Oleokoronal and oleomissional: new major phenolic ingredients of extra virgin olive oil

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Abstract

Extra virgin olive oil contains significant quantities of polar phenolic ingredients. The large majority is made up of esters of tyrosol or hydroxytyrosol with secoiridoid derivatives from oleuropein or ligstroside. In the current study we describe a number of new or incompletely characterized forms of ligstroside and oleuropein aglycons. Two of them which are stable enolic forms are described for the first time as real olive oil ingredients although their presence in olive oil had been postulated. To minimize the confusion with the complicated names of the aglycon isomers we propose the names oleokoronal and oleomissional for the two ingredients. After screening 2000 samples of olive oil from most major varieties we were able to identify samples of olive oil in which oleokoronal and oleomissional were the major phenolic ingredients and could be used as starting material for their isolation. Interestingly, during normal or reversed phase chromatography both compounds were transformed to the known forms of monoaldehydic closed ring aglycons, which offers an explanation as to why those compounds had not been identified so far. Their real presence in olive oil was confirmed by direct NMR observation without the use of any solvent.

Key words

Phenolics, secoiridoids, ligstroside aglycon, oleuropein aglycon, olive oil, NMR.

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Introduction

The traditional Mediterranean diet, which is attracting continuous interest from the scientific community because of its health protecting properties, is based on the daily consumption of olive oil as the major source of lipids. Secoiridoid phenolic derivatives are one of the most important classes of constituents in olive oil which present an increasing potential for health protection. European Union legislation (EU 432/2012) based on the scientific opinion of EFSA has recently permitted specific health claims related to the levels of specific phenolic compounds found in olive oil.

The key compounds that are responsible for the recognized health claim "protection of blood lipids from oxidative stress" are hydroxytyrosol (**1**), tyrosol (**2**) and their derivatives. For this reason, it is very important to obtain accurate knowledge of the chemical identity of all these ingredients and to perform their quantitative measurement in olive oil. As yet there is no officially adopted method for the measurement of the ingredients related with the health claim because of well known technical difficulties. Hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) are found in olive oil mainly in the esterified forms of oleacein (3,4-DHPEA–EDA) (**3**) and oleocanthal (p-HPEA-EDA) (**4**) as well as oleuropein aglycon (3,4-DHPEA-EA) (**5a**) and ligstroside aglycon (p-HPEA-EA) (**6a**), which all have significant biological activities.

However, the terms oleuropein aglycon and ligstroside aglycon are not accurately defined and are often used in a misleading way. In fact, there are many possible isomers of the aglycons and many of them are not well characterized. They are often reported with complicated descriptive names like: hydroxylated form, monoaldehydic form, dialdehydic form, hydrated form, open ring, closed ring, carboxylated, decarboxylated etc. The lack of accurate and official definitions of names and of robust NMR data is the source of several problems in the related literature. In the current paper we discuss the isolation and structure elucidation of a series of previously undescribed forms of oleuropein aglycon and ligstroside aglycon and we clarify the terms and the NMR characterization of the previously described members of this family of compounds.

Materials and methods

General

Almond β-glucosidase was purchased from Sigma-Aldrich. Oleuropein was isolated from leaves of wild olive trees with a high oleuropein content (15% per dry weight) as previously described (Andreadou *et al* 2006). NMR spectra were recorded on an Avance 700 spectrometer; chemical shifts were expressed in ppm and the axes were calibrated on the residual signal of $CDCl_3$. Column chromatography was performed on columns containing RP-18 Si gel 60 (40–63 μm) (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on plates coated with RP-18 Si gel 60 F254 Merck, 0.25 mm.

Olive oil

The isolation of the studied compounds was performed in two types of oil: the first was provided by the Cooperative of Paleopanagia, Lakonia, Greece and was produced in November 2013 from the Koroneiki variety in a three phase mill operating at 25 ºC with a 30 min malaxation period. The second was provided by Berkeley olive grove, Oroville, California and was produced from the Mission variety in November 2013.

Other olive oils used for screening came from the sample database as previously described (Karkoula *et al* 2014).

Extraction and isolation

Olive oil (100 g) was mixed with cyclohexane (400 mL) and acetonitrile (500 mL) and the mixture was homogenized and centrifuged at 4,000 rpm for 5 min. The acetonitrile phase was collected using a separation funnel, and evaporated under reduced pressure using a rotary evaporator. The residue was subjected to reversed phase silica gel column chromatography with acetonitrile 100% to remove the residual lipids. All the collected fractions that were free of lipids were pooled, evaporated and rechromatographed on a reversed phase preparative TLC, (H_2O/acetonitrile 60:40), resulting in the isolation of two zones: A (2.3 mg/ rf = 0.5) and B (3.3 mg/ rf = 0.7). The structure of the isolated compounds (**Figure 1**) was studied using a combination of 1D and 2D NMR spectra and the assigned peaks are presented in **Tables 1-4**.

Table 1. ^{1}H-NMR data of oleokoronal (14) and ligstrodials (12a,b) at concentration 3 mg/0.6 mL. Axis calibration based on $CDCl_3$ = 7.26 ppm

	12a (5S,4R)	12b (5S,4S)	14
1	9.212, d, 2.0	9.218, d, 2.0	9.225, d, 1.7
3	9.68, d, 2.7	9.46, d, 2.7	7.386, dd, 12.6, 0.8
4	4.06, dd, 10.5, 2.7	4.11, dd, 10.5, 2.7	-
5	3.83, m, (overlap)	3.83, m (overlap)	4.16, ddd (overlap)
6a	2.81, (overlap)	2.82, (overlap)	2.97, dd, 16.1, 9.6
6b	2.62, (overlap)	2.58, (overlap)	2.78, dd, 16.1, 6.3
8	6.70, q. 7.1	6.72, q, 7.1	6.56, q, 7.1
10	2.055, d, 7.0	2.062, d, 7.1	2.062, d, 7.0
3-OH	-	-	11.75, d, 12.6
OCH3	3.65, s	3.77, s	3.75, s
1'	4.20, m	4.18, m	4.18, m
2'	2.81, m	2.81, m	2.81, m
4',8'	7.05, d (overlap)	7.05, d (overlap)	7.05, d (overlap)
5', 7'	6.76, d (overlap)	6.76, d (overlap)	6.76, d (overlap)

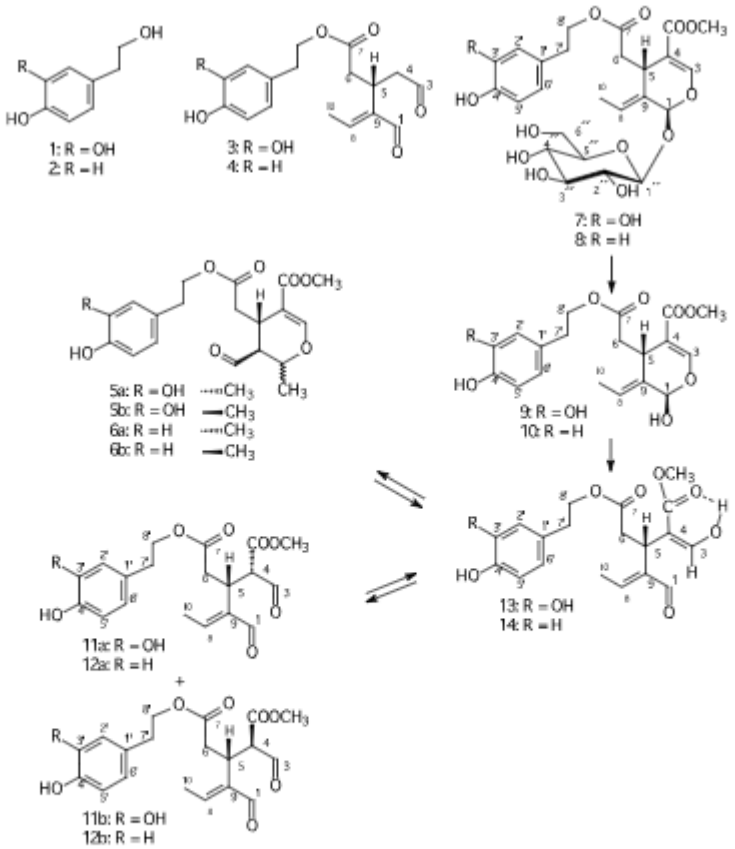

Figure 1: Structures of the studied compounds