LIPOXYGENASE INHIBITORY ACTIVITY OF OXIDIZED RESVERATROL METABOLITE MIXTURES

Orinamhe Godwin Agbadua[1], Attila Hunyadi[1,2,*]

[1] *Institute of Pharmacognosy, University of Szeged, Eötvös str. 6, 6720 Szeged, Hungary*
[2] *Interdisciplinary Centre for Natural Products, University of Szeged, Eötvös str. 6, H-6720 Szeged, Hungary*
**e-mail: hunyadi.a@pharmacognosy.hu*

Abstract
Resveratrol, a natural polyphenol, has numerous biological activities such as anticancer, antioxidant, anti-inflammatory. Resveratrol is highly oxidisable due to its chemical structure, and reacts with reactive oxygen species or free radicals resulting in new metabolites that are also potentially bioactive. Oxidation of resveratrol in various conditions resulted in mixtures that exhibited greater lipoxygenase inhibitory activities compared to the parent compound. This, together with the complex chromatographic fingerprints of the mixtures suggests that several oxidized metabolites of resveratrol may exert greater biological activity than their parent compound.

Introduction
Resveratrol is a well-known polyphenol with plethora of pharmacological activities such as anticancer, antioxidant, anti-inflammatory, cardio-protective and neuroprotective [1,2]. Most polyphenols are not stable, especially under oxidative conditions and often lead to the accumulation of new metabolites in living systems [3]. These new metabolites have been suggested to mediate at least some biological effects of the parent compound [4]. One of such studies showed a greater lipoxygenase (LOX) inhibitory activity of Fe-catalyzed oxidation of resveratrol and led to the identification of some active metabolites that, however, did not fully explain the increase in bioactivity [5].
LOX accelerates lipid oxidation by converting arachidonic, linoleic and other polyunsaturated fatty acids into biologically active metabolites, which are implicated in the pathogenesis of a variety of human diseases. Therefore, it is a promising therapeutic target for the prevention and/or treatment of a wide spectrum of human diseases [6].
Thus, we aimed at preparing oxidized resveratrol metabolites through various chemical reactions including biomimetic approaches, and to study the lipoxygenase inhibitory activity of the mixtures obtained.

Experimental
Resveratrol with a purity 97%, was purchased from Career Henan Chemical Co., China. Several oxidative reactions were carried out on resveratrol as shown in Table 1. The reactions were monitored by TLC at regular intervals. At the end of each reaction, the mixtures were evaporated, extracted with ethyl acetate and evaporated. As a prepurification step, each residue was filtrated through silica with hexane – acetone (1:1, v/v). A ten microliters aliquot of each mixture dissolved in CH_3CN was analized by HPLC (PU-2080 pumps; AS-2055 Plus autosampler; MD-2010 Plus PDA detector, Jasco Co., Tokyo, Japan) under the following conditions: column, Kinetex XB-C18 (250 x 4.6mm, 5_{μ}); solvent system, water (solvent A) and CH_3CN (solvent B): elution, linear gradient from 25% solvent B to 75% solvent B for 25 min and then isocratic mode for 75% solvent B for 2 min; flow rate, $1mlmin^{-1}$; detection, 199nm – 650nm.

Table 1: Oxidative conditions applied on resveratrol.

ID	Oxidants	Experimental conditions[a]
Rox1	2 eq. (Diacetoxyiodo)benzene (PIDA)	EtOH, r.t., 2h
Rox2	2 eq. (Diacetoxyiodo)benzene (PIDA)	CH_3CN, r.t., 5h
Rox3	1 eq. (Bis(trifluoroacetoxy)iodo)benzene (PIFA)	EtOH, r.t., 2h
Rox4	1 eq. (Bis(trifluoroacetoxy)iodo)benzene (PIFA)	CH_3CN, r.t., 5h
Rox5	1.5eq. (Diacetoxyiodo)benzene (PIDA)	CH_3CN, r.t., 2h
Rox6	1 eq. 2,2'-Azobis(2-amidinopropane) dihydrochloride	EtOH, 65°C, 72h[c]
Rox7	1.5 eq. 2,2'-Azobis(2-amidinopropane) dihydrochloride, 1 eq sodium periodate	EtOH/H_2O(3:1), 65°C, 24h[c]
Rox8	1.5eq. 2,2'-Azobis(2-amidinopropane) dihydrochloride, 10eq hydrogen peroxide	EtOH/H_2O (2:1), 65°C, 24h[c]
Rox9	1 eq. Iodine	EtOH/H_2O (1:1), r.t, 24h[b]
Rox10	2 eq. Iodine	EtOH/H_2O (1:1), r.t. 24h[b]
Rox11	1 eq. periodic acid	EtOH/H_2O (3:1), r.t, 24h[b]
Rox12	2 eq. 2,2-diphenyl-1-picrylhydrazyl (DPPH)	EtOH, dark/r.t., 0.5h
Rox13	2 eq. 2,2-diphenyl-1-picrylhydrazyl (DPPH)	CH_3CN, dark/r.t., 0.5h
Rox14	2 eq. 2,2-diphenyl-1-picrylhydrazyl (DPPH)	MeOH, dark/r.t., 0.5h
Rox15	0.001 eq. $FeCl_3$	EtOH, r.t., 20 days
Rox16	0.01 eq. $FeCl_3$	EtOH, r.t., 20 days
Rox17	0.31eq. iron (III) meso-tetra (p-hydroxyphenyl) porphine chloride, 10eq, hydrogen peroxide	MeOH/Acetate buffer (4:1) r.t., 48h

[a]Experimental conditions are given as solvent, temperature, time, respectively.
[b]reactions were stopped using aq. sodium thiosulfate
[c]reactions were stopped using ice-bath.

Lipoxygenase inhibition of the reaction mixtures was determined using the Lipoxygenase Inhibition Screening Assay Kit (760700, Cayman Chemical, USA) according to the leaflet instructions, with absorbance measured using a plate reader (BMG Lambtech GmbH, Germany)

Percentage inhibition was calculated as;

$$\% \text{ inhibition} = (IA - \text{inhibitor})/IA \times 100$$

IA = absorbance of the 100% initial activity wells (containing LOX and solvent used to dissolve the reaction mixtures).

Inhibitor = absorbance of the inhibitor wells (containing LOX and reaction mixture).

Results and discussion

Results show resveratrol had little or no inhibitory activity on 15-LOX, which corroborates several reports [5]. Lipoxygenase is a key pro-inflammatory enzyme with a central role in various diseases; therefore, lipoxygenase inhibitors have attracted much interest in medical science [7]. Our data indicated that various oxidized resveratrol mixtures exhibited significant activities while resveratrol itself was inactive. Similar increase in the increased LOX inhibitory following Fe-catlaysed oxidation was found as previously reported [5]. In our case, mixtures obtained with periodic acid and iodine exerted the greatest LOX inhibitory activities that were ca. 50% at 4mM concentration; chromatographic fingerprints of these samples are shown in Figure 1.

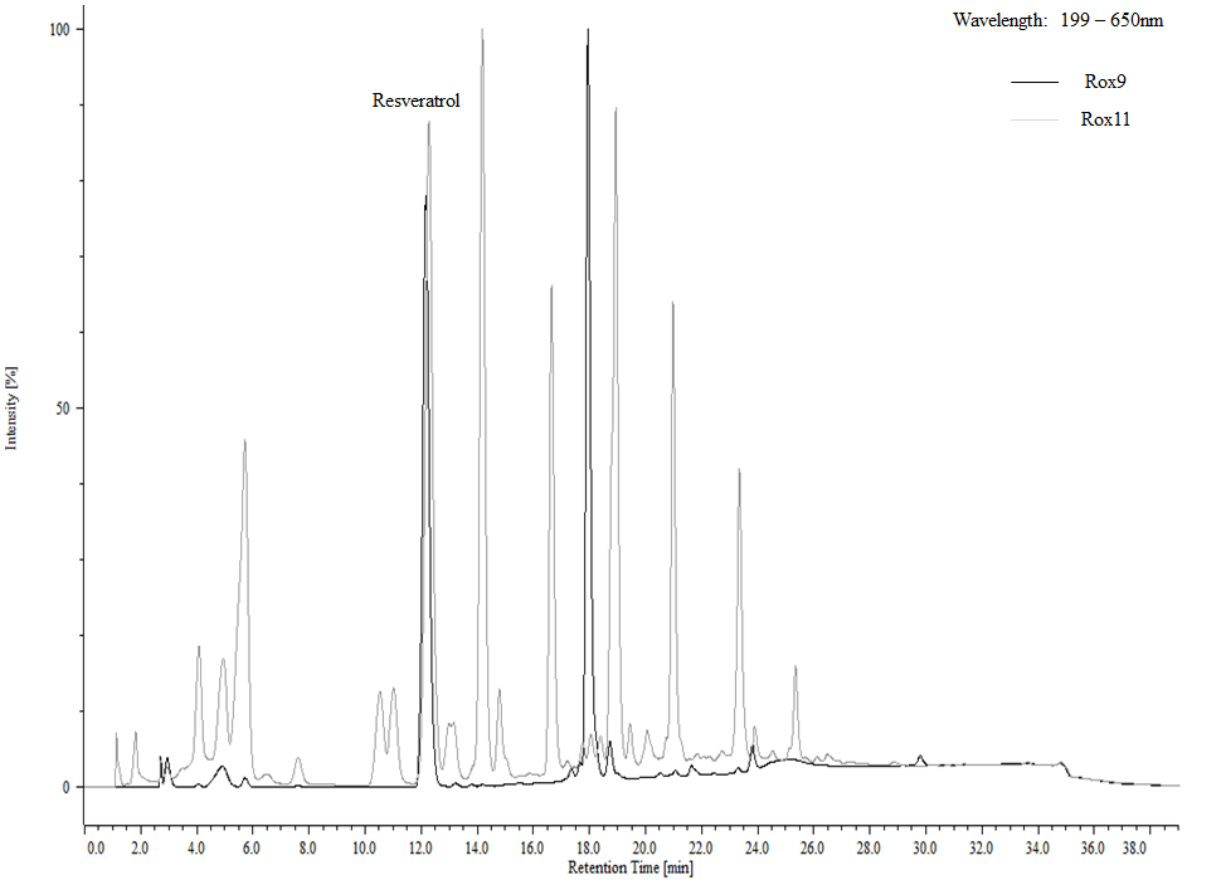

Figure 1. HPLC fingerprint of samples ROX9 and ROX11 that exerted near 50% inhibition of LOX in our experimental setup, while their parent compound resveratrol was inactive at the same concentration.

We plan to perform metabolomic studies of the reaction mixtures in the near future to assist the identification of compounds with a high LOX inhibitory potential.
Previously, resveratrol was found to moderately inhibit cell proliferation and viability in MCF-7 and MDA-MB-231 cancer cell lines [8,9]. Evaluation of the anti-proliferative activities of the oxidized resveratrol mixtures is currently ongoing.

Conclusion

The oxidation of resveratrol led to the accumulation of metabolites that showed favorable LOX inhibitory as compared to the parent compound. Isolation and purification of the individual metabolites in these complex oxidized mixtures could result in the discovery of compounds with potentially greater pharmacological activity.

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