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ISOLATION OF CORNOSIDE FROM *OLEA EUROPAEA* AND ITS TRANSFORMATION INTO HALLERIDONE

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Key Word Index—*Olea europea*; Oleaceae; oleuropein; cornoside; halleridone; secoiridoid.

Abstract—*Olea europaea* contains, besides oleuropein, demethyloleuropein, verbascoside and ligstroside, cornoside which can be easily transformed into halleridone.

INTRODUCTION

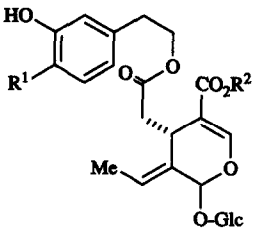
Olea europaea is a tree typical of the Mediterranean area where it is widely cultivated for oil production. Extensive studies have been performed on the chemical composition of the secondary metabolism of this plant with a view both to improving the organoleptic quality of the oil and to identifying the substances of this plant that influence host acceptance to *Dacus oleae* [1, 2]. The olive fruit fly deposits its eggs only in olives, causing the degradation of the mesocarp and consequently a low quality oil.

RESULTS AND DISCUSSION

We examined the phenolic glucosides present in *Olea europaea*, following their qualitative and quantitative changes during the ripening of the fruit. The extraction of these compounds must be accomplished by adding the olives to boiling methanol in order to avoid the action of very active enzymes present in the olives, which produce several transformations, including the hydrolysis of glucosidic linkages. The following compounds were detected.

Verbascoside, which we isolated some years ago from *Verbascum sinuatum* [3] and from olive leaves [4], is present only in traces in olives, but has been investigated by some authors. The changes in the contents of the most important phenolic glucoside of olives, oleuropein (**1**), present in green fruits, are very consistent. The content of **1** decreases as the olives ripen and falls to zero when they are completely black (*O. europaea*, var. *leccino*). Demethyloleuropein **2** [5], the acid derivative of oleuropein, replaces **1** in about the same amount, and is the major constituent of black olives [6, 7]. Quantitatively less important is the presence in small green olives of ligstroside **3** [8], which disappears when green olives reach normal size.

We have observed (TLC) the same trend for another compound, **4**, not yet found in *O. europaea*, as reported here. Compound **4** remains in water when the other glucosides **1–3** are extracted by ethyl acetate and was

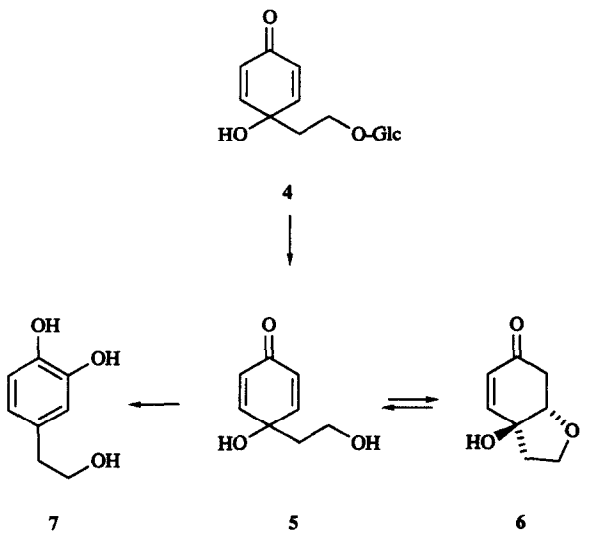


	R^1	R^2
1	OH	Me
2	OH	H
3	H	Me

isolated by RP-MPLC and purified by preparative TLC. Compound **4** is hydrolysed by β-glucosidase but the aglucone **5** is partially converted into another less polar compound **6**. The components of the mixture were separated by preparative TLC, but the same starting mixture was obtained from both of them, showing the existence of a rapid equilibrium. On the basis of ^1H and ^{13}C NMR spectra, glucoside **4** appears to be identical to cornoside [9], found previously in Oleaceae only in the *Forsythia* genus.

We have examined the ^1H and ^{13}C NMR spectra of the purified mixture **5/6**, arising from enzymatic hydrolysis of cornoside. From the analysis of the COSY spectrum it is possible to distinguish the signals of **5** from those of **6**. Compound **5** is the real aglycone corresponding to cornoside. In fact, the doublets relative to the quinol system at δ6.96 and 6.14 ($J = 10.0$ Hz) and the two triplets relative to the β-substituted ethyl-alcohol moiety at δ1.95 and 3.49 ($J = 7.0$ Hz) are clearly recognizable and are closely related to the corresponding signals of cornoside [4] (see Experimental).

The spectrum of the second compound **6** shows a doublet relative to only one α,β-unsaturated carbonyl moiety at δ6.84 and 5.98 ($J = 10.0$ Hz), an ABX_2 system relative to CH_2 in α position to oxygen (δ3.85 and 3.95,



$J_{AB}=8.0$ Hz, $J_{AX}=J_{BX}=7.0$ Hz) and an ABX system relative to a CH_2CH moiety (δ2.57 and 2.76, $J_{AB}=17.2$ Hz, $J_{AX}=5.6$ Hz, $J_{BX}=4.6$ Hz). The comparison of these data with those of ^{13}C NMR-APT spectrum of the same mixture, in which the signals relative to **5** can easily be separated from those relative to **6**, allow us to demonstrate the presence of **6**. A similar equilibrium is well known in flavonoid chemistry (chalcone–flavanone equilibrium) and is obviously due to the presence of the hemiquinone moiety.

Compound **6** (rengyolone [10] or halleridone [11]), described as a natural product is probably an artefact arising from the aglycone of **4**, according to the fact that rengyolone (**6**) is described as a racemic mixture of the *trans* isomers. Hendo and Hikino [10] affirm that rengyolone is a racemic mixture, although in their Experimental they attributed an α_D to this compound. As a result of the addition of the CH_2CH_2OH chain to the conjugated double bond, two asymmetric centres arise from the non-chiral aglycone **5**. Nevertheless the ether function is probably formed only on the side opposite to the tertiary hydroxyl, and the product **6** is the racemic mixture of the compounds with a *cis* junction between the rings.

Cornoside (**4**) seems to be related to the oxyphenylethyl alcohol moiety **7** present in the above secoiridoids. In fact, the aglycone **5** is an isomer of **7**, which could arise from **5** via an allylic transposition. In this connection, Jensen and Nielsen reported [private communication] the occurrence of **7** in several genera where iridoid glycosides linked with phenylpropanoid residues have been detected.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded using Varian XL-300 or Varian Gemini-200 spectrometers; chemical shifts are expressed in δ and coupling constants in Hz.

Isolation of natural compounds. Voucher specimens of *Olea europaea* are in the herbarium of Dipartimento di Biologia Vegetale–Università di Roma "La Sapienza". Green olives (100 g) were extracted with boiling MeOH (100 ml) for 30 min under reflux. After decantation of MeOH, the kernels were taken out and the remaining pulp was extracted with additional 100 ml of MeOH. Collected extracts were evapd *in vacuo* until MeOH elimination: the remaining aq. phase was exhaustively extracted with EtOAc until phosphomolybdic testing was negative. The organic phase was evapd *in vacuo*, affording 1.6 g of residue which, after chromatography on RP-8 silica gel in MeOH–H_2O (1:1) afforded 1.03 g of oleuropein (**1**) as amorphous powder and 450 mg of crude ligstroside (**3**). Purification of **3** was performed by prep. TLC, eluting with $CHCl_3$–MeOH (4:1) which afforded 150 mg of **3** as an amorphous powder. The aq. phase was evapd *in vacuo*, affording 900 mg of residue which was chromatographed on RP-8 silica gel in H_2O–MeOH (7:3) affording 85 mg of crude cornoside (**4**) . This was further purified on a prep. silica gel plate, eluting with $CHCl_3$–MeOH (4:1), giving 25 mg of pure **4**, which was identified by comparison with an authentic sample. ^1H NMR (D_2O), δ: 7.01 (H-2, H-6, *d*, $J=10.0$ Hz), 6.15 (H-1, H-5, *d*, $J=10.0$ Hz), 4.29 (H-1', *d*, $J=7.5$ Hz), 2.02 (2H-7, *t*, $J=7.0$ Hz).

Enzymatic hydrolysis of **4**. Compound **4** (170 mg) was dissolved in H_2O (4.5 ml) and 85 mg of β-glucosidase (EC 3.2.1.21) was added. The hydrolysis was complete after 2 hr at room temp. and the aglycone was extracted with Et_2O and EtOAc. The organic phases were dried over Na_2SO_4 and evapd *in vacuo*, affording 50 mg of residue, which appeared as a mixt. of **5** and **6**. Compound **5** can be sepd from **6** by chromatography on a prep. silica gel plate, eluting with $CHCl_3$–MeOH (9:1) but the equilibrium was rapidly restored. Compound **5** ^1H NMR (D_2O), δ:6.96 (H-2, H-6, *d*, $J=10.0$ Hz). 6.14 (H-3. H-5, *d*, $J=10.0$ Hz), 3.49 (2H-8, *t*, $J=7.0$ Hz), 1.95 (2H-7, *t*, $J=7.0$ Hz). Compound **6** ^1H NMR (D_2O), δ:6.84 (H-2, *d*, $J=10.0$ Hz), 5.98 (H-3, *d*, $J=10.0$ Hz), 4.20 (H-5, *d*, $J=5.0$ Hz), 3.95, 3.85 (2H-8, ABX_2, $J_{AB}=8.0$ Hz, $J_{AX}=J_{BX}=7.0$ Hz), 2.76 (2H-6, ABX, $J_{AB}=17.2$ Hz, $J_{AX}=5.6$ Hz, $J_{BX}=4.6$ Hz), 2.22 (2H-7, *t*, $J=7.0$ Hz).

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