrosol and lignans seem to be among the most stable phenolics during oil storage.

TABLE 4
Phenolic Compounds Concentration (mg/kg) in Extra Virgin Olive Oils of Five Olive Cultivars[a]

	Picual		Picudo		Hojiblanca		Arbequina		Empeltre	
	Oil 1	Oil 2	Oil 1	Oil 2	Oil 1	Oil 2	Oil 1	Oil 2	Oil 1	Oil 2
Hydroxytyrosol	17.8	2.2	7.5	3.5	5.8	8.3	11.4	2.6	1.3	2.9
Tyrosol	10.5	10.5	10.0	9.5	6.0	5.6	8.4	3.5	3.2	1.8
Vanillic acid	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Vanillin	0.6	0.5	0.2	0.5	0.7	0.6	0.3	0.5	0.3	0.2
3,4-DHPEA-AC	12.8	7.4	2.4	2.0	10.1	10.3	7.4	28.4	2.8	14.0
p-Coumaric acid	0.3	0.1	0.1	0.1	0.4	0.1	0.1	0.3	0.1	0.1
3,4-DHPEA-EDA	82.0	26.2	14.0	37.2	132.0	70.0	28.6	107.0	14.3	58.1
p-HPEA-EDA	31.0	32.2	7.3	38.0	27.1	16.6	7.5	18.0	7.0	9.2
1-Acetoxypinoresinol	4.9	2.7	6.8	12.5	3.7	30.5	36.4	66.9	31.5	40.0
Pinoresinol	29.5	36.0	31.2	29.1	30.8	24.4	34.0	41.2	19.0	11.7
3,4-DHPEA-EA	359.7	120.3	40.9	94.2	134.9	72.5	29.0	16.4	15.9	56.6
p-HPEA-EA	48.7	32.2	7.0	22.2	15.5	7.9	4.4	4.1	1.5	6.5

[a]3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon; *p*-HPEA-EA, ligtroside aglycon.

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REFERENCES

1. Willet, W.C., S. Sacks, A. Trichopoulou, G. Drescher, A. Ferro-Luzi, E. Helsing, and D. Trichopoulos, Mediterranean Diet Pyramid a Cultural Model for Healthy Eating, *Am. J. Clin. Nutr. 61*:1402S–1406S (1995).
2. Lipworth, L., M.E. Martinez, J. Angell, C.C. Hsien, and D. Trichopoulos, Olive Oil and Human Cancer, an Assessment of the Evidence, *Prev. Med. 26*:81–190 (1997).
3. Wiseman, S.A., J.N. Mathot, N.J. de Fouw, and L.B. Tijburg, Dietary Non-tocopherol Antioxidants Present in Extra Virgin Olive Oil Increase the Resistance of Low Density Lipoproteins to Oxidation in Rabbits, *Atherosclerosis 120*:15–23 (1996).
4. Visioli, F., and C. Galli, Olive Oil Phenols and Their Potential Effects on Human Health, *J. Agric. Food Chem. 46*:4292–4296 (1998).
5. Montedoro, G.F., M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni, Simple and Hydrolyzable Compounds in Virgin Olive Oil. 3. Spectroscopic Characterization of the Secoiridoid Derivatives, *Ibid. 41*:2228–2234 (1993).
6. Cortesi, N., M. Azzolini, P. Rovellini, and E.I. Fedeli, Minor Polar Components of Virgin Olive Oils: A Hypothetical Structure by LC–MS, *Riv. Ital. Sostanze Grasse 72*:241–251 (1995).
7. Angerosa, F., N. d'Alessandro, F. Corana, and G. Mellerio, Characterization of Phenolic and Secoiridoid Aglycons Present in Virgin Oil by Gas Chromatography–Chemical Ionization Mass Spectrometry, *J. Chromatogr. A 736*:195–203 (1996).
8. Pirisi, F., A. Angioni, P. Cabras, V.L. Garau, M.L. Sanjust di Teulada, M. Kaim dos Santos, and G. Bandino, Phenolic Compounds in Virgin Olive Oils. I. Low-Wavelength Quantitative Determination of Complex Phenols by High-Performance Liquid Chromatography Under Isocratic Elution, *Ibid. 768*:207–213 (1997).
9. Manna, C., P. Galletti, V. Cucciolla, G.F. Montedoro, and V. Zappia, Olive Oil Hydroxytyrosol Protects Human Erythrocytes Against Oxidative Damages, *J. Nutr. Biochem. 10*:159–165 (1999).
10. Giovannini, C., E. Straface, D. Modesti, E. Coni, A. Cantafora, M. De Vincenzi, W. Malorni, and R. Masella, Tyrosol, the Major Olive Oil Biophenol, Protects against Oxidized-LDL-Induced Injury in Caco-2-Cell, *J. Nutr. 129*:1269–1277 (1999).
11. Rovellini, P., N. Cortesi, and E.I. Fedeli, Analysis of Flavonoids from *Olea europaea* by HPLC–UV and HPLC–Electrospray–MS, *Riv. Ital. Sostanze Grasse 74*:273–279 (1997).
12. Bianco, A., R.A. Mazzei, C. Melchioni, G. Romeo, M.L. Scarpati, A. Soriero, and N. Uccella, Microcomponents of Olive Oil-III. Glucosides of 2(3,4-dihydroxyphenyl) Ethanol, *Food Chem. 63*:461–464 (1998).
13. Brenes, M., A. García, P. García, J.J. Rios, and A. Garrido, Phenolic Compounds in Spanish Olive Oils, *J. Agric. Food Chem. 47*:3535–3540 (1999).
14. Montedoro, G.F., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 2. Initial Characterization of the Hydrolyzable Fraction, *Ibid. 40*:1577–1580 (1992).
15. Montedoro, G.F., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 1. Their Extraction, Separation and Quantitative and Semiquantitative Evaluation by HPLC, *Ibid. 40*:1571–1576 (1992).
16. Hidalgo, F.J., R. Zamora, and E. Vioque, Syntheses and Reactions of Methyl (*Z*)-9,10-Epoxy-13-oxo-(*E*)-11-octadecenoate and Methyl (*E*)-9,10-Epoxy-13-oxo-(*E*)-11-octadecenoate, *Chem. Phys. Lipids 60*:225–233 (1992).
17. Servili, M., M. Baldioli, R. Selvaggini, E. Miniati, A. Macchioni, and G.F. Montedoro, High-Performance Liquid Chromatography Evaluation of Phenols in Olive Fruit, Virgin Oil, Vegetation Waters, and Pomace and 1D- and 2D-Nuclear Magnetic Resonance Characterization, *J. Am. Oil Chem. Soc. 76*:873–882 (1999).
18. Pretsch, E., T. Clerc, J. Seibl, and W. Simon, *Tabellen sur Strukturaufklärung Organischer Verbindungen mit Spektroskopischen Metoden,* Springer-Verlag, Berlin, 1976.
19. Tsukamoto, H., S. Hisada, and S. Nishibe, Lignans from Bark of the *Olea* Plants. I, *Chem. Pharm. Bull. 32*:2730–2735 (1984).
20. Harborne, J.B., and H. Baxter, *Phytochemical Dictionary: A Handbook of Bioactive Compounds,* Taylor and Francis, Philadelphia, 1999, pp. 480–490.
21. Lin-gen, Z., O. Seligmann, K. Jurcic, and H. Wagner, Constituents of *Daphne tangutica, Planta Med. 45*:172–176 (1982).
22. Katayama, T., L.B. Davin, and N.G. Lewis, An Extraordinary Accumulation of (−)-Pinoresinol in Cell-Free Extracts of *Forsythia intermedia*: Evidence for Enantiospecific Reduction of (+)-Pinoresinol, *Phytochemistry 31*:3875–3881 (1992).
23. Nikaido, T., T. Ohmoto, T. Kinoshita, U. Sankawa, S. Nishibe, and S. Hisada, Inhibition of Cyclic AMP Phosphodiesterase by Lignans, *Chem. Pharm. Bull. 29*:3586–3592 (1981).
24. MacRae, W.D., and G.H.N. Towers, Biological Activities of Lignans, *Phytochemistry 23*:1207–1220 (1984).
25. Oomah, B.D., and G. Mazza, Flaxseed Products for Disease Prevention, in *Functional Foods,* edited by G. Mazza, Technomic Publishing Company, Inc., Lancaster, 1998, pp. 91–138.
26. Sih, C.J., P.R. Ravikumar, F.-C. Huang, C. Buckner, and H. Whitlock, Isolation and Synthesis of Pinoresinol Diglucoside, a Major Antihypertensive Principle of Tu-Chung (*Eucommia ulmoides,* Oliver), *J. Am. Chem. Soc. 98*:5412–5413 (1976).
27. Kawagishi, S., T. Oosawa, and H. Katsuzaki, Pinoresinol Glucoside, Sesame Seed Extract Containing Glycoside, and Its Use for Preventing Oxidation of Lipids, Jpn. Patent 6116282, 1994, pp. 1–5.
28. Meagher, L.P., G.R. Beecher, V.P. Flanagan, and N.W. Li, Isolation and Characterization of the Lignans, Isolariciresinol and Pinoresinol, in Flaxseed Meal, *J. Agric. Food Chem. 47*:3173–3180 (1999).
29. Tsukamoto, H., S. Hisada, and S. Nishibe, Lignans from Bark of the *Olea* Plants. II, *Chem. Pharm. Bull. 33*:1232–1241 (1985).
30. Tanahashi, T., N. Nagakura, K. Inoue, H. Inouye, and T. Shingu, Sambacolignoside, a New Lignan-Secoiridoid Glucoside from *Jasminum sambac, Chem. Pharm. Bull. 35*:5032–5035 (1987).

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