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Yeast-Mediated Xanthone Synthesis through Oxidative Intramolecular Cyclization

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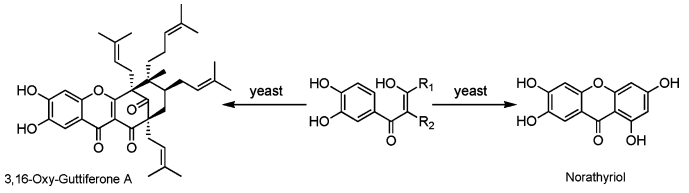
ABSTRACT



Benzoylphloroglucinol derivatives are natural products showing diverse biological activities that could be modulated by structural modifications. For this purpose, we studied the biotransformation of guttiferone A and of maclurin using a combinatorial approach for screening active microorganism strains. We found a novel and unexpected yeast-catalyzed oxidation that has selectively given a new oxy-guttiferone A and norathyriol.

In recent years, biotransformation entities derived from natural products have gained importance in the search for therapeutic drugs.[1] In this context, we have investigated the study of benzoylphloroglucinol derivatives, present in various species and parts of plants. The simplest compounds, like maclurin, are the precursors of polysubstituted compounds such as polycyclic polyprenylated acylphloroglucinols (PPAPs familly), and especially guttiferones.[2]

Guttiferone A **1** was isolated from *Symphonia globulifera* roots[3] and recently from *Garcinia livingstonei*[4] and *Garcinia macrophylla*.[5] This natural product exhibits a wide pharmacological profile, with anti-HIV,[3] cytotoxic,[4–6] trypanocidal,[7,8] antiplasmodial,[8–10] leishmanicidal,[8,11,12] and antibacterial[13] effects. In an interesting way, guttiferone A shows good radical scavenging activity and could

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Table 1. Screening of Active Microorganisms in Guttiferone A Biotransformations

entry	culture of microorganisms	incubations: step 1	incubations: step 2	total number of assays	selected microorganism (yield[a])
1	8 strains	2 mixtures of 4 strains: 1 gave biotransformation	4 assays	6	*Rhodotorula buffonii* MUCL29812 (48%)
2	15 strains	3 mixtures of 5 strains: 1 gave biotransformation	5 assays	8	*Candida pinus* MUCL 27856 (19%)
3	12 strains	4 combinatorial mixtures: 1 gave biotransformation	no assay required	4	*Pichia anomala* NRRL Y40 (17%)

[a] Yield in compound **2** after purification.

be a lead in the search for new antimalarial drugs.[9,14] Several actions of guttiferone A toward cell pathologies and specific enzymatic activities have also been reported.[11,15]

Concerning the antiparasitic activities, it is worth noting that Malaria remains one of the most important infectious diseases causing 1 million deaths every year. Due to the rising prevalence of *Plasmodium falciparum* resistance, the treatment of malaria is becoming increasingly difficult. There is therefore an urgent need to diversify the antimalarial therapeutic arsenal. Most of the antimalarial drugs are natural products and natural product analogues.

For this reason, our group investigated the antiparasitic potency of guttiferone A analogs. Because it is isolated from fruits, guttiferone A is an interesting renewable starting material for semisynthesis. Within the field of natural products, pharmacomodulation has provided significant results.[16]

Then to improve selected activity, we wanted to generate a library of guttiferone A derivatives for performing structure−activity relationship studies. Among the strategies used, we studied the biotransformation to obtain selective modifications, and yeast-mediated transformations have been investigated with the hope to stereospecifically reduce one of the carbonyl groups.

We present here the results of original approaches in the screening of microorganism strain collections, the finding of an unexpected yeast-mediated oxidation reaction, and its use in the efficient synthesis of the natural product and analog.

Microbial reduction of ketones especially with baker yeast is largely documented;[17] however the reduction is strongly influenced by the bulkiness of substituents of the carbonyl group. It turns out that the baker yeast is not effective in benzophenone reduction, so we screened the 35 yeasts in our collection.

Usually a selection of active microorganisms was performed by incubation of an individual strain with a substrate to be transformed resulting in a number of tests equal to the number of strains to be tested. In order to reduce the number of assays, the selection of active yeasts was performed using a new approach, based on the implementation of biomass mixtures (Table 1). At first, we tested mixtures of four (entry 1) and five (entry 2) different strains (step 1), and biotransformations were monitored by HPLC and mass spectrometry. Then the microorganisms that were present in the mixtures able to transform guttiferone A were tested individually (step 2), and *R. buffonii* and *C. pinus* were selected among eight and fifteen strains through 6 and 8 assays, respectively.

To improve our strategy, mixtures were carried out using combinatorial distribution (entry 3). This approach allowed identifying the active microorganisms without further incubation (for details, see Supporting Information), and the yeast *P. anomala* was selected among twelve strains through only four assays. Our combinatorial approach is an efficient strategy; the number of assays is reduced by three compared to traditional screening.

HPLC chromatographic profiles of biotransformation media with the three selected microorganisms showed one peak having the same retention time, which is longer than the one of the starting material. A mass spectrometry spectrum showed a m/z 601 for $[M+H]^+$ corresponding to a loss of two protons regarding guttiferone A. These results suggested an oxidative reaction rather than an expected reduction reaction, and the product has been identified as 3,16-oxyguttiferone **2** by NMR analysis.

The loss of one proton in the aromatic part on the NMR spectra of product **2** leads to the structure of an

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oxy-guttiferone. In fact the phenolic oxidative coupling may engender four compounds due to the enolization of the triketone (C-1 or C-3) and due to both aromatic positions (C-12 or C-16), which could be involved in the reaction. The presence of two singlets (H-12 and H-15) showing no correlation between each other in COSY and HMBC was in agreement with a cyclization in the position 16 on the catechol ring (Table 2).

HMBC (Figure 1) presents a strong correlation between H-7 and carbon 1 at 194.38 ppm. The position of this ketone is also confirmed with the correlation of H-29 with carbon 1. In the other part, the HMBC correlation of H-17 with the specific quaternaries carbon 5 (51.96 ppm) and carbon 3 (179.52 ppm) demonstrated the cyclization with the enol in the position C-3.

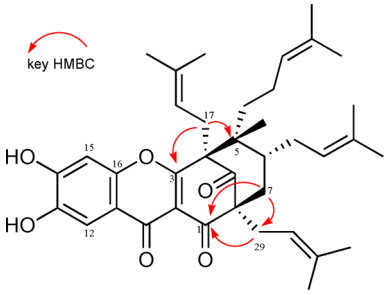
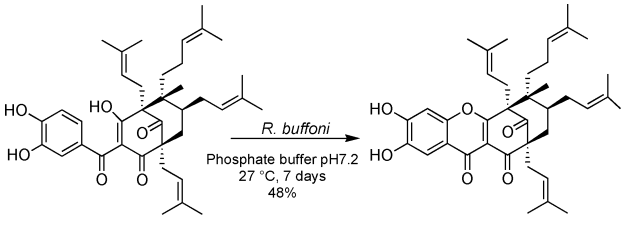


Figure 1. Key HMBC correlation of 3,16-oxyguttiferone **2**.

The active microorganisms were tested on a preparative scale (20 mg, 0.1 mg/mL), and the most efficient, *R. buffoni*, gave **2** in 48% yield (Scheme 1). Four oxy-guttiferones were isolated from plants, but this is the first time that oxy-guttiferone A was obtained. The other strains did not transform guttiferone A in those conditions, and the starting material was recovered unchanged, excluding a spontaneous formation of oxy-guttiferone A.

Scheme 1. Biotransformation of Guttiferone A by *R. buffoni*



Biological activities of compound **2** were evaluated by its ability to inhibit the growth of *Trypanosoma brucei* bloodstream forms[18] and the intraerythrocytic growth of *P. falciparum*.[19] Better activities than those observed for guttiferone A were obtained but with increasing toxicity toward Vero cells (Table 3).

Table 2. Spectral Data of 3,16-Oxyguttiferone A (**2**)[a]

no.	^{13}C δ (ppm)	^{1}H δ (ppm)	J (Hz)	COSY	HMBC
1	194.38				
2	118.91				
3	179.52				
4	65.02				
5	51.96				
6	41.58	1.88 (m, 1H)		7,24	4,8
7	39.24	2.08 (t, 2H)	5.54	6	1,5,6,8,24
8	66.75				
9	207.39				
10	174.49				
11	118.02				
12	151.15				
13	103.92	6.96 (s, 1H)			11,12,14,15
14	154.96				
15	147.15				
16	109.52	7.43 (s, 1H)			10,12,14,15
17	27.40	2.84/2.96 (m/m, 2H)		18	3,4,5,18,19
18	119.67	4.63 (m, 1H)		17	
19	136.17				
20	18.65	1.73 (s, 3H)			18,19,21
21	25.90	1.41 (s, 3H)			18,19,20
22	20.41	1.28 (s, 3H)			4,5,6,23
23	37.89	1.42/1.53 (m/d, 2H)	7.31	34	4,5,6,23,35,36
24	30.42	1.77/1.92 (m/m, 2H)		6,25	5,25
25	124.60	4.85 (m, 1H)		24	27,28
26	134.09				
27	17.95	1.37 (s, 3H)			25,26,28
28	26.10	1.64 (s, 3H)			25,26,27
29	30.30	2.51 (t, 2H)	7.45	30	1,8,30,31
30	120.83	5.21 (t, 1H)	7.59	29	32,33
31	135.54				
32	18.12	1.70 (s, 3H)			30,31,33
33	26.03	1.70 (s, 3H)			30,31,32
34	23.91	1.98 (m, 2H)		23,35	23,35,36
35	124.98	5.10 (t, 1H)	6.73	34	37,38
36	132.98				
37	17.79	1.64 (s, 3H)			35,36,38
38	26.32	1.70 (s, 3H)			35,36,37

[a] ^{13}C (75 MHz), ^{1}H (400 MHz) in MeOD.

Table 3. Trypanocidal, Antiplasmodial, and Cytotoxic Activities of Guttiferone and 3,16-Oxy-guttiferone

	T. brucei IC50 (μM/mL)	*P. falciparum* IC50 (μM/mL)	Vero cells % inhibition 10^{-5} M	Vero cells % inhibition 10^{-6} M
guttiferone A **1**	2.95	3.32	89 ± 1	3 ± 3
3,16-oxy-guttiferone A **2**	2.08	1.25	94 ± 1	17 ± 3

In order to extend the reaction, we investigated the biotransformation of maclurin, which has phenol groups

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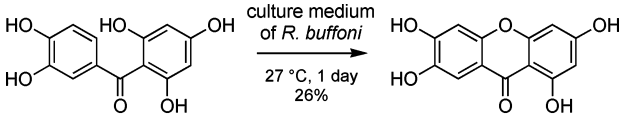
that are able to react in the oxidative cyclization instead of enol groups, by the selected yeasts. Furthermore, as mentioned before, maclurin is the biogenetic precursor of guttiferone A; both compounds could react in the same way. Incubation of maclurin in the presence of *R. buffonii* afforded the natural xanthone derivative, norathyriol **4**, in 16% yield. Investigations of this biotransformation showed that the enzyme was excreted in culture medium. The best yield (26%) was obtained when incubation was performed in culture medium (40 mg, 0.1 mg/mL) after centrifugation (scheme 2), whereas heating of the culture medium before addition of maclurin resulted in a loss of activity.

Scheme 2. Biotransformation of Maclurin by Enzymatic Activity from *R. buffoni*



Maclurin **3** Norathyriol **4**

Norathyriol has been recently described for different biological activities[20,21] and as a safe chemopreventive agent effective against development of UV-induced skin cancer.[22] It is the aglycone of mangiferin, which exhibits a wide spectrum of pharmacological effects.[23] So syntheses of norathyriol, mangiferin, and derivatives have recently received attention,[21,24] and our method could serve as a straightforward alternative to multistep syntheses.

Such chemical cyclizations were reported; benzophenones were converted into xanthone photochemically[25] or by using chemical reageant potassium ferricyanide.[26] A mechanistic study showed that reaction of garcinol with the stable DPPH radical generates a conjugated radical whose cyclization involving the catechol ring leads to the tetracyclic xanthone derivatives.[27]

The natural occurrence of xanthones with the corresponding benzophenones could be in agreement with the mechanism of cyclization proposed by Sang et al. for garcinol.[28] In some cases, xanthones are a mixture of isomers. Maclurin, norathyriol (1,3,6,7-terahydroxy-xanthone), and its isomer 1,3,5,6-terahydroxy-xanthone have been isolated from *Symphonia globulifera*.[29] Likewise, oxy-guttiferones K, K2, I, and M together with their corresponding guttiferones have been isolated from *Garcinia cambogia*.[28]

However oxyguttiferones are not systematically observed in the presence of guttiferone suggesting an enzymatic-catalyzed cyclization. Mechanisms for the cyclization in biosynthetic pathways of xanthones have been postulated.[30] A xanthone synthase has been identified as cytochrome P450.[31] However, cyclization of hydroxybenzophenones by laccase from *Polystictus versicolor* and horse radish peroxidase have been reported, but yields in these enzymatic oxidations were very low (5%) and the enzymes did not act on maclurin.

In this work, we show the relevance in the screening of microorganisms able to transform a molecule since it allows finding unexpected enzymatic activity which could be used in organic synthesis. For this purpose, our combinatorial approach is a suitable strategy to decrease the number of assays. The newly found oxidative activity acts like peroxidase and lacase, and just as those enzymes, it is excreted. It must be noted that no lacase and peroxidase have been described in wild-type strains of yeast. This strategy has been used in the preparation of new oxyguttiferone and in the first enzymatic synthesis of norathyriol.

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Supporting Information Available. Screening of active microorganisms, preparation of oxy-guttiferone A and norathyriol, 1D and 2D NMR spectra of products, and protocols of measurement of biological activities. This material is available free of charge via the Internet at http://pubs.acs.org.

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