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Crude peroxidase from onion solid waste as a tool for organic synthesis. Part III: synthesis of tetracyclic heterocycles (coumestans and benzofuroquinolinones)

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ABSTRACT

In the present study the biocatalytic activity of a crude onion peroxidase extract on the reaction between catechol and heterocyclic 1,3-dicarbonyl compounds is investigated. The crude enzyme preparation promotes effectively the reaction between catechol and 4-hydroxycoumarin, 4-hydroxy-1-methyl-2-quinolinone and 2,4-quinolinediol to afford the corresponding coumestans and benzofuroquinolinones in satisfactory yields. The products of the reaction are fully characterized spectroscopically and are found to possess potent antioxidant activity as evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and luminol-induced chemiluminescence assays.

Coumestans, systematically known as 6*H*-benzofuro[3,2-*c*][1]benzopyran-6-ones, constitute an oxygenated class of aromatic natural products, widely occurring in nature and possessing diverse pharmacological properties. The most recent examples of coumestans from natural sources include glycyrurol, isolated from the ethyl acetate extract of the roots and rhizomes of *Glycyrrhiza uralensis*,[1] 2-(α,α-dimethylallyl)coumestrol isolated from *Pueraria lobat*,[2] and flemicoumestan A isolated from the ethyl acetate extract of the roots of *Flemingia philippinensis*, which exhibits immunosuppressive activity.[3] The bioactivity of natural and synthetic coumestan analogues expands from the myotoxic activities shown by the natural coumestan wedelolactone and its synthetic analogues,[4,5] and the promising anticancer activity exhibited by demethylwedelolactone derivatives[6] to sirtuin deacetylase inhibitory activity shown by a synthetic coumestan analogue,[7] and the anti-inflammatory activity exhibited by psoralidin[8] (Fig. 1).

Benzofuroquinolinones can be considered as the 'aza-analogues' of coumestans, and have been less studied. Interesting bioactivities exhibited by these tetracyclic nitrogen heterocycles are inhibition of osteoporosis[9] and important anticancer activity.[10]

Different synthetic approaches to construct the basic fused-tetracyclic ring of coumestans have been reported, which include intramolecular oxidative annellation of different types of coumarins mediated by anhydrous $FeCl_3$ and SiO_2,[11] and enzymatic regioselective oxidation using 4-hydroxycoumarin and catechol in the presence of oxidative enzymes such as mushroom tyrosinase[12] and laccase.[13]

Plant peroxidases (PODs) are widely distributed enzymes in nature, located inside and outside the cell wall, and in the vacuole of higher plants.[14] PODs are multifunctional enzymes, mainly involved in cell protection against accumulating dangerously reactive compounds such as H_2O_2, by catalyzing a variety of oxidative transformations using H_2O_2 or other peroxides as oxidants.[15] Recently, our research group has been involved in the exploitation of crude onion POD extracts obtained from solid onion waste. This research has shown that, owing to its oxidative nature, crude onion POD can replace current chemical oxidants in several areas, such as bioremediation of waste-waters,[16–19] eliminating phenolics and other pollutants by oxidation, as well as in the organic synthesis of natural products with important biological, structural, and functional activities.[20,21]

In order to further explore the biocatalytic potential of crude onion POD extract, we decided to investigate its ability to perform a domino reaction involving oxidation, 1,4-addition, oxidation and 1,4-addition between 1,3-dicarbonyl compounds and catechol leading to the synthesis of coumestans and benzofuroquinolinones (Scheme 1).

The onion solid waste used as the enzyme source in this study, consisting of the apical trimmings of brown-skin onion bulbs

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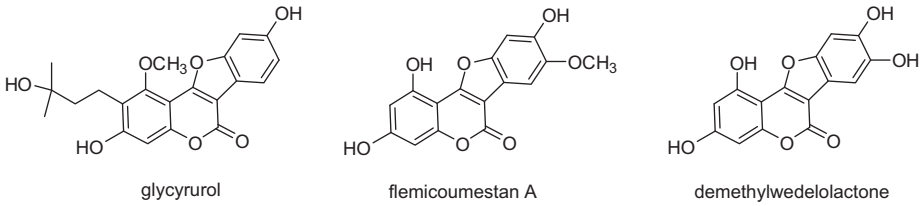
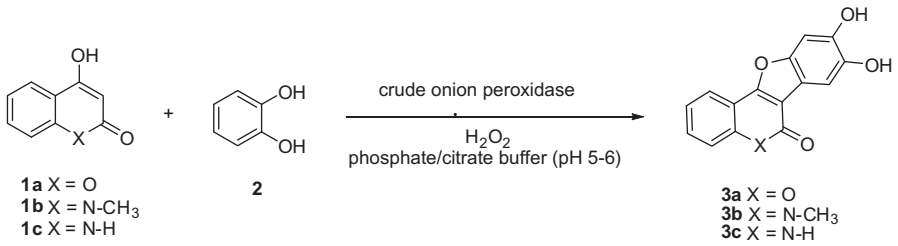


Figure 1. Examples of naturally occurring coumestan analogues.



Scheme 1. Domino reaction between 1,3-dicarbonyl compounds and catechol catalyzed by crude onion peroxidase.

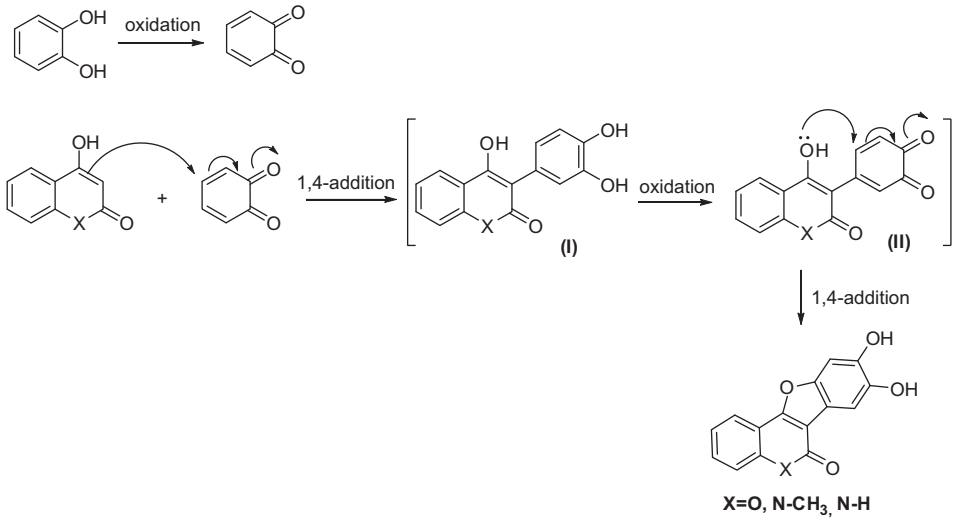
(*Allium cepa*), was obtained from a local catering facility (Chania, Crete). This is the common onion variety that can be found in typical supermarkets in almost every region of Greece. The cell-free, POD-active extract was prepared as previously described.[22]

In order to optimize the conditions that provide a maximum yield of the product, a series of experiments varying in the most important parameters of the reaction (pH, H_2O_2 concentration, and reaction time) were performed. Thus, the conditions for optimal enzyme activity were determined: pH 5 and 6 in a phosphate/citrate buffer, 0.3 mM H_2O_2 as a solution in phosphate buffer, and a 0.15 mM substrate concentration, at room temperature and reaction times of 1.5, 3, and 1.5 h for **1a**, **1b,** and **1c**, respectively. Following optimization of the process, scale-up reactions were carried out in order to obtain the products in amounts adequate for spectroscopic characterization and antioxidant activity evaluation.[23] The reaction progress was monitored by LC–DAD–MS at 278 nm.[24] The reaction of 4-hydroxycoumarin (**1a**) resulted in a compound showing λ_{max} 242, 284, and 348 nm, presenting $[M+H]^+$ at *m*/*z* 269, whereas the reaction using 4-hydroxy-1-methyl-quinolone (**1b**) produced a compound showing λ_{max} 240, 314, 346, and 356 nm and $[M+H]^+$ at *m*/*z* 282. In the case of 2,4-quinolinediol (**1c**) the product showed λ_{max} 242, 312, and 352 nm and $[M+H]^+$ at *m*/*z* 268. These data were indicative that the reaction proceeded exclusively with the formation of the corresponding tetracyclic products **3a**–**3c**.

The crude products **3a** and **3b** were successfully purified using flash column chromatography and their structures were unambiguously assigned using ^1H NMR and ^{13}C NMR spectroscopy,[25,26] and by comparing these data to those reported in the literature.[12,27] Benzofuroquinolinone **3c** was unstable under the purification conditions, and therefore was not isolated in pure form.

The reaction is postulated to proceed through a domino process involving several steps (Scheme 2), in a similar way as has been described for analogous reactions using pure oxidizing enzymes.[12,13] Initially, crude onion POD uses H_2O_2 to oxidize catechol to the corresponding quinone, which in turn undergoes a 1,4-addition by the nucleophilic enol of the heterocyclic 1,3-dicarbonyl compound. The non-isolable intermediate (I) is again oxidized by the enzyme to the quinone intermediate (II) which further undergoes an intramolecular 1,4-addition to produce the final tetracyclic heterocycles.

The antioxidant activity of purified compounds **3a** and **3b**, as well as of the starting materials was determined using three different in vitro assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric



Scheme 2. Suggested reaction mechanism.