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Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids

from *Olea europaea* L.

By

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## **Abstract**

The genus *Olea* contains the economically important European olive tree (species *Olea europaea* L.). This species is also of chemotaxonomic interest because of the presence of various phenol-conjugated oleosidic secoiridoids or oleosides. The chemistry of these phenolic oleosides is diverse and complicated. It is only in recent years that attention has been given to their biosynthesis and biotransformations during processing and storage of olive products. Many questions regarding these processes remain unanswered and, yet, these have significant impact on the quality and value of olive products such as olive oil.

## **Introduction**

*Olea europaea* L. belongs to the Tribe Oleae, Family Oleaceae<sup>1</sup> which comprises approximately 600 species in some 25 genera including *Forsythia*, *Fraxinus*, *Jasminum*, *Ligustrum*, *Olea*, and *Syringa*. Oleaceae are especially abundant in temperate and tropical Asia. They are of economic and aesthetic importance and provide many commercial products such as food, lumber, cosmetics and edible olive oil. Some provide ornamental features such as *Forsythia spp* which have a vibrant yellow spring color and *Fraxinus* (the ashes) with their autumnal colour are also noted for their hardwood timber. At the molecular level, members of the family contain a diverse array of secoiridoid derivatives making them of chemotaxonomic interest.

The secoiridoids are derived from iridoids (Figure 1) via opening of the cyclopentane ring of the iridoids. The latter (eg. loganin) are monoterpenes characterized by a bicyclic fused ring system comprising a 6-membered heterocyclic ring fused to a cyclopentane ring. Iridoids are abundant in Oleaceae and many other plants.<sup>2-5</sup> Jensen et al.<sup>6</sup> have reviewed the distribution and

biosynthesis of iridoids in the *Oleaceae* family; and their terminology for the various biosynthetic pathways is adopted in this review. Although two known routes for the production of iridoids exist, *Oleaceae* is characterised by the presence of iridoids derived only from the pathway presented in Figure 2 (termed route I by Jensen et al.),<sup>6</sup> namely, biosynthesis of deoxyloganic acid from iridodial via iridotrial.

Most of the iridoids in *Oleaceae* are secoiridoids derived from deoxyloganic acid as the common intermediate with many secoiridoids produced directly from route I via loganin and secologanin. However, at least five different subroutes exist within the family, with branching out occurring from deoxyloganic acid. Different pathways are attributed to different genera but a pathway up to and including deoxyloganic acid is probably a common feature within the *Oleaceae* family.<sup>7-9</sup> The secoiridoids characterized by an exocyclic 8,9-olefinic functionality are termed oleosidic secoiridoids or oleosides. Interestingly, the oleosides form several phenolic conjugates that are unique to oleaceous plants.

The genus *Olea* includes the economically important European olive tree (species *Olea europaea* L.). Jensen et al.<sup>6</sup> provided limited data for this genus. This can be attributed to the low rate of water uptake by plants in this genus and the corresponding difficulty of conducting biosynthetic studies. On the other hand, water uptake is more rapid in plants of the Genera *Fraxinus* and *Syringa* and much of our detailed knowledge of biosynthesis in *Olea europaea* is inferred from studies of these genera. From such studies it appears that 7-epi-loganin/7-epi-loganic acid are the key intermediates in the biosynthesis of most of the oleosides. This involves pathways (Figure 3) designated as 1d and 1e by Jensen et al.<sup>6;10</sup>

In the last decade, considerable effort has been expended on identifying new conjugated oleosides in *Olea europaea* L. and determining their chemistry during processing and storage<sup>11-23</sup> of olive oil, and their bioactivity due to increasing interest in the potential health benefits of a Mediterranean diet. This review examines various aspects of the complex chemistry of oleosides in which the conjugated moiety is phenolic. These economically important compounds have a restricted distribution<sup>24</sup> and data relevant to their biosynthesis and degradation in *Olea europaea* L. are examined. The review is very timely as a member of this class of compound has recently been identified as having physiological properties akin to those of the non-steroidal anti-inflammatory drug ibuprofen.<sup>25</sup> It is hoped that the review will stimulate progress in the study of these compounds.

The range of compound types covered by the review is illustrated in Table 1. Oleuropein and ligstroside, the most significant oleosides in *Olea europaea*, are esters of elenolic acid with 2-(3,4-dihydroxyphenyl)ethanol (hydroxytyrosol) and 2-(4-hydroxyphenyl)ethanol (tyrosol), respectively. They are found in all the constituent parts of the olive fruit whereas related compounds such as salidroside, nüzhenide and nüzhenide oleoside appear to be restricted to the seed, albeit at all ripening stages.<sup>26</sup> Oleuropein is not restricted to the *Olea* genus but occurs also in many other genera belonging to the *Oleaceae* family including *Fraxinus excelsior*,<sup>27</sup> *F. Chinensis*,<sup>28</sup> *Syringa josikaea* and *S. vulgaris*,<sup>27</sup> *S. Dilatata*,<sup>29</sup> *Ligustrum ovalifolium*,<sup>27</sup> *Jasminum polyanthum*<sup>30</sup> and *Osmanthus asiaticus*.<sup>31</sup>

As we move to a more mature phase of research on these compounds, the availability of pure materials as reference compounds assumes a greater importance. A limited number of these secoiridoid derivatives are now commercially available. Of these, oleuropein is the most significant and Extrasynthese is a common supplier. However, the material typically generates

three main peaks in reversed phase LC-MS; two of these have identical UV spectra, both fluoresce at 330 nm and have a molecular mass of 540 amu. The first eluting peak, the major component, is oleuropein itself while the later eluting peak has been identified as oleuroside.<sup>21;39;52;53</sup> A third component elutes at an intermediate retention time and is an oleuropein derivative. The presence of more than one compound in a commercial standard may interfere with bioactivity studies, while the availability of suitably labelled precursors is a limitation for biosynthetic studies.

### **Bioactivity**

Secoiridoid conjugates exhibit a diverse range of bioactivities.<sup>54-57</sup> Moreover, natural products such as olive oils exhibit variation in secoiridoid content. For instance, secoiridoid derivatives were the major compounds in Seggianese oils whereas the major compounds of Taggiasca oils were lignan derivatives.<sup>58</sup> Both oils dose-dependently inhibited the copper(II) oxidation of human LDL but the Seggianese oil was more effective.

The dialdehydic form of (-)-deacetoxy-ligstroside aglycon or oleocanthal (see Table 1) is found in extra-virgin olive oil<sup>25</sup> and induces a strong stinging sensation in the throat, not unlike that caused by the non-steroidal anti-inflammatory drug ibuprofen (Table 1). This similar perception seems to be an indicator of a shared pharmacological activity in that both molecules inhibit the same cyclooxygenase enzymes in the prostaglandin biosynthesis pathway.<sup>25</sup> The concern that the active component may have been a minor contaminant was 'eliminated' by the *de novo* synthesis of (-)-oleocanthal. Whether (-)-oleocanthal has *in vivo* bioactivity has been questioned.<sup>59</sup> Very recently, other *in vitro* bioactivities of oleocanthal have been reported: anti-proliferative activity<sup>60</sup> and anti-bacterial activity against helicobacter pylori.<sup>61</sup>

Obied et al.<sup>62</sup> extensively reviewed the bioactivities of secoiridoids derived from *Olea europaea*. The following discussion presents more recent examples of bioactivities. Because of the interest in the potential health benefits of olive oil, several studies imply bioactivity of phenolic conjugated secoiridoids because of their presence in olive oil (e.g.<sup>63</sup>). However in this review, we have focussed on those reports where pure compounds were used. Moreover, we have distinguished tests that are *in vitro*, *in vivo* and *ex vivo*. Of the twelve biological activities listed by Obied et al.<sup>62</sup> for oleuropein and elenolic acid, no new work (as determined by ISI citation searching) has been reported for antihypertensive, endocrinal, enzyme modulation and cytostatic activities for oleuropein; nor for antimicrobial and antiviral activities for elenolic acid.

Antioxidant and related activities of oleuropein have dominated the literature since the Obied review. *In vitro* studies by Briante et al.<sup>64</sup> and Masella et al.<sup>65</sup> reported protection of LDL against Cu<sup>2+</sup> and cell-mediated oxidation. Oleuropein was demonstrated to have *ex-vivo* antioxidant activity in a rat heart.<sup>66</sup> Anti-ischemic, hypolipidemic as well as antioxidant activities were reported *in vivo* (rabbit) by Andreadou et al.<sup>67</sup> The inflammation response is believed to play an important part in cardiovascular disease and anti-inflammatory activity of oleuropein has been reported *in vitro*<sup>68</sup> and *in vivo*.<sup>69;70</sup> Oleuropein aglycon, which is more abundant in olive oil, has been reported to protect against vascular risk through the down-regulation of adhesion molecules involved in early atherogenesis<sup>71</sup> in an *in vitro* assay using human umbilical vascular endothelial cells.

While the above activities represent the majority of studies on oleuropein, various other bioactivities have been reported, including several recent studies on *in vitro* antimicrobial and antiviral activities. Zanichelli et al.<sup>72</sup> reported *Staphylococcus aureus* inhibition, whereas Caturla et al.<sup>73</sup> proposed anti-microbial activity based on oleuropein being membrane active. Anti-viral

activities against HIV (HIV-1 integrase inhibitor)<sup>74</sup> and haemorrhagic septicaemia rhabdovirus<sup>75</sup> have also been reported. Bazoti et al.<sup>51</sup> described the *in vitro* binding of oleuropein to amyloid proteins with implications for Alzheimer's diseases. A study by Al-Azzawie and Alhamdani<sup>76</sup> was able to show *in vivo* hypoglycaemic activity of oleuropein in rabbits. Oleuropein was tested for effects on human Th1 and Th2 cytokine production, but was found to have no *in vivo* activity, whereas kaempferol did.<sup>69</sup>

### **Biosynthesis**

Secoiridoid conjugates such as oleuropein that contain an esterified phenolic moiety result from a branching in the mevalonic acid pathway in which terpene synthesis (oleoside moiety) and phenylpropanoid metabolism (phenolic moiety) merge. This is illustrated schematically in Figure 4. Phenylpropanoid metabolism is generally well documented<sup>77-79</sup> whereas pathways leading to the secoiridoids are not as well established. The majority of the literature concerning secoiridoids relates to the discovery and structural elucidation of new compounds<sup>80</sup> and/or chemotaxonomy<sup>81</sup> rather than biosynthesis. For instance, the presence of oleoside-type secoiridoids in Oleaceae, which is shared by the family Loasaceae, confirm a common ancestry. However, the presence of the oleoside-type iridoids in Loasaceae and the differences of the biosynthetic pathways between Oleaceae and Loasaceae justify the placement of Loasaceae in its own order.<sup>6</sup>

Biosynthetic studies employ a range of techniques to infer metabolic pathways. Common approaches are monitoring of target metabolites and use of radiolabelled substrates. The former approach is probably easier and metabolic inference is then gained by comparison of the analyte fluxes over time. A central issue in metabolic studies is that the level and pattern of a compound is a result of catabolic processes and turnover. Historical approaches such as target analysis and



metabolite profiling<sup>82</sup> suited the traditional linear view of a metabolic pathway. However, their limitations have been recognised with the realisation that metabolic pathways do not act in isolation but rather as part of an extensive network. This has led to development of metabolomics as a holistic comprehensive approach to metabolite analysis.<sup>83</sup>

Given the significance of olive in this family it is not surprising that olive drupes (*Olea europaea*) have been selected to investigate secondary metabolism of oleaceae secoiridoids.<sup>40</sup> A number of new components have recently been identified in olive drupe tissues.<sup>40;46</sup> Several of these new compounds were closely related to oleuropein but others, such as neo-nuzhenide and 2'-hydroxyoleuropein have never been found in olive tissues and were typical of other oleaceae families. Such discoveries may provide further insight into the secondary metabolism of oleaceae secoiridoids.

Oleuropein was the first of the oleosides to be reported<sup>84</sup> and has now been studied extensively.<sup>80</sup> Metabolic profiling has established that the concentration of oleuropein reaches relatively high levels in immature fruit of some cultivars of *Olea europaea* during the growth phase and declines with physiological development of the fruit.<sup>85-88</sup> Although turnover may be simply related to recycling of phenolic moieties into new conjugates, the changes associated with oleuropein appear to involve more extensive degradation. This conclusion is based on the accumulation of demethyloleuropein and elenolic acid glucoside that accompanied oleuropein degradation.<sup>87</sup> Amiot et al.<sup>88</sup> suggested a metabolic relationship between oleuropein and the non-secoiridoidal biophenol, verbascoside, since oleuropein decreased in olive pulp with maturation, whilst the concentration of verbascoside and demethyloleuropein increased with ripening.<sup>23</sup> The inverse relationship between oleuropein and verbascoside was not supported by the findings of Ryan et al.<sup>89</sup> in that metabolism of oleuropein in *Hardy's Mammoth* olive pulp differed as a

result of alternate bearing. In a high fruiting season, oleuropein (and verbascoside) concentrations were found to increase with fruit ripening. This was inversely related to 3,4-DHPEA-EDA concentrations in olive pulp and interestingly, this inverse relationship was also evidenced in olive leaves. In the following low fruiting season, concentrations of both oleuropein and 3,4-DHPEA-EDA in olive pulp were found to decline with fruit ripening, consistent with previous literature. Verbascoside concentrations remained relatively stable. Olive leaves were the only *Hardy's Mammoth* tissue to contain quantifiable amounts of oleuroside, and interestingly, the metabolism dynamics of oleuroside precisely paralleled that of oleuropein. This suggests that the two isomers are not in equilibrium but rather that oleuroside synthesis is sustained from the conversion of oleuropein, which is always in higher concentrations than oleuroside. In both new and old season leaves, oleuropein and oleuroside showed a general increase in concentration in the high fruiting season, followed by declining concentrations in the following low fruiting season.<sup>89</sup> Nüzhenide, the predominant phenolic secoiridoid in olive seeds, was found to exist in higher concentrations in seeds at the beginning of the low-fruiting season compared to that of the high-fruiting season. This phenomenon was also observed for oleuropein and verbascoside in olive seeds. Collectively, such results highlight the significant impact of alternate bearing in oleoside metabolism.

The use of radiolabelled precursors in metabolic studies is one of the most routinely employed methods for elucidating biochemical pathways and metabolic functioning within plants. Labelled precursor may be applied to plant shoots, cell free systems (i.e. a crude mixture of enzymes from the organism) or to tissue cultures (typically undifferentiated callus cells). Early feeding experiments<sup>90;91</sup> on *Olea europaea*, in which secologanin was incorporated into oleuropein at 0.34% suggested a possible route to the oleosides via secologanin followed by oxidation at C-7 and subsequent rearrangement of the 8,10-double bond to oleoside 11-methyl

ester. It has been claimed that the intermediacy of secologanin is supported by the occurrence of oleuroside in an oleaceous plant.<sup>39</sup> However, conclusions from these studies have been rejected<sup>27</sup> because of the low percentage incorporation rates of the label. Low unpredictable incorporation rates are a common problem particularly with many oleaceous plants where the situation is exacerbated by the presence of a multitude of secoiridoids leading to extensive dispersal of the incorporated label.

The problems of low water uptake rate and low incorporation of radiolabel were overcome by working with *Fraxinus* and *Syringas* where there is a more rapid water uptake and incorporation of radiolabelled deoxyloganic acid, 8-epi-deoxyloganic acid, loganic acid and 7-epi-loganic acid.<sup>7-9;27</sup> High incorporations of deoxyloganic acid, loganic acid and its 7-epimer were observed. The incorporation of loganic acid and its 7-epimer to the same extent strongly suggests that 7-ketologanic acid is an intermediate in the biosynthesis of the oleosides. Unlike 'normal' secoiridoids in the Gentianales family produced<sup>6</sup> via loganin in route 1, oleosidic secoiridoids in Oleaceae are produced via 7-epi-loganin or 7-epi-loganic acid (Figure 3).<sup>7;8;27</sup> Two subroutes can be distinguished depending on species (Figure 3) as route 1d in *Syringa josikaea* and 1e in *Fraxinus excelsior*, *Syringa josikaea* and *Syringa vulgaris*.

The proposed biosynthetic pathway for the formation of oleuropein in Oleaceae<sup>27</sup> is shown in Figure 5, with ligstroside a direct precursor to the production of oleuropein. Ryan et al.<sup>21</sup> have proposed a possible alternate biosynthetic pathway for the production of oleuropein and the structurally related 2-(3,4-dihydroxyphenyl)ethyl (3*S*,4*E*)-4-formyl-3-(2-oxoethyl)hex-4-enoate ester (3,4-DHPEA-EDA) in Hardy's Mammoth (Figure 6). 3,4-DHPEA-EDA has been variously described as the deacetoxy dialdehydic form of elenolic acid linked to hydroxytyrosol,<sup>92</sup> oleacin<sup>47;93</sup> and 3,4-dihydroxyphenylethyl 4-formyl-3-formylmethyl-4-

hexanoate.<sup>94</sup> Its presence has been attributed to oil-processing methods<sup>23;43;92</sup> and its derivation from oleuropein. Whilst 3,4-DHPEA-EDA is not oleosidic in structure, its precursory role in the production of oleuropein is significant, and was validated by isotopic labelling of olive shoots.<sup>21</sup> The authors suggested that the bioformation of 3,4-DHPEA-EDA was cultivar, season and environment dependent.

The induction of phenylpropanoid metabolism by boron deficiency is well documented whereas the effects of nutrient deficiencies on the secondary metabolites derived from the mevalonic acid pathway have been neglected.<sup>48</sup> Two novel secoiridoid glycosides, 6'-*E-p*-coumaroyl-secologanoside and 6'-*O*-[(2*E*)-2,6-dimethyl-8-hydroxy-2-octenoyloxy]-secologanoside, were isolated together with three known secoiridoid glycosides, oleuropein, oleoside dimethyl ester, and secologanoside from methanolic extracts of boron-deficient *Olea europaea* leaves.<sup>48</sup> The profile of secondary metabolism is highly affected by boron deficiency<sup>95</sup> which is the most frequent micronutrient disorder in olive orchards. The effects of deficiency were most notable in hydroponically grown plants but the accumulation of the specific secoiridoids occurred in field plants also. Their synthesis was attributed to a physiological response to the mineral deficiency. Nevertheless, at least one of these compounds has recently been identified in olive fruit grown under normal conditions without known boron deficiency.<sup>46;96</sup>

## **Degradation**

The commercial significance of *Olea europaea* has focused attention on processing- and storage-induced changes in the oleosides in virgin olive oil and table olives. For example, oleuropein and ligstroside derivatives plus  $\alpha$ -tocopherol decreased following pseudo-first order kinetics during 8 months storage in the dark of extra virgin olive oils.<sup>97</sup> The oleuropein derivatives were less stable than the corresponding ligstroside derivatives and  $\alpha$ -tocopherol due

to their higher antioxidant activity.<sup>98</sup> In any case, the degradation observed in the oleosides during virgin olive oil processing and oil storage are due to hydrolytic and oxidative reactions.

The appearance of derivatives such as 3,4-DHPEA-EDA in products such as virgin olive oil and olive mill waste raises the issue of the origin of such entities and the enzymatic and/or chemical degradation of the oleosidic secoiridoids. An obvious route for the degradation of oleuropein and related compounds involves cleavage by specific endogenous esterases<sup>99</sup> to either elenolic acid glucoside or demethyloleuropein, which are both found in ripe olives. Alternatively, activation of endogenous  $\beta$ -glucosidases during crushing and malaxation may produce the aglycon from the glycoside (Figure 7). The proposed mechanism that may explain the formation of 3,4-DHPEA-EDA in olive pastes during crushing (see Figure 7) presumes that demethyloleuropein in the fruit acts as a precursor for the formation of 3,4-DHPEA-EDA. However, the occurrence of demethyloleuropein in olives is cultivar dependent,<sup>100</sup> and thus it would be expected that 3,4-DHPEA-EDA would only be found in olive oils produced from olives that contained high concentrations of demethyloleuropein. Contrary to this, many analytical studies demonstrate that 3,4-DHPEA-EDA is one of the most concentrated oleosides in virgin olive oils and by-products (such as vegetation waters and pomaces) produced from fruit cultivars with low concentrations of demethyloleuropein.<sup>100</sup> The enzymatic production of 3,4-DHPEA-EDA from demethyloleuropein is well known and largely studied in a model system,<sup>44</sup> however the formation mechanism(s) of 3,4-DHPEA-EDA and 4-HPEA-EDA from oleuropein and ligstroside, respectively, is/are still unknown. Bianco et al.<sup>45</sup> in studying the hydrolysis of oleuropein glucoside by  $\beta$ -glucosidase in a model system provided evidence for the formation of the dialdehydic form of oleuropein aglycon as the final product of this enzymatic reaction; interestingly, 3,4-DHPEA-EDA was not found. In contrast with the results in a model system, Rovellini & Cortesi<sup>101</sup> found very low amount of the dialdehydic form of

oleuropein aglycon in virgin olive oil, as a potential derivative of oleuropein hydrolysis, while the main compound was the 3,4-DHPEA-EDA. However, the latter compound could be the final product of demethyloleuropein enzymatic hydrolysis.<sup>44</sup> Wide variation in demethyloleuropein concentrations in olive fruit are well known<sup>87;88;102</sup> however these differences do not affect the secoiridoid derivatives' concentration in virgin olive oils. The concentration of these compounds is influenced by the overall concentrations of both demethyloleuropein and oleuropein. As a consequence, it is possible to assume that the enzymatic transformation of oleuropein to 3,4-DHPEA-EDA may include in its biochemical mechanism the activity of a methylsterase.<sup>100</sup>

The effects of processing operations such as malaxation on the oleosidic conjugates<sup>103;104</sup> are complicated by various isomerizations and equilibria<sup>105</sup> between different functionalities. This is illustrated in Figure 7 for oleuropein where the aglycon, II was stable in the solid state for two days<sup>13;45</sup> but was degraded in aqueous solution to III.<sup>45;80</sup> Different mechanisms have been proposed<sup>45;80;106</sup> for the formation of III with diastereoisomer IIIa being formed as the kinetic product but it isomerised slowly to IIIb. Compound V was formed in aprotic solvents via ring opening and keto-enol tautomerism. Hemiacetals VII were formed in methanolic solution.<sup>106</sup> Compound VI was the ultimate product in all cases and its formation was favoured at a lipidic/water interface at which surface conversion from the dialdehyde, V occurred within 5 min.

The final composition of secoiridoid derivatives in virgin olive oil is affected by the activity of  $\beta$ -glucosidase, however peroxidase (POD) and polyphenoloxidase (PPO) are also involved in the oxidative reactions that define the final concentration of the secoiridoid derivatives in the malaxed pastes and their corresponding virgin olive oils. Several papers published during the last ten years<sup>103;107-111</sup> reported the impact of these enzymes in reducing the concentration of

secoiridoid derivatives during malaxation in virgin olive oil. These enzymes show a strong effect to reduce the 3,4-DHPEA-EDA and 3,4-DHPEA-EA concentrations, while the oxidative degradation of the 4-HPEA-EDA was lower. These results can be explained by considering the different efficiencies of the PPO and POD to oxidize *o*-diphenol and monophenol compounds. The control of the oxidative reactions, catalysed by these enzymes, is the most important aspect that, from a technological point of view, defines the concentration of secoiridoid derivatives in virgin olive oil. So far, however, while the enzymatic oxidative mechanism that affects phenols is well known and largely studied in various fruits and vegetables, the compounds that originate from the enzymatic oxidation of secoiridoid derivatives such as 3,4-DHPEA-EDA, 3,4-DHPEA-EA and the 4-HPEA-EDA remain unknown. As a consequence, other iridoid derivatives that can originate not only from the hydrolytic reactions, catalysed by the  $\beta$ -glucosidase, but also from the enzymatic oxidation of the aglycon derivatives of oleuropein, demethyoleuropein and ligstroside may occur in virgin olive oil and in the by-products of the oil mechanical extraction process. The study of the oxidative products of secoiridoid derivatives could be important also in terms of virgin olive oil shelf-life investigations. In fact, as previously reported,<sup>112-115</sup> iridoids in virgin olive oil decrease during storage, but their degradation is related not only to their antioxidant activity. Hydrolytic reactions are also involved in the secoiridoid derivatives degradation during oil storage.<sup>112;113;116</sup> In fact, in the cloudy oils, the hydrolysis of the 3,4-DHPEA-EDA, 3,4-DHPEA-EA and the 4-HPEA-EDA was supported by the loss of these compounds and a corresponding increase of the 3,4-DHPEA and p-HPEA during oil storage.

## **Conclusion**

Oleosides derived from *Olea* are chemically diverse and many questions remain regarding their biosynthesis and degradation. As the world becomes more diet conscious, extra virgin olive oil consumption will increase and these compounds are essential for its quality. Hence, they are of

significant economic importance. Moreover, other olive products such as leaf extracts are increasing in market value. In order to understand their potential health benefits in the diet and in dietary supplements, the pharmacokinetics and pharmacodynamics of these oleosides must be investigated. This, in turn, will require a detailed understanding of their biochemistry and transformation by human enzymes.

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## CAPTIONS

Table 1. Chemical structure and name of relevant phenol-conjugated oleosidic secoiridoids

Figure 1. General structure of iridoid and secoiridoid skeletons showing numbering system.

Figure 2. Biosynthetic pathway to the common precursor deoxyloganic acid. Identified as route I by Jensen et al.<sup>6</sup>

Figure 3. Biosynthetic pathways leading to formation of oleosides. Pathways identified as route Id and Ie by Jensen et al.<sup>6</sup>

Figure 4. Schematic diagram showing the links between phenylpropanoid metabolism and the mevalonic acid pathway

Figure 5. Proposed biosynthetic route for the formation of oleuropein in *Oleaceae*<sup>27</sup>

Figure 6. Possible biosynthetic pathway for the production of oleuropein and 3,4-DHPEA-EDA in *Olea europaea*. From Ref.<sup>21</sup>

Figure 7. Biotransformations of oleosides as illustrated for oleuropein (R = hydroxytyrosol) during maturation (biotransformation), processing, extraction, and sample handling. Compounds are identified as follows: (I) oleuropeindial, enol form, (II) oleuropein aglycon, (III) oleuropein, (IV) demethyloleuropein, (V) demethyloleuropein aglycon, (VI) enol form of

demethyloleuropein aglycon, (VII) demethyloleuropein aglycon dialdehyde, (VIII) 4-noroleuropein aglycon (3,4-dihydroxyphenyl ethyl alcohol decarboxymethyl elenolic acid dialdehyde or 3,4-DHPEA-EDA), (IX) 3,4-DHPEA-EDA acetal, (X) oleoside methyl ester, (XI) elenolic acid, (XII) oleuropeindial (keto form), (XIII) Cannizzaro-like product of oleuropeindial, (XIV) lactone of XIII, (XV) oleuropeindial (monohydrate), (XVI) elenolic acid dialdehyde, (XVII) oleoside, (XVIII) acetal of XIX, (XIX) decarboxymethyl elenolic acid dialdehyde DEDA, (XX) demethyloleuropein aglycon acetal, (XXI) Cannizzaro-like product of XIX, (XXII) lactone form of XXI, (XXIII) demethyl elenolic acid, (XXIV) elenolic acid monoaldehyde (rearrangement product), (XXV) hydroxytyrosol elenolate (oleuropein aglycon aldehyde form or 3,4-DHPEA-EA). Reproduced from Ref.<sup>37</sup>