Pergamon

0031-9422(94)E0208-A

Phytochemistry, Vol. 37, No. 1, pp. 205-207, 1994

0031-9422/94 $7.00+0.00

PENTACYCLIC TRITERPENE ACIDS IN OLIVES*

GIORGIO BIANCHI, NICOLETTA POZZI and GIOVANNA VLAHOV

Istituto Sperimentale per la Elaiotecnica, Contrada Fonte Umano, 65013-Citta S. Angelo(Pe), Italy

(*Received in revised form* 17 *February* 1994)

Key Word Index—*Olea europaea*; Oleaceae; olive fruit; surface layer components; oleanene triterpene acids.

Abstract—The pentacyclic triterpenoids present on the skin of three olive cultivars were studied. Chloroform extraction yielded compounds comprising the surface waxes which were in the range 0.08–0.16% of the total olive weight. Chloroform treated olives were further extracted with methanol to give large quantities of pure pentacyclic triterpene acids (0.08–0.13%). The major surface lipid components were identified as oleanolic acid (31–44%) and maslinic acid (55–68%).

INTRODUCTION

An investigation of the cuticular lipid layer of olive fruits revealed the occurrence of large amounts of oleanolic and maslinic acids in mixture with the common wax hydrocarbons, aliphatic esters, alcohols, aldehydes and fatty acids, accompanied by varying amounts of triacylglycerols, triterpenols and methyl phenyl esters [1]. Contrasting data regarding the presence, and the relative proportions of the two oleanene acids in olives, are reported in the literature. Parisi and De Vito [3] reported the isolation of almost pure oleanolic acid from the residue of an ethereal extract of the whole olive flesh treated with petrol. However, Caglioti *et al.* [4] found that maslinic acid was the dominant triterpene acid from the petrol washed olive husks extracted with ethyl ether. Later, Caputo *et al.* [5] reported that fresh olive husks contained only oleanolic acid, whereas extraction of aged husks gave mixtures of maslinic and oleanolic acids in varying proportions and they considered that maslinic acid could be the product of a microbial hydroxylation of the fruit endogenous oleanolic acid. Finally, Frega *et al.* [6] reported an unidentified pentacyclic substance accompanying maslinic and oleanolic acids.

The contrasting data prompted us to reexamine the whole matter in relation with our previous work on olive fruit lipids [1, 2, 7]. In the present work we characterized the cuticular pentacyclic triterpenoids from three olive varieties and compared their composition with respect to the harvesting date.

RESULTS AND DISCUSSION

Sound healthy olives were exhaustively extracted with cold chloroform and methanol in succession (Experimental). The methanol extract contained large amounts of sugars in addition to cuticular lipids [8]. The yields of the chloroform and methanol extractions are reported in Table 1. GC and GC-mass spectrometry analyses revealed that the extractives comprised two major components which were proved to be oleanolic and maslinic acid (Table 2).

The typical wax classes as reported previously, represented a small proportion of the extract [1]. Triterpenols were present in low proportions, uvaol in trace amount and erythrodiol in only 0.5–1.5% yield.

The methyl ester and the acetyl derivative of maslinic acid was synthesized to obtain the ^{13}C NMR spectrum of the substance. The assignments of carbon signals of the diacetyl methylmaslinate were made by recording 1D and 2D ^{13}C NMR spectra. All the 35 signals expected were well separated in the ^{1}H broad band decoupled ^{13}C spectrum. The CH_n multiplicities were determined by non-selective polarization transfer (DEPT) experiments. The 2D carbon–proton shift correlations were determined via one bond CH couplings (XHCORR) and longer-range CH couplings via two, three bonds (COLOC). The chemical shifts of the carbon signals are reported in Table 3.

The ^{13}C NMR spectral data of the diacetate of methyl maslinate were of interest, particularly the effect of hydroxyl acetylation on the chemical shifts of carbons at positions C-1, C-2 and C-3 as compared to the corresponding resonances in the related substances [9–15].

When maslinic acid was treated with ethereal diazomethane and the reaction mixture was left aside for over one week, the GC analysis of the reaction product revealed the presence in the reaction mixture of a minor component whose mass spectral data were consistent with a monomethyl ether of methylmaslinate. This unusual alkylation product of either of the hydroxyls at positions C-2 or C-3 by diazomethane is possibly the unknown compound described by Frega *et al.* [6].

*Part 4 in the series 'The lipids of *Olea europaea*'. For part 3 see ref. [2].

Table 1. Chloroform and methanol extracts from intact olives

	Cultivars			
	Dritta	Leccino	Cipressino	
Harvesting date	14.10.92	14.10.92	14.10.92	12.11.92
Weight (g) of olive samples	300	300	300	300
Weight (g) of $CHCl_3$ extract	0.24 (0.08)*	0.48 (0.16)	0.37 (0.12)	0.36 (0.12)
Weight (g) of MeOH extract	0.32 (0.11)	0.25 (0.08)	0.38 (0.13)	0.24 (0.08)

*Percentage of total weight of olive sample.

Table 2. Composition (%) of pentacyclic triterpene alcohols and acids found on the olive skin of three cultivars at different dates of the harvest season

	Dritta cv			*Leccino* cv			*Cipressino* cv					
Compounds	14.10.92 $CHCl_3$	14.10.92 MeOH	A*	14.10.92 $CHCl_3$	14.10.92 MeOH	A	14.10.92 $CHCl_3$	14.10.92 MeOH	A	12.11.92 $CHCl_3$	12.11.92 MeOH	A
Erythrodiol	3	—	1.5	1	—	0.5	2	—	1	3	—	1.5
Uvaol	tr	—		tr	—		tr	—		tr	—	
Oleanolic acid	67	20	43.5	52	10	31	56	22	39	67	28	42.5
Ursolic acid	tr	—		tr	—		tr	—		—	—	
Maslinic acid	30	80	55	47	90	68.5	42	78	60	30	72	66

*A = Mean value.

In summary, from the results of the present work it can be concluded that (i) the cuticular lipid compositions of the olives are different from the common plant surface waxes [16]; (ii) the olive surface lipids are typically mainly pentacyclic triterpene acids (90–95%) concentrated on the cuticle of the olive fruit and this corrects our previous findings [1]; (iii) both oleanolic acid and maslinic acid are biosynthesized by the olive fruit in comparable amounts; (iv) olive husks may represent a convenient source of the two triterpene acids.

EXPERIMENTAL

Olives. Olive samples (300 g) were collected from Cipressino, Dritta and Leccino cultivars in the autumn of 1992. The trees were in olive groves of the Institute orchard on the hills in the surroundings of Pescara. Olives were picked up randomly from all parts of the tree canopy and then stored in the freezer (−20°) for further processing.

Extraction. Olives samples from each cultivar and for each harvest were immersed in 200 ml of $CHCl_3$ for 1 min at room temp. to remove most of the $CHCl_3$ soluble common cuticular lipids [1]. The cold extracted olives were air-dried for 20 min during which an exudate was noted to form on the olive skin. A further extraction was carried out by immersing the olives in MeOH for 1 min. The $CHCl_3$ and MeOH extracts were evapd *in vacuo* to dryness in a rotary evaporator at 35°. The MeOH extracts contained large proportions of sugars that were eliminated by repeated washing with H_2O [8].

Methyl esters. Triterpene acids were transformed into the corresponding methyl esters with ethereal CH_2N_2.

Acetyl derivatives. To a pyridine soln of the crude extract (100 mg) was added Ac_2O (pyridine–Ac_2O, 1:2) and the mixt. heated at 100° for 1 hr. Usual work-up gave the acetates in high yields. The crude substance was chromatographed on a silica gel column eluting with $CHCl_3$. The oleanolic acid derivative eluted first followed by the maslinic acid derivative. Products were visualized on TLC plates by a 3% soln of CrO_3 in H_2SO_4 (1:1) spray followed by heating at 120°.

All substances isolated were identified either by co-chromatography or by comparison of their GC-MS fragmentation patterns and ^1H and ^{13}C NMR spectra with the literature data.

Trimethylsylyl derivatives. Aliquots (*ca* 10 mg) of dry sample were derivatized with pyridine, hexamethyldisilazane and trimethylchlorosilane (2:1:1) at 60° for 30 min, and the derivatized extract dissolved and kept in isooctane (4 ml). Aliquots were analysed by GC and by GC-MS. The capillary column was a DB-5 (30 m, 0.32 mm i.d., 0.25 μm film thickness, J and W Scientific) with He as carrier gas, injector 'on column', 90° for 1 min, and then the oven temp. was raised to 310° at a rate of 10° min^{-1} and held at this temp. for 20 min. Mass spectra were recorded at 70 eV at 1 scan sec^{-1}, *m/z* (rel. int.) range 40–750. Oleanolic acid: *m/z* (rel. int.) 600 $[M]^+$ (14), 585

Table 3. ^{13}C NMR spectral data of maslinic acid **1**, maslinic acid methyl ester **2** and its diacetate **3**

C	1*	2†	3
1	48.2	46.4	43.9
2	69.0	68.8	70.1
3	84.2	83.8	80.7
4	39.9	39.1	39.4
5	56.3	55.3	54.9
6	19.3	18.3	18.2
7	33.7	32.6	32.5
8	40.0	39.1	39.4
9	48.6	47.5	47.6
10	38.7	38.3	38.2
11	24.4	23.5	23.5
12	122.9	122.0	122.0
13	145.0	143.6	143.9
14	42.4	41.7	41.7
15	28.7	27.6	27.6
16	24.2	23.1	23.0
17	46.8	46.6	46.7
18	42.4	41.3	41.3
19	46.6	45.8	45.9
20	30.0	30.7	30.7
21	34.7	33.8	33.9
22	30.5	32.3	32.4
23	29.8	28.6	17.6
24	17.3	16.8	28.4
25	17.9	16.8	16.4
26	18.1	16.8	16.8
27	26.6	26.0	25.9
28	180.0	178.0	178.2
29	33.7	33.1	33.1
30	24.1	23.5	23.6
CO_2Me		51.5	51.5
$2MeCO_2$			170.5
$3MeCO_2$			170.8
$2\underline{Me}CO_2$			21.2
$3\underline{Me}CO_2$			20.9

*Ref. [15].
†Refs [11, 14].

(21), 482 (60), 320 (44), 203 (100), 189 (38), 73 (81); maslinic acid *m/z* (rel. int.): 688 $[M]^+$ (2), 673 (13), 598 (17), 570 (44), 320 (43), 203 (89), 189 (28), 73 (100); ursolic acid *m/z* (rel. int.): 600 $[M]^+$ (1), 585 (8), 482 (12), 320 (79), 203 (100), 189 (37), 133 (46), 73 (63); erythrodiol *m/z* (rel. int.): 572 (4), 497 (100), 216 (98), 203 (47), 73 (58); uvaol *m/z* (rel. int.): 572 (4), 497 (100), 216 (43), 203 (48), 133 (24), 73 (54). Olean 12-en-28-oic acid, -methyl ester *m/z* (rel. int.): 512 $[M]^+$ (2), 453 (11), 262 (61), 203 (100) 189 (87), 43 (28); olean-12-en-28-oic acid, 2,3-(dihydroxy)-methylester *m/z* (rel. int.): 570 $[M]^+$ (3), 511 (9), 262 (52), 203 (100), 189 (30), 43 (39).

Acknowledgements—This study was partially supported through a grant from CNR (Roma). The Italian Ministero delle Risorse Agricole, Alimentari e Forestali is thanked for a fellowship to N.P.

REFERENCES

1. Bianchi, G., Murelli, C. and Vlahov, G. (1992) *Phytochemistry* **31**, 3503.
2. Bianchi, G. and Vlahov, G. (1994) *Fat. Sci. Technol.* **96**, 72.
3. Parisi, E. and De Vito, G. (1931) *Ann. Chim.* **21**, 323.
4. Caglioti, L., Cainelli, G. and Minutilli, F. (1961) *Gazz. Chim It.* **91**, 1387.
5. Caputo, R., Mangoni, L., Monaco, P. and Previtera, L. (1974) *Phytochemistry* **13**, 1551.
6. Frega, N., Bonaga, G., Lercker, G. and Bartolomeazzi, R. (1989) *Riv. Ital. Sost. Grasse* **66**, 107.
7. Bianchi, G., Vlahov, G., Anglani, C. and Murelli, C. (1993) *Phytochemistry* **32**, 49.
8. Bianchi, G. and Pozzi, N. (1994) *Phytochemistry* **35**, 1335.
9. Kulshreshtha, D. K. and Rastogi, R. P. (1971) *Phytochemistry* **10**, 2832.
10. Kojima, H. and Ogura, H. (1989) *Phytochemistry* **28**, 1703.
11. Seo, S., Tomita, Y. and Tori, K. (1981) *J. Am. Chem. Soc.* **103**, 2075.
12. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4227.
13. Furuya, T., Orihara, Y. and Hayashi, C. (1987) *Phytochemistry* **26**, 715.
14. Kojima, H. and Ogura, H. (1986) *Phytochemistry* **25**, 729.
15. Pambou Tchivounda, H., Koudogbo, B., Besace, Y. and Casadevall, E. (1991) *Phytochemistry* **30**, 2711.
16. Bianchi, G. (1987) *Gazz. Chim. It.* **117**, 707.