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ON THE GREEN CATALYTIC SYNTHESIS OF PURPUROGALLIN

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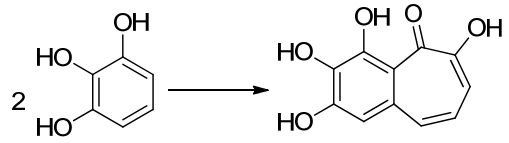
The catalytic oxidation under green conditions of pyrogallol into purpurogallin was performed by air with laccase model copper complexes or by plant extracts containing peroxidases in the presence of hydrogen peroxide. A study was conducted for turnip, depending on the amount of extract, the method of introduction and the pH. Optimization of these conditions made it possible to easily obtain pure purpurogallin with a yield of 78%.



INTRODUCTION

Benzotropolone is a bicyclic polyphenol system with pseudo-aromatic compound properties. The most studied member of this family is purpurogallin a natural red dye found in the bark of oak and gall nuts in the glucoside state.[1] Purpurogallin is known for its antibacterial,[2] antioxidant,[3] anticancer[4] activities. In addition, purpurogallin can prevent the methylation of hydroxyestradiol by catechol-O-methyltransferase.[5] It has been shown to specifically prevent the system activation pathway TLR1 / TLR2.[6]

The structure of purpurogallin was established by single crystal X-ray analysis[1] to be a bicyclic molecule comprising a phenolic ring fused with a seven-membered ring in a highly planar conformation.

Purpurogallin derivatives such as Goupiolone A and B, are known for their significant DNA-damaging activity and were tested for their anti-cancer activity[7,8] while Fomentariol isolated from the tree sponge *Fomes fomentarius*, display anti-oxidant and antidiabetic activities.[9] Crocipodin is a natural pigment from the fruit bodies of mushroom Leccinum crocipodium.[10]

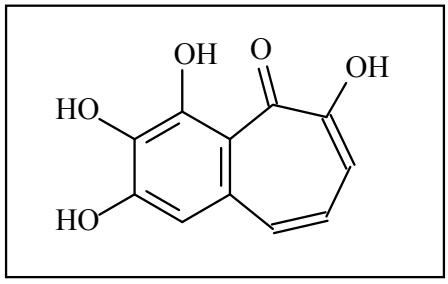


Fig. 1 – Structure of purpurogallin.

EXPERIMENTAL

1. Materials and Methods

All the reactions were carried at room temperature with magnetic stirring and monitored by TLC silica plates. IR spectra were obtained as solids with Fourier transform Perkin-Elmer. "Spectrum One" with ATR accessory. Only significant absorptions are listed.

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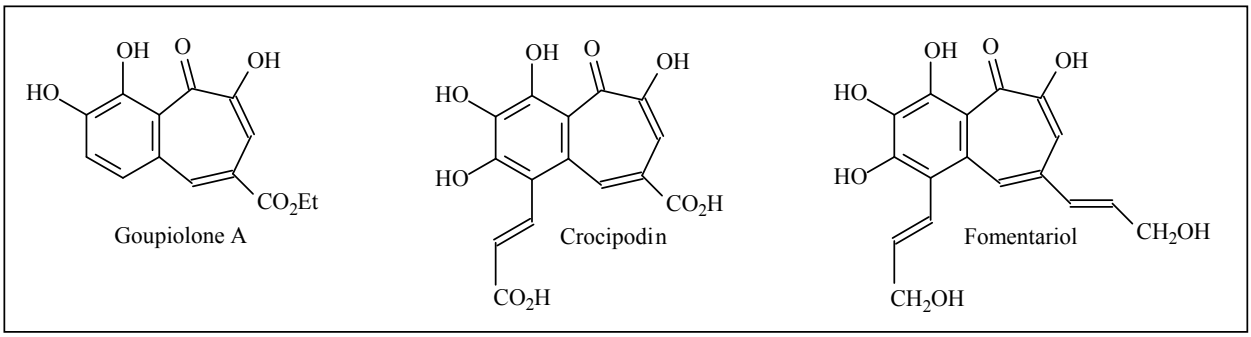


Fig. 2 – Purpurogallin derivatives.

The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6, with a Bruker AC 400 spectrometer. The chemical shifts δ are expressed in parts per million, conventional abbreviation are used chemical and were referenced to the internal solvent signal namely TMS. Mass spectra were recorded on a QTOF Micro (Waters) spectrometer with electrospray ionization (ESI, positive mode), lock spray PEG.

Horseradish was obtained from a market in France. Red radish *(Raphanus sativus var. sativus)*, Red onion (*Allium Cepa*), clove garlic (*Allium sativum*) were purchased from local Market in Tlemcen (Algeria). Bitter turnip or left el mora or left el mahfoura (*Brassica rapa L.*) was collected from a local farm in west Algeria.

2. Aerobic oxidation of pyrogallol into purpurogallin using copper catalysts

The copper complex $[CuClOH\ (TMEDA)]_2$ was prepared according to the literature.[16]

A Cu (I) / PEI suspension was prepared by stirring PEI (MW = 300, 50% in water) (0.43g of PEI) with cuprous chloride (5 mmol, 0.494g) in 100 mL of ethanol.

2.1. Aerobic oxidation catalysed by complex $[CuClOH\ (TMEDA)]_2$

A mixture of pyrogallol (504 mg, 4 mmol) and $[CuClOH\ (TMEDA)]_2$ (74 mg, 0.16 mmol) in ethanol (20 mL) are stirred in an open flask in the air for 4 days at room temperature. The solid formed is isolated by filtration and recrystallized from acetic acid.

2.2. Aerobic oxidation catalysed by Cu (I)/PEI

Pyrogallol (504 mg, 4 mmol) was dissolved in a Cu(I) / PEI (30 mL) suspension and was stirred in an open flask in the air for 4 days at room temperature (20 °). The reaction mixture was dissolved in CH_2Cl_2, and washed with 10% aqueous hydrochloric acid solution, the organic phase was dried over Na_2SO_4, and evaporated under vacuum. The solid formed is isolated by filtration and recrystallized from acetic acid.

(6E,8Z)-2,3,4,6-tetrahydroxy-5H-benzo[7]annulen-5-one: (Purpurogallin) : deep red needles. Yield 76%. M.p. =274-275°C / m.p. (lit.) = 275°C [11]. FT-IR (cm^{-1}): 3446 (OH_{free}), 3316 (OH_{assoc}), 2925 ($C\text{-}H_{arom}$), 1694 (C=O); 1589. ^{1}H NMR δ (400 MHz, DMSO d6, ppm): δ 15.23 (1H, s, OH), 10.55 (1H, s, OH), 9.40 (1H, s, OH), 9.36 (1H, s, OH), 7.32 (1H, d, J=11.2 Hz, H7), 7.11 (1H, d, J=9.3 Hz, H9); 6.73 (1H, s, H1), 6.72 (1H, dd, J= 9.3 and 11.2 Hz, H8); 13C NMR (100 MHz, DMSO d6, ppm): δ 182.6 (**C**5), 155.1 (**C**6), 152.2 (**C**2), 151.9 (**C**4), 135.1 (**C**3), 134.7 (**C**9), 133.4 (**C**10), 124.1 (**C**8), 116.3 (**C**7), 115.2 (**C**11), 110.68 (**C**1). HRMS: for $C_{11}H_9O_5$, (M+1): calculated mass 221.0450, found 221.0420.

3. Oxidation of pyrogallol into purpurogallin using vegetables enzymes

3.1. Preparation of enzymes extract from Horse radish, Red Radish, Red onion and Garlic

Vegetables were washed with water then with distillate water. After cautious peeling, they were cut into small pieces and then mixed in cold distilled water (5°C). The juice obtained was centrifuged (6000 rpm for 12 min) then filtered. The solutions used are prepared at different concentrations (0.2 g/mL; 0.4 g/mL; 0.6 g/mL) relative to the plant material. For the onion and garlic we only used a single concentration of 0.4 g/mL.

3.2. Enzymes oxidation of pyrogallol into purpurogallin

Different tests have been carried out depending on the type of enzyme.

* *With a solution of H_2O_2 (detection of peroxidase)*

In a 250 mL Erlenmeyer flask fitted with a magnetic bar are introduced: pyrogallol (1 g, 0.008 mol), 20mL distilled water, a 20 ml hydrogen peroxide solution (0.147 M) and 20 mL of a solution of vegetable extract per 5 mL portion every 30 min.

The reaction mixture was stirred for 12 h to 16 h at room temperature, and then the precipitate formed was filtered and washed with cold distilled water (5-10°C). More products were recovered by treating the filtrate by extraction with ethyl acetate.

* *Without H_2O_2 solution (detection of tyrosinase and laccase)*

In this synthesis the same steps previously described were followed except that no hydrogen peroxide was added.

4. Extraction of bitter turnip and purpurogallin syntheses:

A systematic study, taking into account various parameters, was carried out for this bitter turnip, in order to optimize yields. The same procedures for the preparation of vegetable extracts and the synthesis of purpurogallin were adopted.

4.1. Variations in the turnip extract concentration

The same steps for preparing turnip extracts were used. Solutions of 0.2 g/mL, 0.3 g/mL, 0.4 g/mL, 0.5 g/mL and 0.6 g/mL were used at the pH of distilled water.

The synthesis of purpurogalline was carried out for 18 hours, at room temperature, the pyrogallol (1g, 0.008 mol) dissolved in 20 mL of distilled water, the hydrogen peroxide solution (20 mL, 0.147 M) and the different concentrations of turnip extract. The precipitate obtained is filtered and washed with cold distilled water. We obtain for each case the

purpurogallin in solid form with variable yields (11-25%) presented in Table 1.

Table 1

Optimization of concentration of turnip extract

Concentration g/mL	Yield %
0.2	11
0.3	15
0.4	25
0.5	25
0.6	11

4.2. Variation of the time and frequency of introduction of bitter turnip extract and H_2O_2 solutions

In an Erlenmeyer flask fitted with a magnetic bar dissolved the pyrogallol (1g, 0.008 mol) in 20 ml of distilled water. The solutions of H_2O_2 (0.147 M) and turnip extract (0.4 g/mL) were added:

* Method 1: every 2 hours for 6 hours (3 x 20 mL of H_2O_2 + 3 x 20 mL of turnip extract)
* Method 2: every hour for 6 hours (6 x 10 mL of H_2O_2 + 6 x 10 mL of turnip extract)
* Method 3: every 30 minutes for 6 hours (12 x 5 mL of H_2O_2 + 12 x 5 mL of turnip extract)

Purpurogallin was obtained with yields varying between 25% and 36% Table 2.

Table 2

Optimisation of introduction methodology of hydrogen peroxide solution

Method 1	Yield = 34 %
Method 2	Yield = **36 %**
Method 3	Yield = 25 %

4.3. Change in the pH of the bitter turnip extract solution

Depending on the pH ranges, buffer solutions was prepared using 0.1 N hydrochloric acid, 0.1 N citrate, KH_2PO_4 (9.078 g/L) and Na_2HPO_4 (11.875 g/L) solutions.

The vegetable extract was prepared in these buffer solutions (20 g in 50 mL of buffer solution) with a concentration of 0.4 g/mL.

The synthesis of purpurogallin was carried out according to method 2 described above. Purpurogallin was obtained with yields ranging from 20-78% Table 3.

RESULTS AND DISCUSSION

One molecule of purpurogallin was formed by the oxidation of two molecules of pyrogallol.

The purpurogallin formation mechanism involves the coupling of two aromatic nuclei followed by the opening of one of the rings by oxidation and then the formation of the tropolone nucleus as highlighted by Haworth. [11]

Oxidases are also capable of oxidizing pyrogallol to purpurogallin, such as laccase,[12] tyrosinase,[13] peroxidases (from horseradish (HRP),[14] from Ginkgo,[15] from *Agaricus bisporus* mushroom[16]) using oxygen from the air or hydrogen peroxide as oxidants.

Although purpurogallin can be obtained from stoichiometric oxidation of pyrogallol with potassium iodate [17] or potassium ferricyanide as oxidant. These old methods are not very clean and the purification processes are quite tedious, so, a green catalytic reaction is desirable.

So we have studied two approaches:

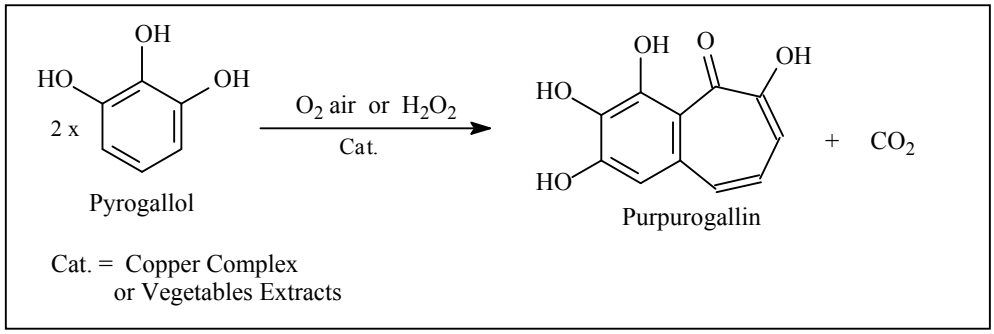
An aerobic process that mimic laccase using air as an oxidant and a copper catalyst.

A process using vegetable peroxidases close to HRP, readily available, using hydrogen peroxide as an oxidant.

Table 3

Optimization of pH with the bitter turnip extracts solution

pH	2.6	3.36	4.15	5.57	6.21	6.97	7.7	8.36
Yield %	20	24	26	49	**78**	54	52	39



Scheme 1 – Synthesis of purpurogallin from pyrogallol.

Table 4

Oxidation of pyrogallol into purpurogallin in the presence of copper catalysts.

Catalyst	Solvent	T°C	Yield (%)
$[CuCl(OH)\ TMEDA]_2$ **[a]**	EtOH	20	43
Cu(I)/PEI **[b]**	EtOH	20	0
$[CuCl(OH)\ TMEDA]_2$ /NHPI **[c]**	CH_3CN	70	0

Conditions: reagent (2.10^{-3} mol), **[a]** cat $[CuCl(OH)\ TMEDA]_2$ (8 % mol), 20°C, EtOH (25 ml), **[b]** cat Cu(I)/PEI, 20 ° C, EtOH (25 ml), **[c]** cat $[CuCl(OH)\ TMEDA]_2$ (8% mol) / NHPI (10 %), 70°C, CH_3CN, 96 h.

Table 5

Comparison of vegetable activities in the synthesis of purpurogallin

Vegetable	Horse radish	Red radish	Red onion	Garlic	Bitter turnip
Concentration g/mL	0.4	0.4	0.4	0.4	0.4
pH	5.2	5.2	5.2	5.2	6.21
Yield (%)					
With H_2O_2	14	13	14	30	**78**
Without H_2O_2	/	/	18 **(a)**	48 **(b)**	

(a) Purpurogallin was obtained with a better yield without using an H_2O_2 solution
(b) The oxidation product of pyrogallol without H_2O_2 was a green solid

Aerobic oxidation catalysed by copper complex as model of laccase

Laccase is known to catalyse the oxidation of pyrogallol.[12] It is an oxidase containing copper atoms in its active oxidation site. Moreover a number of copper-amine complexes are known to mimic laccase.[18] One of the most active complexes is the copper complex with tetramethylethylene diamine[19] $[CuCl\ (OH)TMEDA]_2$ .We have used this complex in the aerobic coupling of naphthol to binaphthol[20]or spiro derivatives.[21] PEI-copper in the laboratory has similar properties in many oxidations of phenols. [22] We tested the effectiveness of the two $[CuCl(OH)TMEDA]_2$ and Cu(I)/PEI catalysts in the synthesis of purpurogallin by aerobic oxidation of pyrogallol. The reactions were carried out in ethanol at room temperature (20°C). We also studied the possibility of pyrogallol oxidation in the presence of N-Hydroxylphtalimide (NHPI) in acetonitrile at 70°C.[23-25] The results are presented in table 4.

The tests for the oxidation of pyrogallol to purpurogallin, reported in Table 4, show that the Cu (I)/PEI and the $[CuCl(OH)TMEDA]_2$/NHPI[20] mixture do not catalyse the oxidation reactions of pyrogallol.

Enzymatic oxidation by vegetables extracts

Peroxidases are known to oxidize pyrogallol into purpurogallin in the presence of hydrogen peroxide as an oxidant.[14] The best known is horseradish peroxidase (*HRP*) found in horseradish and related brassicas.

In order to compare and optimize the yields of the oxidation reaction, we used other vegetable extracts like: red radish (*Brassica rapa, var.sativa*), red onion (*Allium Cepa*), clove garlic (*Allium sativum*) and bitter turnip (left el mora or left el mahfoura (*Brassica rapa, var. Arabum*) as a source of enzymes. Garlic (*Allium sativum*) is also known to contain peroxidase,[26] it has shown interesting oxidative activity on pyrogallol. The results are presented in Table 5.

Horseradish and red radish gave the expected results, although in our case with horseradish extract, we did not adjust the pH of solution.

Red onion and garlic extracts showed oxidative activity both in the presence of H_2O_2 (presence of peroxidases), and without H_2O_2 solution (presence of other oxidases).

We chose the bitter turnip, the most active, to conduct a study with different parameters: concentration of vegetable extract (variation in the mass of the plant material), time and frequency of introduction of the vegetable extract and the H_2O_2 solution, pH of the vegetable extract solution.

For the bitter turnip, we optimized the conditions of concentration and acidity and at pH = 6.21 we obtained a maximum yield of 78% (See experimental part).

CONCLUSIONS

Two efficient green catalytic oxidation approaches to the synthesis of purpurogallin from pyragallol are reported. The first was the aerobic oxidation catalysed by the copper complex $[CuCl(OH)\ TMEDA]_2$. A second approach was the used of vegetable extract as source of peroxidase with hydrogen peroxide as oxidant. Oxidation with extracts of bitter turnips gives a good yield with a simplified purification method.

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