Pergamon

PII: S0031–9422(97)01066–2

Phytochemistry, Vol. 49, No. 5, pp. 1311–1315, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

TWO GLUTARIC ACID DERIVATIVES FROM OLIVES

MATILDE GIL, ALÍ HAÏDOUR and JUAN L. RAMOS*

Department of Biochemistry and Molecular and Cellular Biology of Plants, Estación Experimental del Zaidín. CSIC, 18008 Granada, Spain

(*Received* 13 *October* 1997)

Key Word Index—*Olea europea*; Oleaceae; semisolid olive oil production cake; 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid; 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid; oleuropein.

Abstract—Two linear compounds were isolated from the ethyl acetate extract of residues resulting from olive oil processing. These compounds were characterized by NMR and identified as 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid and 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid. Spectra of the products resulting from reduction and saponification confirmed the proposed structures. These products are structural components of a more complex molecule, oleuropein, which confers a bitter taste to olives. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The Mediterranean basin is an area of relatively low rainfall,which has a notable influence on the agricultural practices in the area. Traditionally, olive trees (*Olea europea*) have been grown in many Mediterranean countries. The well-known nutritional properties of olives and olive oil have led to the development of many different cultivars in this part of the world.

The natural bitterness of olives is caused by a molecule known as oleuropein (Fig. 1) [1–3]. Generally, bitterness is eliminated before human consumption by treating the fruit with sodium hydroxide (2% w/v). Alkaline hydrolysis of oleuropein yields various products (Fig. 1), among which are oleuropein aglycone, *β*-3,4-dihydroxyphenyl ethyl alcohol, glucose and elenolic acid [1, 4]. Oleuropein aglycone also yields further transformation products upon opening of the nonaromatic elenoic ring. These are dialdehyde derivatives, namely compound A and the diastereoisomers B and C in Fig. 1 [2, 5].

The ethyl acetate extract of solid residues resulting from olive pressing for olive oil extraction is the most interesting fraction for the identification of chemicals present in olive fruit [6–8]. In this extract a number of aromatic compounds substituted with hydroxyl and methoxyl groups and alkyl chains have been identified [9, 10]. In the present study, we have isolated and identified two compounds with the aid of NMR, 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid and 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid, which probably act as backbone elements providing the link between the aromatic part of oleuropein and the sugar.

RESULTS AND DISCUSSION

Semisolid residues resulting from olive pressing for olive oil production were macerated in water and filtered as described in the Experimental. The aqueous phase was acidified and then a series of sequential extractions using solvents of increasing polarity were carried out. The most abundant compounds identified in the hexane extract were triterpenes and fatty acids [11]. The methylene chloride and ethyl ether extracts contained mainly aromatic compounds substituted with aldehyde, methoxyl and hydroxyl groups (data not shown). ^{1}H NMR analysis of the ethyl acetate extract revealed that it contained mainly aromatic compounds substituted with carboxylic acid groups (e.g. syringic, ferulic, veratric, 2,4- and 2,3-dihydroxybenzoic acids and *p*-hydroxyphenyl ethanol). In addition, a series of signals were tentatively assigned to nonaromatic compounds, which have not been described previously in this extract.

To analyze the nonaromatic compounds, the ethyl acetate extract was methylated to decrease the polarity of the components and then chromatographed under pressure on a silica gel column using mixtures of hexane and ethyl acetate as eluent. Several fractions were collected and analyzed by ^{1}H NMR, TLC and GC.

The products that eluted with hexane–ethyl acetate

* Author to whom correspondence should be addressed.

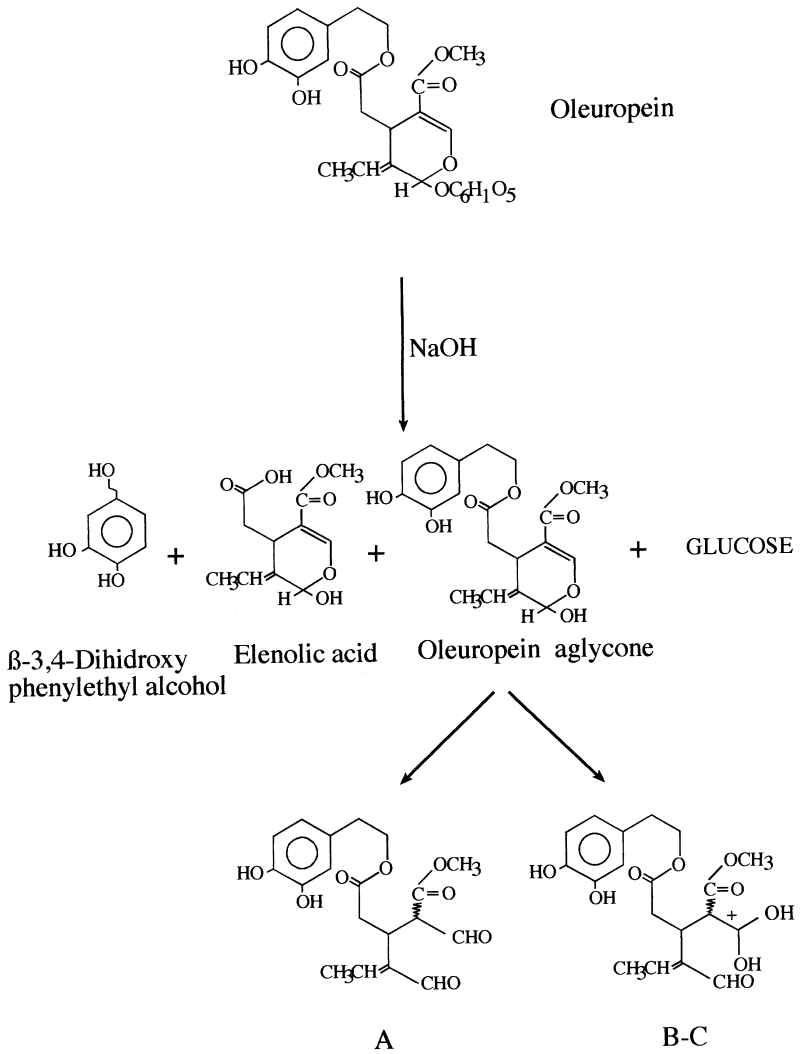


Fig. 1. Structure of oleuropein resulting from its alkaline hydrolysis [1–4].

(3:2) contained products whose ^{1}H NMR signals clearly revealed they were not aromatic compounds. TLC and GC analyses revealed two pure compounds in two different fractions and a mixture of the two in another fraction. To determine the structure of these compounds we carried out analyses of ^{1}H NMR, ^{13}C NMR, HETCOR, COSY 90°, NOE, IR and EI mass spectrometry of each of the fractions containing a pure compound. The molecular formula $C_{11}H_{16}O_5$ for compound **1** was assigned based on the mass spectrum (see Experimental) and ^{13}C NMR data (Table 1). The IR spectrum of the compound revealed absorption bands at 1734 and 1215 cm^{-1}, which were attributed to the presence of a methoxycarbonyl group; another absorption band at 1683 cm^{-1} suggested the presence of a conjugated aldehyde. The ^{1}H NMR spectrum showed a doublet at δ 9.26 (J = 2.0 Hz), typical of an aldehyde group coupled with an olefinic proton, which appeared as a broad quadruplet at δ 6.66 (J = 7.1 Hz) due to the doublet methyl at δ 2.06 (J = 7.06 Hz). These data, together with the results of ^{13}C NMR, suggested that **1** contains a vinylaldehyde substituent. ^{1}H NMR analysis also revealed two *dd* at δ 2.62 (2H, J_1 = 5.83 Hz, J_2 = 16.08 Hz) and δ 2.76 (2H, J_1 = 8.9 Hz, J_2 = 16.08 Hz) (Table 1) and a multiplet at δ 3.50–3.69. The methoxycarbonyl group was assigned to δ 3.58. ^{1}H NMR, ^{13}C NMR and mass spectroscopic data support that the structure of compound **1** is 3-[1-(hydroxymethyl)-(*E*) 1-propenyl] glutaric acid.

For compound **2**, the EI mass spectrum exhibited an ion at m/z 230 [M$^+$]. Its spectroscopic data were similar to those of **1**. The IR spectrum revealed the presence of a hydroxyl group (3685, 3615 cm^{-1}), whereas ^{1}H NMR revealed a *dd* at δ 4.66 (J_1 = 12.8, J_2 = 69.96 Hz) and the corresponding carbon appeared with a chemical shift at δ 72.1 (Table 2), in accordance with the allylic hydroxymethylene group. This establishes that in compound **2** C-1′ bears a hydroxymethyl substituent rather than an aldehyde. To corroborate this observation, compound **1** was

Table 1. ^{1}H and ^{13}C NMR data for compound **1**

	^{1}H	^{13}C
1	—	172.5
2a*, 4a*	2.62 *dd* (5.83; 16.08)	36.7
2b*, 4b*	2.76 *dd* (8.9; 16.08)	36.5
3	3.50–3.69 *m*	30.0
5	—	172.5
1′	—	143.4
2′	6.66 *bq* (7.1)	154.2
3′	2.06 *d* (7.06)	15.1
HCO	9.26 *d* (2.0)	195.0
OCH_3	3.58 *s*	51.4
OCH_3	3.58 *s*	51.4

Chemical shifts in ppm relative to TMS; coupling constants *J* in Hz. * Exchangeable data.

Table 2. ^{1}H and ^{13}C NMR data for compound **2**

	^{1}H	^{13}C
1†	—	171.9
2a*	2.43 *dd* (9.26; 16.14)	38.4
2b*	2.55 *dd* (4.54; 16.11)	38.4
3	3.32 *quintuplet* (6.7)	29.6
4a	2.63 *dd* (6.68; 15.8)	35.2
4b	2.74 *dd* (6.47; 15.81)	35.2
5†	—	171.5
1‡	—	132.3
2‡	5.64 *q* (6.83)	125.1
3‡	1.68 *d* (6.98)	15.5
CH_2OH	4.66 *dd* (12.8; 69.96)	72.1
OCH_3	3.67 *s*	51.9
OCH_3	3.67 *s*	51.9

Chemical shifts in ppm relative to TMS; coupling constants *J* in Hz. * and ‡ exchangeable data.

reduced with $NaBH_4$ and as expected compound **2** was obtained. Other differences were observed between the chemical shifts in the ^{1}H NMR analyses between compound **1** and compound **2** (Tables 1 and 2). The *trans*-configuration of the trisubstituted double bond was assigned by means of NOE difference experiments, where irradiation of the methyl frequency resulted in the NOE effect on the aldehyde group in compound **1**.

Table 3. ^{1}H and ^{13}C NMR data for compound **3**

	^{1}H	^{13}C
1′a*	2.51 *dd* (9.5; 16.5)	35.1
1′b*	2.64 *dd* (4.15; 16.45)	35.1
1″	5.64 *q* (6.5)	125.6
2	—	175.8
2′	—	172.1
2″	1.74 *d* (7.0)	14.2
3a*	2.71 *dd* (6.7; 15.8)	38.1
3b*	2.82 *dd* (6.4; 15.8)	38.1
4	3.6 *m*	30.4
5	—	132.1
6	4.71 *dd* (12.9; 66.0)	72.1

Chemical shifts in ppm relative to TMS; coupling constants *J* in Hz. * Exchangeable data.

The structure of compound **2** was confirmed after chemical modification. Compound **2** was saponified and after acidification product **3** was identified. The spectroscopic data of compound **3** (Table 3) revealed that this compound corresponded to a lactone, whose structure is given in Fig. 2. This lactone was methylated with CH_2N_2 to yield compound **4**, which contained a methoxycarbonyl group (spectroscopic data in Table 4).

The products resulting from the chemical modification of compounds **1** and **2** support the fact that the structures of the compounds present in the ethyl acetate extract were 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid (**1**) and 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid (**2**).

Both compounds may thus be derived from oleuropein, a more complex molecule (Fig. 1), which confers bitterness to olives.

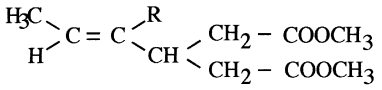
EXPERIMENTAL

General

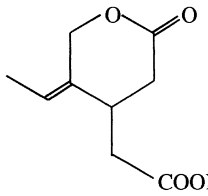
NMR were recorded on Bruker 500 AMX and 400 ARX instruments and referenced to residual solvent signal. The number of attached protons for ^{13}C signals was determined from DEPT 135 assays.

Extraction and isolation

Olive oil production residues were collected from an olive oil mill in Villacarrillo, Jaén, Spain. The residue (1.3 kg) was immersed in 13.750 l of H_2O. The suspension was kept at room temp. for 5 days, with occasional agitation to facilitate maceration. The sam-



	Compound 1	Compound 2
R =	CHO	CH_2OH



	Compound 3	Compound 4
R =	H	CH_3

Fig. 2. Molecular structure of 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid, 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid, the lactone resulting from the saponification and reduction of the first product and its methyl ester derivative.

Table 4. ^1H and ^{13}C NMR data for compound **4**

	^1H	^{13}C
1′a*	2.45 *dd*/2.5 *dd* (9.40; 16.16)/(5.74; 15.3)	35.1/35.0
1′b*	2.56 *dd*/2.61 *dd* (4.43; 16.21)/(4.5; 16.87)	35.1/35.0
1″	5.65 *q* (6.5)	125.2/125.1
2	—	175.3
2′	—	172.1/171.6
2″	1.7 *d*/1.69 (6.50)/(6.19)	14.2/13.6
3a*	2.65 *dd*/2.72 *dd* (6.62; 15.82)/(6.62; 10.96)	38.3/38.0
3b*	2.79 *dd*/2.79 *dd* (6.44; 15.36)/(6.44; 15.36)	38.3/38.0
4	3.32 *m*/3.65 *m*	30.4/29.3
5	—	132.2/132.0
6	4.67 *dd*/4.68 *dd* (12.84; 91.01)/(12.84; 88.43)	72.1
7	3.69 *s*	52.0

Chemical shifts in ppm relative to TMS; coupling constants *J* in Hz. Chemical shifts of two isomer products are separated by a slash. * Exchangeable data.

ple was then decanted and filtered through a wire funnel to remove solids. The pH of the filtered soln was 4.5, which was acidified with 2 N HCl to pH 3. The acidified aq. phase was successively extracted with different solvents of increasing polarity. The solvents used were hexane, CH_2Cl_2, Et_2O and EtOAc. The EtOAc extract was kept for further studies and after evapn *in vacuo*, 2.89 g of EtOAc extract was recovered. This was methylated with CH_2N_2–Et_2O and after usual work-up, 3.11 g of methylated extract was obtained. This extract was then chromatographed under pressure on a silica gel column using silica gel 60 (Merck, 70–230 mesh) and products were eluted with mixts of hexane–EtOAc (10:0–0:10). The frs obtained (25 ml) were analyzed by TLC, ^1H NMR and GC. Three frs that eluted from the column with hexane–EtOAc (3:2) appeared to be free of aromatic compounds and were kept for further analysis. The first fr. (9 mg) contained a pure compound, which was identified as 3-[1-(hydroxymethyl)-(*E*)1-propenyl] glutaric acid (**1**). The third fr. (17 mg) also contained a pure compound, identified as 3-[1-(formyl)-(*E*)1-propenyl] glutaric acid (**2**). The second fr. (6 mg) contained a mixt. of these two compounds.

3-[1-(Hydroxymethyl)-(*E*)1-propenyl] glutaric acid (**1**). EIMS *m/z* (rel. int.): 228 $[M]^+$ ($C_{11}H_{16}O_5$), 227 (8.09), 213 (27.26), 200 (20.85), 198 (11.57), 197 (19.09), 185 (18.01), 182 (6.44), 181 (12.77), 169 (13.79), 166 (37.79), 154 (12.91), 153 (79.21), 141 (12.45), 138 (46.04), 137 (19.32), 123 (67.15), 110 (24.03), 109 (46.58), 108 (45.19), 95 (62.84), 94 (27.32), 81 (56.79), 79 (80.40), 67 (89.05), 66 (31.32), 59 (100), 51 (46.92), 43 (98.55). IR ν_{max} cm^{-1}: 3020, 1734, 1683, 1483, 1215, 757.

3-[1-(Formyl)-(*E*)-1-propenyl] glutaric acid (**2**). EIMS *m/z* (rel. int.): 230 $[M]^+$ ($C_{11}H_{18}O_5$) (5.16), 229 (29.21), 215 (3.43), 199 (20.78), 198 (16.46), 197 (68.10), 183 (19.49), 179 (63.19), 168 (17.38), 167 (60.75), 165 (38.61), 150 (81.96), 139 (29.11), 137 (61.84), 121 (73.78), 119 (27.42), 111 (27.00), 107 (72.71), 93 (48.67), 91 (64.24), 81 (76.83), 80 (17.91), 79 (85.67), 67 (66.38), 59 (87.59), 53 (39.32), 43 (100). IR ν_{max} cm^{-1}: 3685, 3615, 1377, 3019, 2976, 2927, 1736, 1437, 1215, 1080, 1047, 1022, 929, 756, 668.

Reduction of compound **1**

Compound **1** was reduced with $NaBH_4$ in MeOH at room temp. After usual work-up, 11 mg of a mixt. of products was obtained, which were identified as compounds **2** and **3**.

Saponification of compound **2**

Compound **2** was saponified in a 2 N KOH–MeOH soln. After usual work-up, the lactone derivative (**3**) was obtained.

Methylation of compound **3**

Upon methylation of compound **3** with CH_2N_2–Et_2O, compound **4** was obtained.

Acknowledgements—This work was supported by grants from the CICYT, BI097-0641 and PTR93-010.

REFERENCES

1. Brenes-Balbuena, M., García, P. and Garrido-Fernández, A., *Journal of Agricultural and Food Chemistry*, 1992, **40**, 1192.
2. Gariboldi, P., Jommi, G. and Verotta, L., *Phytochemistry*, 1986, **25**, 865.
3. Tsukamoto, H., Hisada, S. and Nishibe, S., *Shoyakugaku Zasshi*, 1985, **39**, 90.
4. Amiot, M. J., Fleuriet, A. and Macheix, J. J., *Phytochemistry*, 1989, **28**, 67.
5. Limiroli, R., Consonni, R., Ottolina, G., Marsilio, V., Bianchi, G. and Zetta, L., *Journal Chemistry Society*, *Perkin Transactions I*, 1995, 1519.
6. Capasso, R., Cristinzio, G., Evidente, A. and Scognamiglio, F., *Phytochemistry*, 1992, **31**, 4125.
7. Fleming, H. P., Walter, W. M. Jr. and Etchells, J. L., *Applied Microbiology*, 1969, **18**, 856.
8. Maestro Durán, R., Borja Padilla, R., Martín Martín, A., Fiestas Ros de Ursinos, J. A. and Alba Mendoza, J., *Grasas y Aceites*, 1991, **42**, 271.
9. Balice, V. and Cera, O., *Grasas y Aceites*, 1984, **35**, 178.
10. Vázquez Roncero, A., Maestro Durán, R. and Graciani Constante, E., *Grasas y Aceites*, 1974, **25**, 341.
11. Gil, M., Haïdour, A. and Ramos, J. L., *Journal Agricultural and Food Chemistry*, 1997, **45**, 4490.