Identification of Two Triterpenoids in Solid Wastes from Olive Cake

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Solid wastes resulting from olive oil production were macerated in ethanol and then extracted with hexane. In this fraction, in addition to typical olive oil triglycerides, two oleanane derivatives were identified. These chemicals belong to the olean-12-ene family, and their ^{1}H-NMR and ^{13}C-NMR signals were confidently assigned with the aid of high-resolution two-dimensional nuclear magnetic resonance spectroscopy. These compounds were identified as methyl 3β-acetoxyolean-12-en-28-oate and methyl 2α,3β-diacetoxyolean-12-en-28-oate.

Keywords: *Olive wastes; oleanane triterpene; 3β-hydroxyolean-12-en-28-oic acid; 2α,3β-dihydroxyolean-12-en-28-oic acid*

INTRODUCTION

The extraction of olive oil produces large amounts of liquid and solid wastes, which constitute the main pollutant in agricultural areas in southern Spain and the Mediterranean basin (Hamdi, 1993). Although many approaches have been suggested to deal with these wastes, no practical solution has yet been found (Hamdi, 1993; Fiestas Ros de Ursinos *et al.*, 1992; Ranalli, 1992; Martínez Nieto *et al.*, 1992). After an in-depth review of research on pollution by olive oil, production wastes showed that the chemical composition of these wastes is practically unknown (Martínez Nieto *et al.*, 1992; Balice and Cera, 1984; Vazquez Roncero *et al.*, 1974), although the presence of aromatic compounds substituted with hydroxyl or methoxyl groups has been reported (Balice and Cera, 1984; Vazquez Roncero *et al.*, 1974). As a strategy to design a series of biological processes to deal with the removal of these wastes, we analyzed their chemical composition with sequential extractions with solvents of increasing polarity. In this paper we present our analysis of the hexane extract of these wastes. This study allowed us to identify typical fatty acids present in olive oil and two triterpenoids of the oleanane family. The spectroscopic data of the triterpenoid are confidently assigned by means of one- and two-dimensional NMR spectra.

EXPERIMENTAL PROCEDURES

General. Nuclear magnetic resonance (NMR) spectra were recorded on a 500 AMX and 400 ARX Bruker, and referenced to the residual solvent signal. The number of attached protons for ^{13}C signals were determined from DEPT135 assays. For COSY 90°, COSY double quantum filter, heteronuclear correlation H−C (optimized for $^1J_{CH} = 140$ Hz), HMBC (optimized for $^nJ_{CH} = 8.1$ Hz), and phase sensitive NOESY, we used the pulse sequences provided by the Bruker Library.

Collection, Extraction, and Isolation of Products from Wastes from Olive Oil Production. Samples of olive oil production solid wastes were collected from the Jimena S.A. olive oil mill in Pinos Puente, Granada (Spain). The solid olive oil waste (5 kg) resulting from olive fruit pressing was immersed in 10 L of 96% vol/vol ethanol. The mixture was kept at room temperature and occasionally agitated to facilitate the maceration process. After 48 h, the sample was decanted and filtered through a wire funnel.

The ethanolic solution was thoroughly extracted with hexane. The hexane extract was evaporated *in vacuo* to dryness in a rotary evaporator at 35 °C; this yielded 3.368 g of products.

Analysis of the ^{1}H-NMR of the hexane extract revealed signals of triglycerides and triterpenoids. The hexane extract was then methylated with ethereal CH_2N_2, the methylated sample was chromatographed under pressure on a silica gel column eluted with mixtures of hexane−ether described below, and three fractions were collected.

Fraction 1 resulted from thorough elution with hexane. This yielded 1.500 g, consisting mainly of triglycerides. After saponification, the fatty acids were transformed into the corresponding methyl esters with ethereal CH_2N_2 and analyzed by gas chromatography−mass spectrometry (GC-MS).

Fractions 2 and 3 (79 and 173 mg, respectively) were eluted with a mixture of hexane/ether 6:4 (vol/vol) and 2:8 (vol/vol), respectively. These were independently acetylated with acetic anhydride (2.52 mL) in pyridine (2.52 mL), and after the usual workup, 250 mg of the acetylated sample was chromatographed on silica gel column, using a mixture of CH_2Cl_2/ethylacetate. We isolated 79 mg of compound **1**, which was identified as methyl 3β-acetoxyolean-12-en-28-oate, and 17 mg of compound **2**, which was identified as methyl 2α,3β-diacetoxyolean-12-en-28-oate.

*Methyl 3β-Acetoxyolean-12-en-28-oate (Compound **1**).* EIMS [relative intensity (rel int)] m/z: 512 $[M]^+$ ($C_{33}H_{52}O_4$), 470 (2.12), 455 (10.21), 454 (18.95), 453 (82.81), 439 (2.94), 437 (11.77), 411 (18.75), 410 (1.41), 262 (34.70), 248 (2.45), 207 (4.69), 203 (100), 189 (28.36), 175 (12.69), 149 (10.26), 133 (31.72), 119 (27.24), 105 (22.48), 49 (63.06), 43 (90.67). IR ν_{max} (cm^{-1}): 2945, 2863, 2357, 2330, 2298, 1725, 1652, 1460, 1385, 1362, 1303, 1262, 1235, 1201, 1190, 1162, 1125, 1094, 1034, 996, 815, 789, 756, 656. MP: 190−192 °C (uncorrected).

*Methyl 2α,3β-Diacetoxyolean-12-en-28-oate (Compound **2**).* EIMS (rel int) m/z: 570 $[M]^+$ ($C_{35}H_{54}O_6$), 569 (3.86), 571 (4.40), 555 (2.44), 511 (12.55), 495 (1.67), 481 (1.31), 466 (1.41), 465 (3.78), 451 (69.96), 436 (1.58), 435 (4.55), 407 (1.53), 393 (2.47), 391 (9.36), 307 (1.32), 262 (34.16), 249 (14.40), 206 (1.55), 203 (32.92), 190 (4.99), 189 (26.34), 177 (7.61), 167 (7.20), 149 (23.35), 113 (11.21), 97 (10.29), 85 (10.70), 83 (15.33), 71 (25.10), 69 (18.00), 61 (86.83), 59 (12.04), 57 (41.98), 55 (19.44), 45 (19.96), 43 (100). IR ν_{max} (cm^{-1}): 2946, 1741, 1460, 1432, 1368, 1253, 1162, 1127, 1042, 918, 823, 755, 664. MP: 112−115 °C.

RESULTS AND DISCUSSION

Hexane Extract from Olive Oil Cake. Samples of solid olive oil waste were macerated in ethanol and

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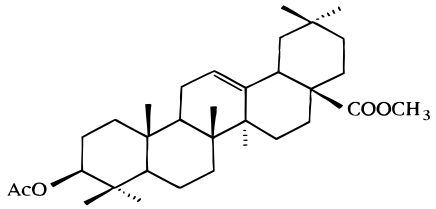
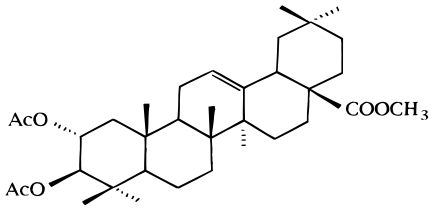


Compound 1



Compound 2

Figure 1.

then filtered as described under Experimental Procedures. The liquid phase was extracted with hexane. The hexane extract was subjected to ^{1}H-NMR analysis, which revealed the presence of triglycerides as the main compounds, and other series of signals typical of triterpenoids. The latter finding comprised seven methyl signals as singlets with δ in the range 0.8−1.2 ppm.

To unequivocally identify the compounds giving rise to these signals, we purified them by treating the hexane extract with CH_2N_2 and then fractionating through silica gel columns as described under Experimental Procedures. Three fractions were recovered.

The first fraction consisted mainly of triglycerides. This fraction was saponified, and the resulting fatty acids were methylated and analyzed by GC-MS. The fatty acids identified by comparison with authentic samples were typical of olive oil: oleic acid (C18:1; 76.5% of total); linoleic acid (C18:2; 17.6% of total); palmitic acid (C16:0; 3.8% of total); stearic acid (C18:0; 0.9% of total), and palmitoleic acid (C16:1; 0.8% of total).

The second and the third fractions each exhibited a main compound and minor products. To further purify the main products, these fractions were acetylated and rechromatographed on a silica gel column; this yielded compounds **1** and **2** (Figure 1), identified as methyl 3β-acetoxyolean-12-en-28-oate (compound **1** in Figure 1) and methyl 2α,3β-diacetoxyolean-12-en-28-oate (compound **2** in Figure 1).

The structure of these compounds was determined by ^{1}H-NMR, ^{13}C-NMR, COSY 90°, COSY dqf, HETCOR, HMBC, NOESY, ROESY, and HOHAHA.

Evidence for Oleanane Structure in Compounds 1 and 2. ^{1}H-NMR spectra of compounds **1** and **2** revealed seven angular methyl signals (δ 0.701, 0.841, 0.851, 0.866, 0.912, 0.917, 1.113 ppm for compound **1**, and δ 0.702, 0.879, 0.890, 0.909, 0.984, 1.035, 1.104 ppm for compound **2**) (Tables 1 and 2). In addition, each compound showed one signal triplet on a trisubstituted double bond located on C-12 (δ 5.368 ppm for compound **1** and δ 5.250 ppm for compound **2**) (Tables 1 and 2). These data suggested the presence of an oleanane skeleton. Furthermore, a singlet due to a carboxymethyl group at δ 3.607 ppm (compound **1**) and δ 3.611 ppm (compound **2**) was found, and a double doublet was detected at δ 2.850 ppm (compound **1**) and δ 2.843 ppm (compound **2**) for the one-proton signal produced by allylic H-18 (Tables 1 and 2). These data suggested that

Table 1. ^{1}H, ^{13}C, and HMBC Data of Methyl 3β-Acetoxyolean-12-en-28-oate (1)[a]

			correlated C-atom	
	α	β	^{13}C (HETCOR)	HMBC
H-1	1.602 m	1.037	38.18	C-10
H-2	1.612 m	1.032 m	27.754	
H-3	4.48 dd (6.2; 9.8)		81.013	C-2; C-4; C-26; OAc(3)
H-4			37.765	
H-5	0.812 m		55.373	C-4; C-10
H-6	1.509 m (2.9; 10.8)	1.382 ddd	18.294	
H-7	1,434 ddd (3.2; 12.2)	1.278 m	32.668	
H-8			39.354	
H-9	1.553 dd (7; 10.4)		47.626	C-4; C-8; C-10
H-10			37.005	
H-11	1.866 m	1.866 m	23.481	C-12
H-12	5.268 t (3.5)		122.351	C-9; C-11; C-14
H-13			143.883	
H-14			41.708	
H-15	1.604 m	1.604 m	23.603	
H-16	1.959 ddd (4.1; 13.38)	1.607 m	23.137	C-28
H-17			46.803	
H-18	2.85 dd (4.05; 14.0)		41.358	
H-19	1.132 m	1.59 m	45.914	
H-20			30.778	
H-21	1.171 m	1.328 ddd (3.45; 13.1)	33.927	
H-22	1.68 ddd (4.35; 13.4)	1.467	32.456	C-28
H-23	0.851 s		28.114	C-4
H-24		0.841 s	16.763	C-4
H-25		0.917 s	15.448	C-8; C-9; C-10
H-26		0.701 s	16.909	C-8; C-9; C-14
H-27	1.113 s		25.974	C-8; C-11; C-13; C-14
H-28			178.406	
H-29	0.866 s		33.189	C-20
H-30		0.912 s	23.721	C-20
$COCH_3$(3)	C	2.033 s	21.415	$COCH_3$(3)
$COCH_3$(3)	C	C	171.135	C
$OCOCH_3$	C	3.611 s	51.621	C-28

[a] Chemical shifts in parts per million relative to TMS; coupling constants J in hertz.

Table 2. ^{1}H, ^{13}C, and HMBC Data of Methyl 2α,3β-Diacetoxyolean-12-en-28-oate (2)[a]

			correlated C-atom	
	α	β	^{13}C (HETCOR)	HMBC
H-1	1.998 dd (4.5; 12.3)	1.043 t (12.43)	43.95	C-2; C-3; C-5; C-10; C-25
H-2		5.08 t (3.6)	70.11	C-1; C-3; OAc(2)
H-3	4.73 d (10.5)		80.70	C-1; C-2; C-4; C-23; C-24; OAc(3)
H-4			39.39[b]	
H-5	0.952 dd (3; 11.5)		54.96	C-4; C-6; C-10; C-24; C-25
H-6	1.529 dq (3; 13.7)	1.410 dddd (3; 12.4)	18.30	
H-7	1.448 ddd (3.5; 12.4)	1.291 m	32.43	C-5; C-8; C-9
H-8			39.42[b]	
H-9	1.593 dd (6.6; 11.4)		47.62	
H-10			38.22	
H-11	1.826 ddd (3.8; 6.6; 11.3)	1.907 ddd (3.8; 10.7; 11.3)	23.71	
H-12	5.25 t (3.6)		122.02	C-9; C-11; C-14; C-18; C-27
H-13			143.97	
H-14			41.73	
H-15	1.580 m	1.030 m	27.67	C-13
H-16	1.953 ddd (4.2; 13.4)	1.600 m	23.01	
H-17			46.76	
H-18	2.843 dd (4.3; 13.7)		41.31	C-12; C-13; C-14; C-16; C-17; C-19; C-28
H-19	1.604 t (13.7)	1.124 dd (4.7; 13.7)	45.90	
H-20			30.78	
H-21	1.171 dq (2.5; 13.5)	1.345 ddd (4; 13.7)	33.91	C-20; C-30
H-22	1.672 ddd (4.4; 17.7)	1.501 dt (3; 13.7)	32.50	C-16; C-17; C-20; C-21; C-28
H-23	0.879 s		28.50	C-4; C-5
H-24		0.890 s	17.71	C-4; C-5; C-23
H-25		1.035 s	16.50	C-1; C-5; C-9; C-10
H-26		0.702 s	16.87	C-7; C-8; C-9; C-14
H-27	1.104 s		25.96	C-8; C-13; C-15; C-14
H-28			178.34	
H-29	0.909 s		33.19	C-19; C-20; C-21; C-30
H-30		0.984 s	23.71	C-19; C-20; C-21; C-29
$COCH_3$(2)	C	2.039 s	21.01	$\underline{C}OCH_3$(2)
$CO\overline{C}H_3$(3)	1.964 s	C	21.25	$\overline{C}OCH_3$(3)
$\underline{C}O\overline{C}H_3$(2)	C	C	170.65	$\overline{C}$
$\overline{C}OCH_3$(3)			170.96	
$\overline{O}CO\underline{C}H_3$		51.65 s	51.65	C-28

[a] Chemical shifts in parts per million related to TMS; coupling constants *J* in hertz. [b] Exchangable data.

compounds **1** and **2** were methyl olean-12-en-28-oate (Akasbi *et al.*, 1993).

One acetyl signal was observed with ^{1}H-NMR for compound **1** (δ 2.033) and two acetyl signals were observed with ^{1}H-NMR for compound **2** (δ 2.039 ppm and δ 1.964 ppm) (Tables 1 and 2). This suggested that most likely one and two hydroxyl groups were present on the nonacetylated original form that gave rise to compound **1** and compound **2**, respectively.

The mass spectra of the two acetylated compounds revealed the presence of M + 1 ion (m/z = 513 and 571 for compounds **1** and **2**, respectively). In addition, the fragmentation pattern showed intense peaks at m/z 262 and 203. These ions were the result of typical retro Diels−Alder cleavage of ring C of olean-12-en with a methoxycarbonyl group on C-17, and the absence of hydroxyl groups on rings D and E (Ali *et al.*, 1990; Biessels *et al.*, 1974; Budzikiewicz *et al.*, 1963, 1964; Razdan *et al.*, 1983). These data, together with the number of signals from ^{13}C-NMR, suggested that the molecular formulas of these compounds were $C_{33}H_{52}O_4$ for compound **1** and $C_{35}H_{54}O_6$ for compound **2**.

Assignment of Protons and Carbon in Compounds 1 and 2 and Configuration. Proton and carbon signals of compounds **1** and **2** were assigned by recording 1D and 2D NMR spectra.

COSY and NOESY analysis of compounds **1** and **2** allowed the full assignment of protons of the two molecules under study, and HETCOR analysis allowed us to assign the ^{13}C-NMR signal spectra (Tables 1 and 2). A signal of protons attached to the carbon bearing an oxygen atom of compound **1** was observed at δ 4.48 ppm (t, J = 8 Hz, H-3). For compound **2**, the signals of protons geminal to the carbons bearing an oxygen atom were δ 5.08 ppm (t, J = 3.6 Hz, H-2) and δ 4.73 ppm (d, J = 10.5 Hz, H-3) (see Tables 1 and 2). Other proton signals are shown in Tables 1 and 2.

In ^{13}C-NMR spectra, we observed signals for C-3 at δ 81.013 ppm for compound **1** (Table 1) and for C-2 at δ 70.11 ppm and C-3 at 80.70 ppm C-3 for compound **2** (Table 2); other C signals are shown in Tables 1 and 2.

On the basis of the assignation of the signals of the 2D-NOESY spectra listed in the Tables 3 and 4, we established the conformation of these oleanane derivatives, as well as the configurations of chiral centers. NOESY assays revealed that the configuration of acetyl groups in C-2 and in C-3 were α and β, respectively. This was deduced from the fact that the H-2 was NOESY with H-1β, Me-24, and Me-25, which unequivocally confirms that the acetyl group C-2 was α (Table 4). The H-3 was NOESY with H-1α, H-5, and Me-23; therefore, the acetyl group in this position was β (Table 4). For compound **1**, we found that the acetyl group in C-3 was β (Table 3).

Together with these results, the multiplicity pattern of geminal proton(s) of acetyl groups allowed us to corroborate the configuration established for C-3 in compound **1** and C-2 and C-3 in compound **2**.

The two-dimensional HMBC spectra allowed us to establish the connections between different parts of the molecule and to unequivocally determine the structure of compounds **1** and **2** as methyl 3-acetoxyolean-12-en-28-oate and methyl 2,3-diacetoxyolean-12-en-28-oate (Tables 1 and 2). For compound **2**, we observed coupling

Table 3. Scalar and Spatial Correlation of the Protons of Methyl 3β-Acetoxyolean-12-en-28-oate (1)

H	COSY	NOESY	H	COSY	NOESY
1α		H-3, H-5, H-9, H-23	16α	H-15α, H-15β, H-16α	H-7β, H-27
1β			16β		
2α		H-3, H-5	17		
2β		H-1α, H-2α	18	H-19α, 19β	H-19α, H-19β, H-27, H-29
3	H-2α, H-2β	H-5	19α	H-18, H-19β	
4			19β	H-18, H-19α	
5	H-6α, H-6β	H-6α, H-9, H-23	20		
6α	H-6β, H-7α, H-7β		21α	H-21β, H-22α, H-22β	
6β			21β	H-21α, H-22α, H-22β	H-30
7α			22α	H-21α, H-21β, H-22β	H-21α, H-29
7β	H-6α, H-6β, H-7α		22β	H-21α, H-21β, H-22	
8			23		
9	H-11α, H-11β		24		
10			25		H-26
11α	H-9		26		H-6β, H-11β, H-25
11β	H-9		27		H-16α
12	H-11α, H-11β	H-11α, H-11β, H-18, H-19α	28		
13			29		
14			30		H-19α, H-19β, H-21α, H-21β
15α			$COO\underline{C}H_3$	C	H-26
15β			$OCO\underline{C}H_3$	C	H-24

Table 4. Scalar and Spatial Correlation of the Protons of Methyl 2α,3β-Diacetoxyolean-12-en-28-oate (2)

H	COSY	NOESY	H	COSY	NOESY
1α	H-1β	H-2, H-3	16α	H-15α, H-15β, H-16β	
1β	H-1α	H-2, H-3	16β	H-15α, H-15β, H-16α	
2	H-1α, H-1β, H-3	H-1β, H-24, H-25	17		
3	H-2	H-1α, H-5, H-23	18	H-19α, H-19β	H-19α, H-29, H-22α, H−12, H−27
4			19α	H-18, H-19β	
5	H-6β	H-9, H-7α, H-23	19β	H-18, H-19α	
6α	H-6β, H-7α		20		
6β	H-6α, H-7β	H-26, H-23, H-24, H-25	21α	H-21β, H-22α, H-22β	H-19α, H-29
7α	H-6α, H-7β	H-27	21β	H-21α, H-22α, H-22β	
7β	H-6α, H-6β, H-7α		22α	H-21α, H-21β, H-22β	
8			22β	H-21α, H-21β, H-22α	H-29, H-18
9	H-11α, H-11β		23		H-6α, H-5
10			24		H-6β, H-25
11α	H-9, H-11β, H-12		25		H-26, H-6β, H-24
11β	H-9, H-11α, H-12	H-25, H-12	26		H-11β, H-25, H-6β, H-7β, H-15β
12	H-11α, H-11β	H-11α, H-11β, H-18, H-27	27		H-7α
13			28		
14			29		H-22α
15α	H-15β, H-16α, H-16β		30		
15β	H-15α, H-16α, H-16β	H-26			

of H-2 with C-1, C-3, and the acetyl group in C-2 (Table 2) and H-3 with C-1, C-2, C-4, C-23, C-24, and the acetyl group in C-3 (Table 2). Other data for compounds **1** and **2** are present in Tables 1 and 2. From these data we concluded that compound **1** and **2** can be assigned as methyl 3β-acetoxyolean-12-en-28-oate and methyl 2α,3β-diacetoxyolean-12-en-28-oate (Alam *et al.*, 1996; Bianchi *et al.*, 1994; Furuya *et al.*, 1987; Kojima and Ogura, 1986, 1989; Seo *et al.*, 1981; Tchivounda *et al.*, 1991).

Arguing against the assignment published by others (Chang *et al.*, 1979; Seo *et al.*, 1975; Tori *et al.*, 1974), we substituted the chemical shift at C-7 for the chemical shift at C-22, and the chemical shift of C-11 for the chemical shift at C-16 in both compounds (Tables 1 and 2). This is based on the findings that for compound **2** H-7α was NOESY with Me-27 (Table 4) and HMBC with C-5, C-8, and C-9 (Table 2), whereas H-22β was NOESY with Me-29 and H-18 and HMBC with C-16, C-17, C-20, C-21, and C-28 (Tables 2 and 4). Moreover, H-11β was NOESY with H-25 and H-12 (Table 4).

This study shows that typical olive oil fatty acids and triterpenoids are the main compounds present in the hexane fraction of olive oil production wastes. Utilization of fatty acids and triterpenoids as the sole C-source by a wide variety of living microorganisms is well established (Mandelstam *et al.*, 1982). We therefore suggest that these compounds should be easily degraded in waste water treatment plants designed for treatment of these specific wastes. Initial biotreatment assays with a wide battery of Gram-positive and Gram-negative bacteria support this conclusion (our unpublished results).

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LITERATURE CITED

Akasbi, M.; Showman, D. W.; Saari Csallany, A. High-performance liquid chromatography of selected phenolic compounds in olive oils. *J. Am. Org. Chem. Soc.* **1993**, *70*, 367−370.

Alam, M. S.; Chopra, N.; Ali, M.; Niwa, M. Oleanen and stigmasterol derivatives from *Ambroma augusta*. *Phytochemistry* **1996**, *41*, 1197−1200.

Ali, M.; Bhutani, K. K.; Srivastava, T. N. Triterpenoids from *Symplocos racemosa* bark. *Phytochemistry* **1990**, *11*, 3601−3604.

Balice, V.; Cera, O. Acidic phenolic fraction of the olive vegetation water determined by a gas chromatographic method. *Grasas Aceites* **1984**, *35*, 178−180.

Bianchi, G.; Pozzi, N.; Vlahov, G. Pentacyclic triterpene acids in olives. *Phytochemistry* **1994**, *37*, 205−207.

Biessels, H. W. A.; van der Kerk-van Hoof, A. C.; Kettenes-van den Bosch, J. J.; Salemink, C. A. Triterpenes of *Prunus serotina* and *P. lusitanica*. *Phytochemistry* **1974**, *13*, 203−207.

Budzikiewicz, H.; Wilson, J. M.; Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. XXXII. Pentacyclic Triterpenes. *J. Am. Chem. Soc.* **1963**, *85*, 3688−3699.

Budzikiewicz, H.; Djerassi, C.; Williams D. H. *Structure Elucidation of Natural Products by Mass Spectrometry*; Holden-Day: San Francisco, 1964; Vol. II, p 122.

Chang, C. W. J.; Flament, I.; Matson, J. A.; Nishida, T.; Ohloff, G.; Wehrli, F. W.; Weinheimer, A. J. The use of carbon-13 nuclear magnetic resonance spectroscopy in natural products chemistry. *Progress in the chemistry of organic natural products*. Springer-Verlag: New York, 1979, Vol. 36, p 98.

Fiestas Ros de Ursinos, J. A.; Borja Padilla, R. Use and treatment of olive mill waste water. Current situation and prospects in Spain. *Grasas Aceites* **1992**, *43*, 101−106.

Furuya, T.; Orihara, Y.; Hayashi, Ch. Triterpenoids from *Eucalyptus perriniana* cultured cells. *Phytochemistry* **1987**, *26*, 715−719.

Hamdi, M. Future prospects and constraints of olive mill waste waters use and treatment: A review. *Bioprocess Eng.* **1993**, *8*, 209−214.

Kojima, H.; Ogura, H. Triterpenoids from *Prunella vulgaris*. *Phytochemistry* **1986**, *25*, 729−733.

Kojima, H.; Ogura, H. Configurational studies on hydroxy groups at C-2,3 and 23 or 24 of oleanene and ursene-type triterpenes by NMR spectroscopy. *Phytochemistry* **1989**, *28*, 1703−1710.

Mandelstam, J.; McQuillen, K.; Dawes, I. *Biochemistry of Bacterial Growth;* Blackwell Scientific Publications: Oxford, U.K., 1982; p 447.

Martínez Nieto, L.; Ramos-Cormenzana, A.; García Pareja, M. P.; Garrido Hoyos, S. E. Biodegradación de compuestos fenólicos del alpechín con *Aspergillus terreus*. *Grasas Aceites* **1992**, *43*, 75−81.

Ranalli, A. Microbiological treatment oil mill waste waters. *Grasas Aceites* **1992**, *43*, 16−19.

Razdan, T. K.; Harkar, S.; Kachroo, V.; Koul, G. L.; Waight, E. S. Triterpenoids from *Phytolaccaacinosa*, three oleanane derivatives. *Phytochemistry* **1983**, *22*, 1797−1800.

Seo, S.; Tomita, Y.; Tori, K. Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus hara* tissue cultures. *Tetrahedron Lett.* **1975**, *1*, 7−10.

Seo, S.; Tomita, Y.; Tori, K. Biosynthesis of oleanene- and ursene-type triterpenes from [4-^{13}C]mevalonolactone and [1,2-$^{13}C_2$] acetate in tissue cultures of *Isodon japonicus* Hara. *J. Am. Chem. Soc.* **1981**, *103*, 2075−2080.

Tchivounda, H. P.; Koudogbo, B.; Besace, Y.; Casadevall, E. Triterpene saponins from *Cylicodiscus gabunensis*. *Phytochemistry* **1991**, *30*, 2711−2716.

Tori, K.; Seo, S.; Shimaoka, A.; Tomita, Y. Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect ^{13}C, ^{1}H spin couplings. *Tetrahedron Lett.* **1974**, *48*, 4227−4230.

Vázquez Roncero, A.; Maestro Durán, R.; Graciani Constante, E. Componentes fenólicos de la aceituna. II. Polifenoles del alpechín. *Grasas Aceites* **1974**, *25*, 341−345.

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