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SYNTHESIS AND CHARACTERISATION OF BENZO(H)CHROMEN-2-ONE3-METHYL CARBOXYLATE USING PHOSPHOTUNGSTICACID ($H_3PW_{12}O_{40}$) AS AN EFFECTIVE CATALYST AND STUDY ITS ANTIMICROBIAL AND ANTI-OXIDANT PROPERTIES

***Anna R. Anthony[1], Abhay Choudhary[2], Shrikant Gajbhiye[2] and A. S. Bobade[2]**

[1]Associate Professor, Department of Chemistry, Maharshi Dayanand College Parel, Mumbai -12, Maharashtra, India.

[2]Haffkine Institute Parel, Mumbai- 12.

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***Corresponding Author'**
Dr. Anna R. Anthony
Associate Professor,
Department of Chemistry,
Maharshi Dayanand
College Parel, Mumbai -
12, Maharashtra, India.

ABSTRACT

Coumarins and their derivatives are very important organic compounds, They are biologically active and widely occur in nature. They are the structural unit of several natural products. Their applications range from pharmaceuticals, optical brighteners, and laser dyes. Also, coumarins and functionalized coumarins have shown activity as antimicrobials and chemotherapeutics. Some Coumarin derivatives have been widely used as an important chemical in perfume, cosmetic as well as pharmaceutical industrial preparation. Coumarins act as in intermediate for the synthesis of various biologically active molecule such as coumarones, and fluorocoumarins. These properties have made coumarins interesting targets for organic chemists. Thus the synthesis of coumarins is of continuing interest. Phosphotungsticacid ($H_3PW_{12}O_{40}$) is a heteropoly acid environmentally benign non-toxic widely used heterogeneous catalyst. It is the strong acid of the hetero poly acid. The hetropoly anion ($PW_{12}O_{40-3}$keggin unit) represent the structure of HPW, has been investigated by Jalil.*et al* using different spectroscopic and chemical techniques such as X-ray diffraction studies (XRD) and infrared (IR) spectroscopy indicated the acid stability and Izumi *et al reported HPW* molecule is highly stable up to 600^{oC}. This paper focuses is to develop environment friendly reactions, simple, highly efficient and high yielding protocol for the

synthesis of Benzo(h)chromen-2-one3-methyl carboxylate using a Phosphotungsticacid ($H_3PW_{12}O_{40}$) as catalyst. This compound showed antimicrobial and anti oxidant properties.

KEYWORDS: Anti microbial, antioxidant Phosphotungsticacid, microwave irradiation.

INTRODUCTION

Coumarins are nowadays an important group of organic compounds that are used as additives to food and cosmetics[1], optical brightening agents[2], and dispersed fluorescent and laser dyes.[3] The derivatives of coumarin usually occur as secondary metabolites present in seeds, root, and leaves of many plant species. Their function is far from clear, though suggestions include waste products, plant growth regulators, fungistats and bacteriostats.[4] It is therefore of utmost importance that the synthesis of coumarin and its derivatives should be achieved by a simple and effective method. Coumarins can be synthesised by one of such methods as the Claisen rearrangement, Perkin reaction, Pechmann reaction as well as the Knoevenagel condensation.[5] It was recently shown that the Pechman reaction could be quickly achieved using microwave irradiation of the reagents in household microwave oven.[6] Since the solvent free phase-transfer catalytic reactions under microwave irradiation has prompted us to present our results of the synthesis of coumarins by the Knoevenagel condensation under such conditions.

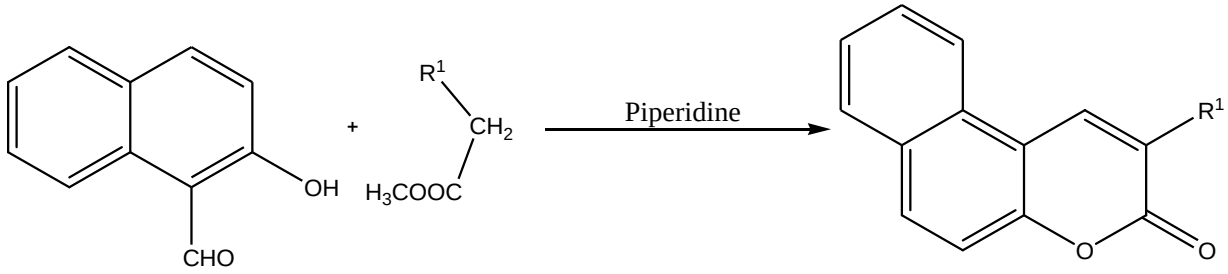
Phosphotungsticacid ($H_3PW_{12}O_{40}$) a commercially available environmentally benign catalyst non-toxic widely used for the synthesis of the substituted coumarin The scope of this catalyst has not been fully explored, but can be used as buffer, neutralizing agent. Owing to the numerous advantages associated with cheap and non-hazardous catalyst, and also realizing g the importance of coumarin herein we would like to focus the eco –friendly method for his synthesis of derivatives of coumarin using cheaper and commercially available acid catalyst Phosphotungsticacid ($H_3PW_{12}O_{40}$) and also by the Knoevenagel condensation under microwave irradiation. This paper focuses is to develop environment friendly reactions, simple, highly efficient and high yielding protocol for the synthesis of new derivative of coumarine using Phosphotungsticacid as a catalyst.[7-8]

RESULTS AND DISCUSSION

The aim of the present paper is to show that under the microwave irradiation the Knoevenagel condensation can be successfully applied to the synthesis of a number of coumarins, and the scope of the method is much broader. Here we report a very simple, fast

and general procedure where the Condensation of 2-hydroxy naphthaledyde with and dimethyl malonate in presence of Potassium dihydrogen phosphate catalyst leads to the synthesis of derivatives of coumarins(Figure-1).

Scheme--A

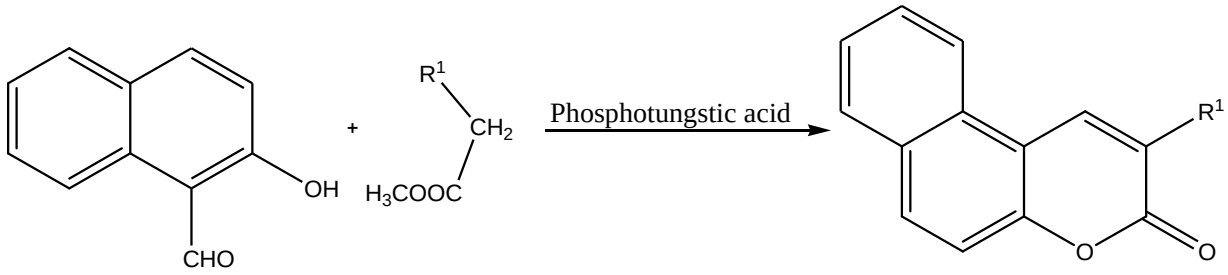


1 1 2 3-k

Where R^1 is $COOCH_3$

Figure-1: Synthesis of coumarin derivatives by Knoevenagel condensation under microwave irradiation & using Potassium dihydrogen phosphate as catalyst.

Scheme--B



1 2 3k

Where R^1 is $COOCH_3$

Figure-1Synthesis of derivatives of coumarin using phosphotugstic acid catalyst

RESEARCH METHODOLOGY

A mixture of 2- hydroxyl naphthaledyde (1) (1mmol), carbonyl compound (dimethyl m,alonate)(2) (1 mmol), and Potassium dihydrogen phosphate (20mol%)in ethanol(5ml)was stirred at room temperature for one hour .It is then neutralised with ammonium chloride solution extracted with ether. Ether layer was dried with sodium sulphate and evaporated to dryness to get the product.

Table-1

Compound	M.Pt °C	Yield %	IR(KBr)	NMR: ϑ (ppm)
3k	215	68	1750,1210,1720, 3080,1604,1450,	3.9(s,3H)8.12(s,1H) 7.68(m,4H)8.57 (d,1H)8.33(d,1H)

Frist experiment focused to carry out the reaction in piperidine in microwave under normal condition, in the second stage the reaction was carried out in presence of potassium dihydrogenphosphate catalyst with conventional heating and in modified microwave and compared their yield with first part. Under modified microwave heating offers a convenient environmentally friendly alternative to conventional reactions. Clearly, the reaction time by microwave heating has been reduced with higher yield than conventional heating (86% versus 65 %,)Monitoring of the reactions and analysis can be accomplished by using standard methods (thin layer chromatography, ^{1}HNMR, FT-IR spectroscopy.). Finally, the product is isolated by crystallization. The formation of coumarin was evidenced by the absence of two peaks at 2880cm^{-1} (Ar-CHO) and 3550cm^{-1}(Ar-OH)but the appearance of two prominent peaks due to C-O-C at 1275-1210cm^{-1} and lactone C=Oat 1720-1700cm^{-1}, rest all the substituents peaks are shown as per literature .The detailed data is as shown in the Table -1 The proton nuclear magnetic spectral analysis (^{1}HNMR) of the compound showed signals corresponding to the multiplicity for different types of protons were consistent with assigned structure.

Anti-microbial studies

Pharmacology analysis.

In Vitro Antibacterial Assay

Anti bacterial testing is carried out at Haffkine Institute for Training Research & Testing.

The anti-microbial activity of newly syntheisied Coumarins was conducted against Escherichia coli, (ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungi species used are Asp ergilliusniger(ATCC 16404) and Candida albicans (ATCC 10231) Ampicillin was employed as reference to compare the results. Nutrient broth was used for the preparation of inoculation of the bacteria and nutrient agar was used for the screening methods. The synthesized coumarin derivatives were screened in Vitro anti-microbial efficacy testing. In vitro anti-microbial efficacy testing

was carried out by broth dilution method as mentioned in "Pharmaceutical Microbiology" Edited by W. BHugo & A. D. Russel, Sixth Edition, Blackwell Science publication. The concentration of the samples used were 25 ppm, 50ppm, 100ppm, & 150 ppm. Initially, Dimethylsulphoxide solvent was used to prepare stock solution of 1000ppm. of all samples separately, thenfurther required dilutions were done in respective broth medium i.e Muller Hinton medium. For anti-bacterial activity, Muller Hinton broth was used as the nutrient media. Test bacterial species used are Escherichia coli, (ATCC 10148), Staphylococcus aureus (NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungi species used are Aspergilliusniger(ATCC 16404) and Candida albicans (ATCC 10231) in different concentrations starting from 25ppm. All the coumarin derivatives are active against the test bacteria and fungai in different concentrations. The four different concentrations of the samples 25 ppm, 50 ppm,100 ppm, &150 ppm per ml. were prepared and taken in Muller Hinton broth separately in sterile test tube and to each individual test tube 0.1 cm^3 of above mentioned bacterial suspension was added (having approximately1.0 x 106 *CFU). These tubes were then kept for incubation at 37^oC for 48 hours. To check the growth if any.

Compound Name 3k.

Table-2.

Sr.No	Test bacterial species	Standard reference sample, Ampicillin/ fluconazole (MICppm)	Inhibition\ Viability of the test bacterial species after 48 hours of incubation in the concentration of			
			25ppm	50ppm	100ppm	150ppm
01	Pseudomonas Aeroginosa (Fisher's immunotype-IV	150	V	V	**N	N
02	Escherichia coli, (ATCC 10148),	100	V	V	N	N
03	Staphylococcus aureus(NCTC 3750),	100	V	V	N	N
04	Aspergilliusniger (ATCC 16404)	150	V	V	N	N
05	Candida albicans (ATCC 10231)	100	V	V	N	N

Compound labelled as' 3k,' kills /inactivates the test organism Escherichia coli, (ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher' Immunotype IV), test fungai species used are Aspergilliusniger(ATCC 16404) and Candida albicans (ATCC 10231) in the concentration of 100 ppm, In other words the compound 3k have shown the anti-bacterial/antifungal activities in the concentration of 100 ppm, against the

above mentioned test bacterial/fungal species. whereas Standard reference sample ampicillin/fluconazole (MIC) at 100ppm in the same condition against Escherichia coli, (ATCC 10148) and Staphylococcus aureus(NCTC 3750), but against Pseudomonas aeruginosa is150ppm. Standard reference sample fluconazole shows MIC at 100ppm against Candida albicans (ATCC 10231) but 150 ppm against Aspergillius Niger(ATCC 16404)

Anti-oxidant activity

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidativestress. DPPH Radical Scavenging Activity.10 ml of the different concentrations of samples /standard was centrifuged at 3000 rpm using a centrifuge for 10 minutes and collected. The supernatant of the extract (1 ml) was added to 3 ml of methanolic solution of ofof DPPH (20 mg/l) in a test tube. The reaction mixture was kept at 250C for one hour in anincubator. The absorbance of the residual(1,1-Diphenyl-2-picrylhydrazy)l DPPH solution was determined at 517 nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. The standard used was BHT Butyrated Hydroxy Toluene as positive control. The inhibition was calculated in following formula, I (%) = 100 x $(A_0-A_1)/A_0$Where A_0 is the absorbance of the control; A_1 is the absorbance the extract/standard, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined and expressed as IC50 value. The lower the IC_{50} value indicates high antioxidant capacity. The result is as shown in table-3.

Table-3

Antioxidant activity			
Sr.No	**standard**	**Sample Code**	**IC_{50}±SD**
1	BHT(ButyratedHydroxyToulene)	BHT	8.25±0.336
2		3k	16.00+1.77

CONCLUSION

- Mild reaction conditions, short reaction time, simple experimental work up cheapness of the reagents are the noteworthy advantages of this environment friendly protocol.
- A practical method for an efficient synthesis of product (3k) using an inexpensive catalyst at ambient temperature has been described. High yields along with simple reaction condition auger well for the application of this strategy for the synthesis of derivative of coumarin.

- The synthesised compound is found to possess good anti-bacterial/anti-fungal activity when compared with the standard. This compound exhibits better anti-bacterial &antifungal activities compared to the standard reference sample ampicillin & fluconazole respectively. It is found to be good antioxidants too.
- Thus the development of an efficient and versatile method to synthesis of coumarin derivatives is an active ongoing research and there is a scope further improvement towards milder reaction condition and yield.

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