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Crude peroxidase from onion solid waste as a tool for organic synthesis. Part I: Cyclization of 2′,3,4,4′,6′-pentahydroxy-chalcone into aureusidin

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ABSTRACT

The potential of a crude peroxidase (POD) from onion solid waste as a biocatalyst for the synthesis of a naturally occurring aurone is described. The crude enzyme preparation effectively promotes the cyclization of 2′,3,4,4′,6′-pentahydroxy-chalcone (which is not a natural substrate of onion POD) into aureusidin.

Aurones, (*Z*)-2-benzylidenebenzofuran-3-(2*H*)-ones (Fig. 1) represent a minor category of flavonoids, both in terms of occurrence and burden in plant tissues, yet their biochemical properties might be of significant value. Studies on the bioactivity potential of natural and synthetic aurones have shown that these heterocyclic compounds possess insect antifeedant activity,[1] anticancer,[2–4] antileishmanial,[5] and antibacterial properties,[6] and are potent antioxidants[7,8] and inhibitors of enzymes such as tyrosinase,[9] AChE,[10] and lipoxygenase.[8] In this regard, their examination with respect to structure, synthesis, and activity merits further attention.

Aurones are responsible for the bright yellow color of some popular ornamental flowers including snapdragon, cosmos, and dahlia.[11] Representative naturally occurring aurones include aureusidin,[12] sulfuretin,[13] maritimetin,[14] and bracteatin[15] which possess various hydroxylation patterns.

In plant tissues, aurones are biosynthesized from chalcone precursors, utilizing the enzyme *Aureusidin synthase* (AS). This peculiar biocatalyst has been characterized as a chalcone-specific polyphenol oxidase (PPO) homologue, while its action upon classic PPO substrates (such as phenolic derivatives, for example, caffeic acid, L-tyrosine, L-DOPA, and eriodictyol) is either particularly low or non-existent.[16–19]

The biosynthesis of aureusidin from 2′,3,4,4′,6′-pentahydroxy-chalcone (PHC) catalyzed by AS has been proposed to involve three steps, with only the first being enzyme-dependent (Scheme 1).[19] In this step, the enzyme-catalyzed reaction generates a chalcone with an *o*-quinonoid B-ring (intermediate **2a**). Cyclization of **2a** via formation of a benzofuranone ring followed by rearomatization yields aureusidin (**1**) as the thermodynamically more stable *Z*-geometric isomer.

Peroxidases (PODs) are common enzymes that catalyze a variety of oxidative transformations using hydrogen peroxide or other peroxides as oxidants. Very few plant tissues have been examined as sources of peroxidases, the most studied enzyme in this regard being horseradish peroxidase.

The exploitation of food residuals as sources of crude enzymes could contribute not only to a reduction of the polluting load of food industry wastes but also to the development of high value added products. In this context, we have previously developed a method for the preparation of a peroxidase-active crude extract from onion solid wastes for use as an alternative source of inexpensive POD. The biocatalytic potential of the crude homogenate obtained from onion solid wastes has been studied using a variety of phenyl-propenoic acids.[20–25]

In view of the previous encouraging results, we herein present the use of a crude onion peroxidase homogenate as a biocatalyst for the synthesis of the natural aurone, aureusidin, using PHC as the substrate.

The natural product PHC was synthesized starting from 2,4,6-trihydroxyacetophenone, as described previously.[8] The onions used as the enzyme source in this work are brown skinned onions

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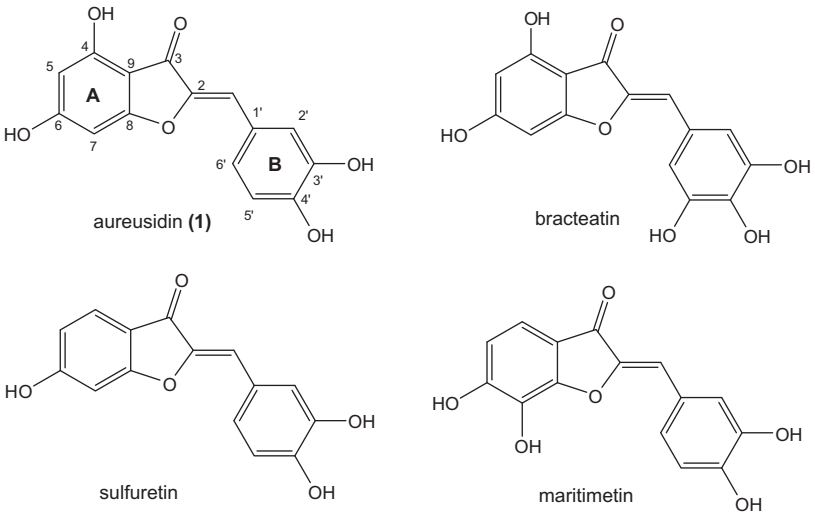
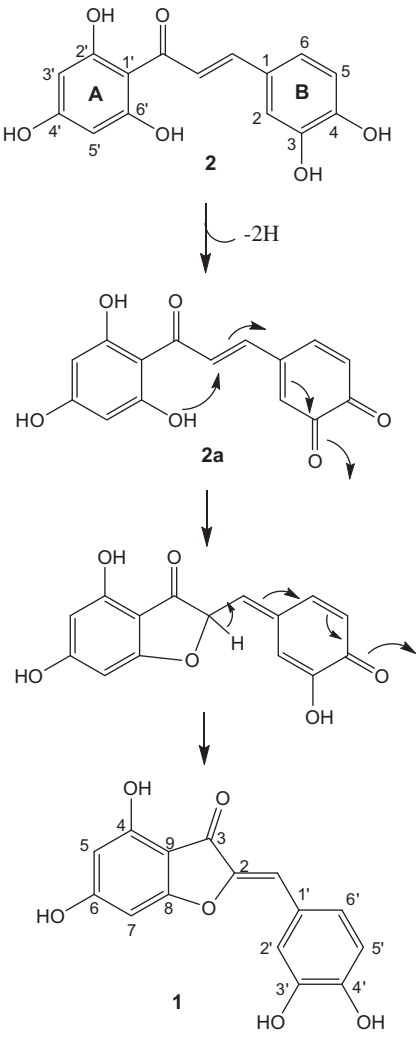


Figure 1. Representative naturally occurring aurones.



Scheme 1. Aureusidin biosynthesis.[13]

(*Allium cepa*). This is the most widely cultivated horticultural crop in Europe, and is the common onion variety that can be found in typical supermarkets in almost every region of Greece. To prepare the crude onion POD extract, two parts of ground onion solid waste (apical trimmings and outer dry layers) were dispersed in 15 parts of phosphate buffer (pH 7) and centrifuged at 3000 rpm, treated with polyvinylpyrrolidone (PVPP), and then filtered and used as such.[26] Increased enzyme activity was observed at pH 4, but considerable activity was also retained at pH up to 7.[27]

To the crude enzyme extract were added H_2O_2, as a solution in phosphate buffer pH 4, and a solution of PHC in methanol, and the mixture was stirred at room temperature for 60 min. The reaction was quenched by adding hydrochloric acid, and after evaporation of methanol, the aqueous residue was extracted with ethyl acetate.[28]

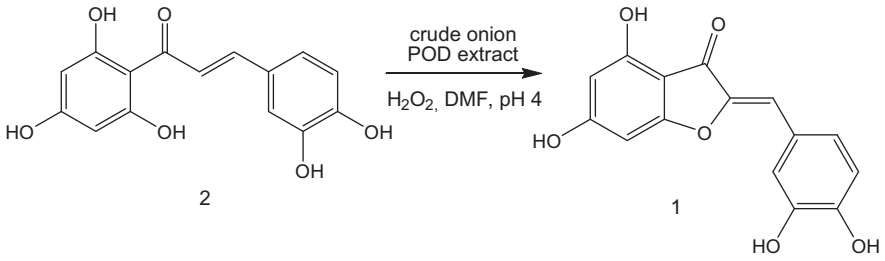
We were gratified to find that the crude onion peroxidase extract cyclized PHC to aureusidin (Scheme 2), mimicking *Aureusidin synthase*, at a yield of 55% under the reaction conditions. In order to achieve optimal solubility of the enzyme and the substrate in an aqueous environment, a few drops of DMF were added to the mixture. In this case, the oxidative cyclization of PHC to aureusidin proceeded smoothly with a significantly higher yield (95%).

Aureusidin obtained via this enzymatic procedure was purified using RP18 preparative column chromatography and the pure sample was identified by comparison with chemically synthesized aureusidin.[8]

A blank reaction was carried out under the same conditions and concentrations, but with the exclusion of enzymic extract. The reaction was monitored by LC-DAD-MS and formation of the aureusidin aurone ring was not observed. Moreover, as is postulated in the work of Detsi et al.,[8] the oxidative cyclization of chalcones into aurones can be achieved under harsh reaction conditions, involving the use of metal salts, strongly acidic or basic reagents, and heating.

It important to mention that, although PHC is not a natural substrate for the onion POD as known to date,[31] this methodology allows it to be cyclized exclusively into aureusidin, thus it can be postulated that this crude enzyme preparation manifests an as yet uncharacterized PPO activity. On the other hand, cyclization of PHC using a crude *Aureusidin synthase* extract from yellow snapdragon flowers has been reported to produce aureusidin/bracteatin in a ratio of 6:1.[19] This study proved that onion POD can mimic AS (a PPO analogue) as far as the oxidative cyclization of PHC is concerned.

In this work we have presented the use of crude onion peroxidase homogenate as a biocatalyst for the oxidative cyclization of a 2′-hydroxy-chalcone possessing a catechol moiety on ring B into the corresponding aurone scaffold; specifically PHC into aureusi-



Scheme 2. Enzymatic synthesis of aureusidin from PHC.

din. The scope of the biocatalytic activity of crude onion POD is currently being investigated using a variety of polyhydroxylated chalcone substrates and phenyl-propenoic acids.

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26. *Preparation of the crude onion peroxidase (POD) extract*: Parts of the onion bulb considered as waste (non edible) material, consisting of the apical trimmings, were used for preparing the crude POD extract.[29] The plant tissue was cut and an aliquot of approximately 20 g was homogenized in a domestic blender with 200 mL of acetate buffer (pH 4) and 2 g of PVPP. The homogenate was centrifuged at 3000*g* for 20 min and then filtered. The clear filtrate was used as the crude enzyme source.
27. *Determination of POD activity*: An aliquot of 0.1 mL of quercetin solution (1 mM in DMF) was mixed with 0.1 mL of crude enzyme extract and 0.8 mL of hydrogen peroxide (3 mM in acetate buffer, pH 4) and the reaction was followed by measuring the decrease in absorbance at 370 nm.[20] The decrease in absorbance was monitored at 370 nm against a suitable blank (the above mixture without the enzyme extract). One enzyme unit is defined as $\Delta A_{370}\ s^{-1}$. Control reactions omitting H_2O_2 or using heat-inactivated homogenate were also carried out. The protein content of the homogenate was determined according to Bradford,[30] using bovine serum albumin as the standard. For all determinations, a computer-controlled HP 8452A diode-array spectrophotometer was used.
28. *Aureusidin synthesis using POD*: 50 mg of PHC was dissolved in 10 mL of MeOH and mixed with 19 mL of H_2O_2 (3 mM prepared in buffer, pH 4) to obtain a final concentration of 0.86 mM. Then 3 mL of crude onion POD extract was added to the mixture, which was left to stir for 1 h. The mixture was subsequently acidified with HCl to pH 1 and MeOH was removed in vacuo. The remaining aqueous solution was extracted with EtOAc (3×20 mL). The organic phase was dried over $MgSO_4$, then filtered, and evaporated. The solid residue was purified by preparative RP18 flash column chromatography (15×2 cm, 40–63 μm, using a H_2O–MeOH gradient from 100% to 0% in steps of 10% and 50 mL of eluent per step. The elution was monitored by RP-18 TLC). Yield 27.3 mg (55%).
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