Synthesis of Lophirone C using coconut water peroxidase

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Received: 16/09/2018 / Accepted: / Published:

Abstract: Lophirone C is a lignan with several pharmacological activities reported such as anticarcinogenicity and antioxidation activities. The bark of Lophira spp contains that lignan. Due to those important properties, the purpose of this work is the organic and enzymatic synthesis of Lophirone C. Starting from the methylation of resorcinol with methyl iodide using potassium carbonate as base, the product was acylated by a mixture of acetic anhydride and trifluoroacetic acid, so providing the acetophenone. Following step was the aldol condensation of this ketone with 4-methoxybenzaldehyde, using ethanol as solvent and potassium tert-butoxide as base. With the chalcone formed, the deprotection of the phenolic hydroxyls and subsequent oxidative coupling provides the final product with synthetic Lophirone C.

Keywords: aldol reaction; Lophirone c; lignin; chalcone dimers; stem bark; coconut peroxidase; organic synthesis.
1. Introduction

The biological activity of lophirone C has been reported in literature and it exhibits a range of interesting activities. These include anticancer, antioxidant, anti-inflammatory, analgesic, and antibacterial/microbial activity. Lophirones C is a member of numerous chalcone dimers found in Lophira spp, a tree which is widely distributed in the woody savanas of tropical Africa and is used in folk medicine. Since vegetable extractivism provides small amounts of this substance and compromises the preservation of the plant, synthetic products can make medium and long-scale production feasible, cheaper, and offers the flexibility for the preparation of analogues as well.

2. Results and Discussion

The H and C nuclear magnetic resonance data provided confirmed that the isolated compounds are the acetophenone and first chalcone expected, as these data are the same as those obtained in literature.

In the step for deprotection of the phenolic hydroxyls, surprisingly, another molecule was formed, most probably 4',7-Dimethoxyflavanone. For the preparation of the chalcone 1-(2,4-dimethylphenyl)ethanone first we tried to use distilled water as solvent however the reaction seemed stuck, so we used only ethanol (EtOH). In the deprotection of the phenolic hydroxyls. dichloromethane created emulsion in the extraction, so ethyl acetate showed as better solvent.

**Acetophenone (3):**

δ 7.82 (d, J = 8.7 Hz, 1H), 6.50 (dd, J = 8.7, 2.3 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 2.56 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 198.22, 164.70, 161.22, 132.75, 120.98, 105.12, 98.30, 55.53, 55.45, 31.77.

**Chalcone (4):**

δ 7.73 (d, J = 8.6 Hz, 1H), 7.64 (d, J = 15.7 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 15.7 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 6.55 (dd, J = 8.6, 2.2 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 190.74, 164.05, 161.32, 160.34, 142.15, 132.78, 130.05, 128.22, 125.09, 122.52, 114.38, 105.22, 98.75, 55.83, 55.60, 55.44.

![Figure 1. Methylation of resorcinol, preparation of acetophenone and preparation of chalcone](image-url)
3. Materials and Methods

1 - Preparation of 1,3-dimethoxybenzene
To a stirred solution of resorcinol (10g, 90mmol) and acetone (100 ml) in a 500ml flat-bottomed flask in an ice bath was added K$_2$CO$_3$ (31g, 220mmol) and methyl iodide (13ml, 200mmol) drop wise (using a Pasteur pipette) over a period of 10 min. After complete addition, the solution was stirred overnight at room temperature (r.t.). After the completion of the reaction, acetone was evaporated under reduced pressure (rotavap). The crude was diluted with water and extracted two times with chloroform. The organic phase extracts were dried (Na$_2$SO$_4$) and evaporated. The title compound was obtained with 100% yield. The mechanism of this reaction is based on Williamson ether synthesis.

2 – Preparation of acetophenone
Dimethylated resorcinol (5g, 36mmol), acetic anhydride (7ml, 102mmol) and F$_2$CCOOH (TFA) (37ml) were all put to mix in a flask, on magnetic stirrer at r.t. After 1.5 hours, an aqueous solution of NaHCO$_3$ 10% (50ml) was added and stirred. Thin-layer chromatography was performed (using hexane 90% and ethyl acetate 10% as mobile phase), showing a more polar compound (R: 0,5) if compared to dimethylated resorcinol (R: 0.625) and the sample was immersed in 2,4-dinitrophenylhydrazine, testing positive for carbonyl groups of the new compound, 1-(2,4-dimethylphenyl)ethanone. The crude was diluted with water and extracted with ethyl acetate. The organic phase was dried (Na$_2$SO$_4$) and concentrated, the product was purified by column chromatography using only hexane as mobile phase.

3 – Preparation of chalcone
Using a 50ml round-bottomed flask, it was put ethanol (30ml) as solvent to 1g of the acetophenone, 755,64mg of p-anisaldehyde (1 e.q.) and 622,69mg of potassium tert-butoxide (1 e.q.). The solution was stirred 24x7. It was added 1g of Na$_2$SO$_4$ then filtered with filter paper, then concentrated at rotavap. The chalcone was purified by column chromatography using only hexane 90% and ethyl acetate 10% as mobile phase. At room temperature it appeared a yellow powder the compound, as the literature refers.

3 – Deprotection of the phenolic hydroxyls and continuation
Using dichloromethane as solvent (20ml), AlCl$_3$ (1 e.q.) and the chalcone, it was stirred for 24h at r.t. In the extraction, for the organic phase was used ethyl acetate. Surprisingly, a less polar compound was showed in TLC, so the studies are on going. The next step will be oxidative coupling using coconut peroxidase.

4. Conclusions
Our attempt to shorten pathways to achieve the synthetic Lophirone C has been successfull so far, however the studies are still on going. We achieve to synthetize a chalcone that is a pre-molecule to the Lophirone C.

Conflicts of Interest
State any potential conflicts of interest here or “The authors declare no conflict of interest”.

References and Notes
1. Gopinath Gudipudi, G.G.; Someswar R. Sagurthi, S.R.S; Shyam Perugu S.P. Rational design and synthesis of novel 2- (substituted-2H-chromen-3-yl)-5-aryl-1Himidazole derivatives as an anti-angiogenesis and anti-cancer agent. RSC Advances 2014, 4(99), 56489-56501.
2. Guangchang Liu, G.L.; Bo Xu, B.X.; Hydrogen bond donor solvents enabled metal and halogen-free Friedel–Crafts acylations with virtually no waste stream. Tetrahedrom Letters 2018, 59, 869-872.
3. Hossay Abas, H.A.; et al. Total Synthesis of (+)-Lophirone H and Its Pentamethyl Ether Utilizing an Oxonium–Prins Cyclization. Organic Letters 2017, 19, 2486-2489.