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Roberto Lo Scalzo, and Maria Luisa Scarpati

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A NEW SECOIRIDOID FROM OLIVE WASTEWATERS

ROBERTO LO SCALZO and MARIA LUISA SCARPATI*

Department of Chemistry, "La Sapienza" University, P.le A. Moro 5, 00185 Rome, Italy

ABSTRACT.—The structure of a new compound **5**, isolated from the juice of ripe black olives (*Olea europaea*, cv. Leccino), was elucidated. In unripe green olives oleuropein [**1**] is present as the major *o*-diphenolic compound, while in ripe olives demethyloleuropein [**6**] predominates. Both these glucosides disappear from olive juice, as they are hydrolyzed by native β-glucosidases. Demethyloleuropein aglycone [**7**] rapidly eliminated the carboxyl group, giving **5**. Acid methanolysis of **5** gives **8**, which supports the assigned structure.

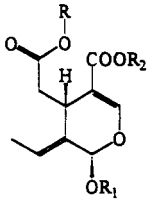
Olive mill wastewater (WW) disposal is a source of problems in olive-oil-producing countries, mainly due to WW toxicity. The WWs show a strong chemiotactile repellency (1) toward the main olive parasite, the olive fly *Dacus oleae*, but this action falls off rapidly when WWs are sprayed on trees in the field (2). We have shown that the repellency is mainly due to the olive *o*-diphenolic components oleuropein [**1**] (3), the aglycone **2** (3), and β-(3,4-dihydroxyphenyl)ethanol [**3**] (4). Secoiridoid **4** (Scheme 1) (3), the nonphenolic aglycone subunit of oleuropein, is also repellent (5). We were interested in the isolation of the phenolic components of olive WWs, either to identify active compounds or to find the conditions to stabilize repellent action over time. In the latter case the WWs could be used instead of pesticides in the fight against the olive fly.

Our research was carried out on fresh olive juice, suitably prepared in the laboratory, because olive mill WW does not have a constant composition. While known compounds **2**, **3**, and **4** are present in green olive juice, the juice obtained from ripe black olives (Leccino cultivar) contains an *o*-diphenolic compound **5**, not previously found in *Olea europaea* L. (Oleaceae), which is present in considerable amounts. We report now the isolation and structure determination of this new compound.

RESULTS AND DISCUSSION

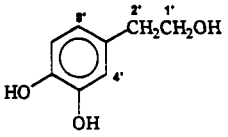
Oleuropein [**1**] is present in green olives; with the ripening of the drupes **1** is substituted by its acid derivative, demethyloleuropein [**6**] (6). In ripe black olives only **6** is present. The preferred way to isolate these glucosides is to subject the fresh intact olives to extraction with boiling solvents (i.e., MeOH) since the glucosides disappear rapidly from olive juice owing to rapid action of β-glucosidases. To isolate **5**, the fresh juice of recently picked black olives was shaken with hexane to remove fats, triterpenic acids, and other slightly polar compounds, then extracted with Et_2O.

The new compound was first thought to be the acid demethyloleuropein aglycone **7**. Accordingly the Et_2O extracts were treated with an aqueous solution of $NaHCO_3$. However **5** remained in the organic solution, indicating that further transformations had occurred. After



R=$CH_2CH_2C_6H_3(OH)_2$

1 R_1=glc, R_2=Me
2 R_1=H, R_2=Me
6 R_1=glc, R_2=H
7 R_1=R_2=H



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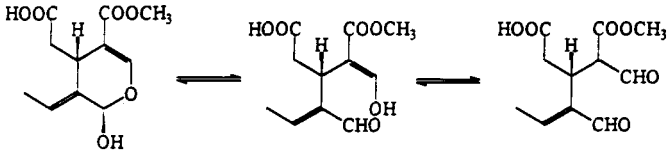

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SCHEME 1

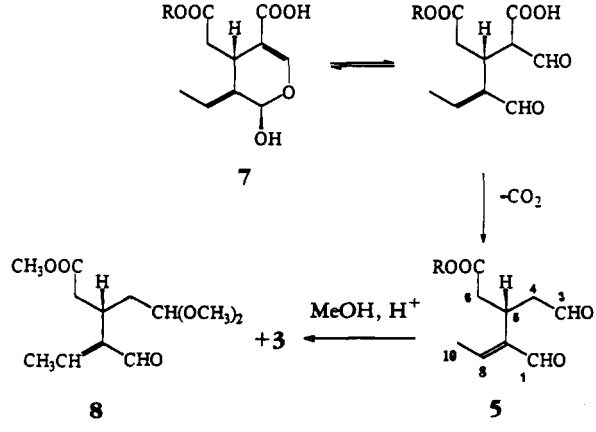
purification (reversed-phase cc, preparative tlc), **5** was obtained as an amorphous powder, $[\alpha]^{25}D$ +6.5°.

Alkaline hydrolysis of **5** under N_2 gave **3**, suggesting the possible origin of **5** from demethyloleuropein [**6**] in the juice. In fact, β-glucosidase hydrolysis of **6** gave a compound identical with **5** (tlc, hplc). The compound was then submitted to acid methanolysis. After the usual workup, **3** was separated from ester **8** with aqueous NaOH. The structure of **8** was elucidated via uv, ir, eims, and ^{13}C- and ^{1}H-nmr spectra.

Compound **5**, unlike the oleuropein aglycone **2**, does not possess a cyclic structure, as demonstrated by its ^{1}H-nmr spectrum: the two aldehydic groups are free (signals at δ 9.20 and 9.60), and the ethylidene double bond has not undergone additions, as shown by signals of vinyl CH, δ 6.64, q, and of Me, δ 2.00, d, J = 7 Hz.

Compounds **5** and **8** do not contain the carboxy group at C-4 (^{13}C-nmr spectra). It appears that **7**, formed from **6**, rapidly undergoes spontaneous decarboxylation. This assumption is consistent with the properties of a β-formyl carboxy acid (Scheme 2).

The structure assigned to **5** was also confirmed by comparison of its ^{1}H-nmr spectrum with the ^{1}H-nmr spectrum of **8**. In the case of **8**, only one formyl group was present (δ 9.30, s); the other had undergone acetalization (CH signal 4.10, two MeO δ 3.20, s and 3.22, s). The ethylidene group was still present and had the same signals as in the spectrum of **5**. The remaining signals were in accordance with the proposed structure. The geometry proposed for the ethylidene group of **5** is the same as that demonstrated for oleuropein [**1**] (7), from which **5** was derived. The uv spectrum of **8** yielded evidence that the nonacetalized formyl group is conjugated [λ max = 226 nm (17000)]. This was further supported by the ir signals at 2710 and 1685 cm^{-1} relative to a conju-



7 8 5

SCHEME 2

gated CHO. The eims also supported the assigned structure (weak $[M-Me]^+$ as *m/z* 229, base peak at *m/z* 75, typical of dimethylacetals).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{13}C- and ^1H-nmr spectra were determined with a Varian Gemini 200 instrument with chemical shifts recorded in ppm downfield from internal TMS and coupling constants in Hz. Analytical tlc: E. Merck Kieselgel 60 F-254 (0,2 mm thickness), spots visualized either by uv light or by spraying with $FeCl_3$ (3% in EtOH) or phosphomolybdic acid (10% in EtOH); preparative tlc was performed on E. Merck Kieselgel plates 60 F-254 (film thickness 2 mm). Cc was performed with a Duramat 80 IP31 apparatus on a Merck Lobar (310-25) Lichroprep RP-8, (1–3 bars) column (40–63 μm). Uv spectra were determined using a Varian CORY 210 spectrophotometer and ir spectra were obtained on a Shimadzu IR 470 apparatus. Mass spectra were taken on a HP model 5971A GC/MS spectrometer. Optical rotations were measured on a Perkin-Elmer model 241 Digital Polarimeter.

For enzymatic hydrolysis almond β-glucosidase (Fluka, 49290) was used.

COMPOUND **5** FROM OLIVE JUICE.—The homogenate was prepared at 5° from 400 g of recently picked black olives deprived of the stones. (*O. europaea* cv. Leccino, characterized by small dimension and used for oil production, collected in December 1991 in the Latina region, Italy). A voucher specimen is on deposit in the herbarium of Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza." The homogenate was centrifuged at 5°. The cold juice (120 ml) separated from the oil was immediately shaken with hexane and extracted with Et_2O (3 × 120 ml). The combined extracts were shaken with aqueous $NaHCO_3$ to remove acid components, washed with H_2O, and dried with anhydrous Na_2SO_4.

After removal of the solvent in vacuo, the residue showed by tlc [$CHCl_3$-MeOH (8:2)] an intense spot ($FeCl_3$ or phosphomolybdic acid positive), with an R_f slightly lower than the oleuropein aglycone **2**, near small amounts of other compounds.

Compound **5** was purified by reversed-phase cc on a Lobar column with a medium pressure apparatus [MeOH-H_2O (4:6)], monitoring the fractions by hplc. MeOH was removed in vacuo from the combined fractions, containing pure **5**, and the H_2O solution extracted with Et_2O. After drying with Na_2SO_4 and solvent removal in vacuo, **5** was obtained as an amorphous powder (0.750 g): $[\alpha]^{25}D$ +6.5°; ^1H nmr ($CDCl_3$) 9.58 (H-1, d, J = 1.4 Hz); 9.19 (H-3, s), 6.50–6.80 (H-8, H-4′, H-7′, H-8′, m), 5.60 (O*H*, br s, exchanges with D_2O), 4.15 (H-1′, m, J = 6.5 Hz), 2.50–3.00 (H_2-6, H_2-4, H_2-2′, m), 2.00 (H_3-10, d, J = 7 Hz).

ENZYMATIC HYDROLYSIS OF DEMETHYLOLEUROPEIN [**6**].—β-Glucosidase (20 mg) was added to a solution of **6** (50 mg) in H_2O (1 ml) at 35°. After 2 h the reaction was complete (tlc). The aglycone extracted (Et_2O) was identical with **5** (tlc, hplc, $[\alpha]^{25}D$).

METHANOLYSIS OF **5**. TRIMETHYLETHER **8**.—To a stirred solution of **5** (300 mg) in 10 ml of MeOH, 0.4 ml of H_2SO_4 (96%) was added at 25° (Scheme 2). After 12 h the acid was eliminated by stirring with $NaHCO_3$ (powder), and the solution was filtered and evaporated in vacuo. H_2O was added (10 ml), and the mixture was extracted with Et_2O (3 × 20 ml). The combined extracts were shaken with 1 N NaOH (1 ml) to remove **3** and washed with H_2O. The Et_2O solution, dried with anhydrous Na_2SO_4, was evaporated in vacuo, and the residue was purified by preparative tlc [$CHCl_3$-MeOH (95:5)] and by RP-8 cc Lobar (40-63 μm) [MeOH-H_2O (1:1)], obtaining 185 mg of **8**: uv λ max (MeCN) 226 (17000); ir (CCl_4, cm^{-1}) ν max 2985, 2945, 2825, 2710, 1735, 1685, 1636; eims *m/z* (rel. int.) $[M-Me]^+$ 229 (0.7), 181 (18), 152 (11), 139 (27), 123 (9), 107 (11), 79 (11), 75 (100), 58 (16); ^1H nmr ($CDCl_3$) 9.30 (H-1, d, J = 1.4 Hz), 6.64 (H-8, q, J = 6.9 Hz), 4.12 (H-3, dd, J = 7.7 Hz), 3.56 (COOMe, s), 3.22 (OMe, s), 3.20 (OMe, s), 2.67 (H_2-6, m, Js = 9 and 6.2 Hz), 2.01 (H_3-10, d, J = 6.9 Hz), 1.87 (H_2-4, m, Js = 6.4 and 5.6 Hz). *Anal.* calcd $C_{12}H_{20}O_5$, C 59.00%, H 8.25%; found C 58.83%; H 8.50%.

β-(3,4-DIHYDROXYPHENYL)ETHANOL [**3**].—The phenolic solution, obtained via NaOH extraction, was acidified with 2 N HCl. Compound **3** was extracted with EtOAc and identified by comparison with an authentic sample (tlc, hplc).

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