SHORT COMMUNICATION

SYNTHESIS AND APPLICATIONS OF COUMARIN

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ABSTRACT

In this work, coumarin was synthesized by Perkin reaction using salicylaldehyde, acetic acid and sodium acetate. Due to the misuse of acetic anhydride in narcotics synthesis, acetic acid was substituted for acetic anhydride in Perkin reaction. On the basis of this substitution a hypothesis was proposed that "acetic acid could be substituted as an acetylating agent in place of acetic anhydride in coumarin synthesis via Perkin reaction". In the present research project, salicylaldehyde was prepared from phenol, sodium hydroxide and chloroform for further procedure. Then four different coumarin samples were synthesized by changing the parameter of reactants proportions. From this parameter, we designed a trend of high product yield. Yields of Coumarin samples will lead towards either acception or rejection of the above proposed hypothesis. In the next step, these Coumarin samples were characterized by age yield (%), solubility and melting points. At last Antibacterial activities of all the four coumarin samples were evaluated against two bacterial strains; *E.coli* and *S.aureus*. As a consequence of all above, it was inferred that the yields of all coumarin samples obtained were low as compared to the yield obtained by the use of acetic anhydride in previous reports. This led to the rejection of proposed hypothesis. Among four Coumarin samples, sample-4 obtained by taking equal amounts of all the reactants had shown maximum yield, best characterization and excellent antibacterial activity. In spite of low yields obtained, the remarkable antibacterial activities of Coumarin samples have enabled us to suggest coumarin as a strong antibacterial agent and it must be employed for further applications.

Keywords: Acetylating agent, salicylaldehyde, acetic acid, sodium acetate, *E. coli*, *S. aureus.*

INTRODUCTION

Coumarin is a phytochemical (benzopyrone); a toxin found in many plants, notably in high concentration in the tonka bean, vanilla grass, woodruff, mullein, lavender, licorice, strawberries, apricots, cherries, cinnamon, sweet clover and bison grass having vanilla like flavor and is a oxygen heterocycle as shown in fig. 1.

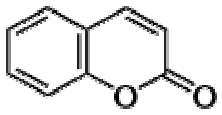

Fig. 1: structure of coumarin

Coumarin can occur either free or combined with the sugar glucose (coumarin glycoside). It has a sweet scent, readily recognized as the scent of newly-mown hay, and has been used in perfumes since 1882. Coumarins are naturally occurring polyphenolics distributed widely in plants, fungi, and bacteria and have found applications for centuries in traditional medicine (Kumar *et al*., 2008).The biosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclization of cinnamic acid. Coumarin has appetite suppressing properties, suggesting its widespread occurrence in plants, especially grasses, is because of its effect of reducing the impact of grazing animal (Laposata *et al.,* 2007).

Due to the potential applications in fragrance, pharmaceutical, and agrochemical industries, coumarins occupy an important position in natural and synthetic organic chemistry. Synthesis of coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus. They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, dispersed fluorescent and laser dyes, insecticides and in optical brighteners (Fulchand *et al*., 2008). Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which had been used in traditional medicine for thousands of years. Coumarins constitute an important class of compounds with several types of pharmacological agents possessing anticancer, anti-HIV, anticoagulant, spasmolytic and antibacterial activity among others. Of the many actions of coumarins, antioxidant and antiproliferative effects standout. A large number of structurally novel coumarin derivatives had shown substantial cytotoxic activity *in vitro* and *in vivo*. Moreover, the inhibitory action on inflammatory cells appeared to surpass any other clinically available compounds. Given that certain substituents are known to

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be required to increase their actions, the therapeutic potential of selected coumarins is fairly obvious (Irena, 2005).

In view of the natural occurrence, useful range of biological activity and diverse pharmacological properties associated with coumarins, many strategies have been developed for the synthesis of coumarins. Classical routes to coumarins incorporate Pechmann, Perkin, Knoevenagel, Reformatsky and Wittig reactions (Rajhita *et al.*, 2006). A number of coumarin derivatives have been isolated from natural sources, and their pharmacological and biochemical properties depend upon the patterns of substitutions (Irena, 2005).

Different methods and conditions can be employed for the synthesis of coumarin and its derivatives. Perkin reaction provides a useful method for the synthesis of α,β-unsaturated aromatic acids and involves the condensation of a carboxylic anhydride with an aromatic aldehyde in presence of a weak base like sodium or potassium acetate or triethylamine (Majumder and Suman, 2007). In our study, a simple and convenient method of unsubstituted coumarin is described by the reaction of salicylaldehyde, sodium acetate and acetic acid. A new approach in Perkin Reaction is described in which acetic acid was replaced for acetic anhydride to suggest a substitute for acetic anhydride as anhydrides are banned due to their misuse in narcotics synthesis. We had employed different amounts of reactants to prepare four coumarin samples. Salicylaldehyde was first prepared in laboratory and then used for further coumarin samples preparation. Then, samples were characterized by age yield (%), solubility and melting point determination. At last, antibacterial activities of all the four coumarin samples were explored against *E.Coli* and *S.aureus. Escherichia coli* are one of many species of bacteria living in the lower intestines of mammals, known as gut flora. *E. coli* can generally cause several intestinal and extra- intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and Gram-negative pneumonia (Ojoo *et al*., 2007). *Staphylococus* is a bacterium that causes a multitude of diseases. These are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Under a microscope, *Staphylococus* bacteria are round and bunched together. They can cause illness directly by infection or indirectly through product they make, such as toxins responsible for food poisoning and toxic shock syndrome. The best known member of the *Staphylococus* family is *Staphylococus aureus. Staphylococus* are the main culprit in the hospital-acquired infections and cause thousands of deaths every year. *Staphylococci* cause abscesses, boils and other infections of the skin such as impetigo. *Staphylococus* organisms also generate toxins and enzymes that can destroy white blood cells (Nikadio and Vaara, 1985).

MATERIALS AND METHODS

Chemicals

Acetic acid, sodium hydroxide, phenol, calcium chloride and methanol were obtained from Merck. Sodium acetate and chloroform were obtained from Farco. All other chemicals and solvents were of the highest available commercial grade and used as they were received without further purification except salicylaldehyde which was prepared in laboratory. Commercially available antibiotic chloramphanicol of Remington Pharmaceutical Industries (PVT) LTD. Pakistan. was taken from local medical store whereas nutrient Agar was obtained from Fluka Industriestrasse.

Bacterial strains

The microorganisms (*E.Coli* and *S.aureus*) used for the study of antimicrobial activity were obtained from Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad.

Synthesis of Salicylaldehyde

Salicylaldehyde was synthesized in the laboratory through Reimer-Tiemann reaction as described by Kar (2004), in which phenolic aldehydes are formed by the reaction of phenol, chloroform and alkali as given in fig. 2.

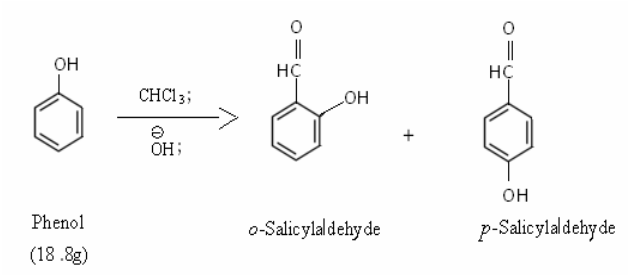

Fig. 2: Synthesis of salicylaldehyde by Reimer-Tiemann reaction.

60 g sodium hydroxide, 80 mL of water and18.8 g of phenol were heated at 60-65°C. 30 mL chloroform was added step by step. Finally reaction mixture was heated for one hour. Liquid layer containing salicylaldehyde was separated through suction pump.

Experimental Protocols

Three sets of experiments were conducted;

(i) Synthesis of Coumarin samples with different proportions of reactants.
(ii) Characterization of coumarin samples including Melting point determination, Solubility and calculations of age yields (%) of four coumarin samples.
(iii) Applications of coumarin samples in the evaluation of antibacterial activity.

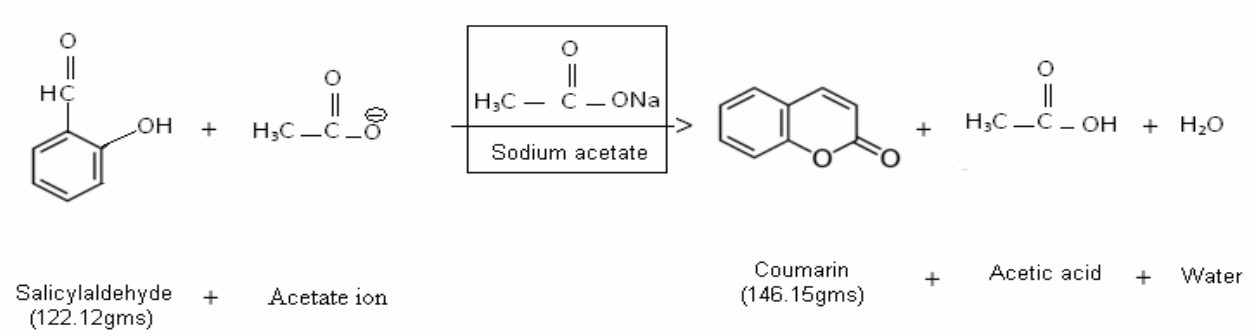

Fig. 3: Synthesis of coumarin by Perkin reaction.

(i) Synthesis of Coumarin samples

Coumarin samples were prepared by Perkin Reaction which involves the interaction of salicylaldehyde and acetic anhydride in the presence of sodium acetate as described by Kar (2004). In our work, acetic anhydride was substituted by acetic acid. Acetic acid and water were also obtained as side products in addition to coumarin as shown in fig. 3.

8 mL salicylaldehyde, 10 g fused sodium acetate and 20 mL acetic acid was transferred to a 250 mL Erlenmeyer flask duly installed with an air reflux condenser; the top end of which was provided with $CaCl_2$. guard tube and heated the mixture in an oil-bath for a duration of 6 hours between 180-190^{0}C.Then cooled the contents of flask and ground them finely with the help of mortar and piston.

Weighed out the crude product and dissolved the crude product in petroleum ether to get pure coumarin crystals, separated after the evaporation of ether.

Sample-2, Sample-3 and Sample-4 were prepared by varying the amounts of different reactant components according to the table 1.

Table 1: Amounts of different reactant components used in Coumarin samples preparation

Sample No.	Salicylaldehyde	Sodium acetate	Acetic acid
Sample-1	8 mL	10g	20 mL
Sample-2	20 mL	10g	20 mL
Sample-3	20 mL	10g	10 mL
Sample-4	20 mL	20g	20 mL

(ii) Characterization of Coumarin Samples

Characterization of all the four coumarin samples were made through melting point determination, Solubility in ether, paraffin oil, ethanol, Sodium hydroxide solution, chloroform etc, and calculation of age yield (%).

(iii) Antibacterial activity

Antibacterial activities of different samples of coumarin were investigated by Agar disc diffusion technique (Conventry and Allan, 2001). Coumarin Samples were tested *in vitro* against two bacterial strains; *Escherichia coli* and *Staphylococcus aureus.* Both of the strains were incubated at 30±0.1^{o}C for 24 hours by inoculation into inoculum. Nutrient agar sterilized in the flask and cooled to 45-50^{o}C was distributed by the pipette (8 mL) into each medium sized petri plate and 35 mL into big sized Petri plate. Then swirled to distribute the medium homogenously. Sterilized petri plates already poured with nutrient- agar were inoculated with 1 mL of above cultured media (105-106 bacteria/mL). Discs already autoclaved were applied on solid agar medium by pressing tightly. Then 20µ L of coumarin solution (1mg/mL) was poured on individual separate disc. The treated Petri plates were placed at 4^{o}C for 1-2 hours and then incubated at 37^{o}C for 18-24 hours. At the end of period, the inhibition zones formed on the media were measured with zone reader in mm expressed as mean diameter of the inhibition zone produced by samples. Commercially available antibiotic chloramphanicol (80 mg/mL) was used as standard reference, by placing with any one sample.

RESULTS

Salicylaldehyde obtained had the following physical and chemical characteristics, similar to one, described by Kar (2004).

Physical characteristics of Salicylaldehyde

- Orange oily liquid
- B.P : 196-197^{o}C
- Slightly soluble in water and fairly soluble in ether and ethanol.

Chemical characteristic of Salicylaldehyde

- Produced deep violet coloration with $FeCl_3$ solution.

Characteristics of Coumarin samples

The age yield (%) and different characteristics of coumarin samples are given in the table 2.

Table 2: Different Characteristics of Coumarin samples

No. #	Characteristics	Sample-1	Sample-2	Sample-3	Sample-4
1.	Age yield (%)	7.41	3.39	4.02	10.60
2.	Melting point	69°C	67°C	73°C	67°C
3.	Solubility in NaOH	Soluble	Soluble	Soluble	Soluble
4.	Solubility in ethanol	Soluble	Soluble	Soluble	Soluble
5.	Solubility in paraffin oil	Soluble	Soluble	Soluble	Soluble
6.	Solubility in $CHCl_3$	Soluble	Soluble	Soluble	Soluble

Antibacterial Activity of Coumarin

All the four coumarin samples showed remarkable antibacterial activity. The values of inhibition zones produced by coumarin samples against two pathogenic bacterial strains in comparison with standard reference chloramphanicol are shown in table 3 and fig. 4.

Table 3: Antibacterial Activity of Coumarin samples

Bacterial strains	Zone of inhibition (mm) at concentration (1mg/ mL)				
	Standard	Sample-1	Sample-2	Sample-3	Sample-4
E. coli	50	25	22	30	48
S. aureus	45	35	40	29	38

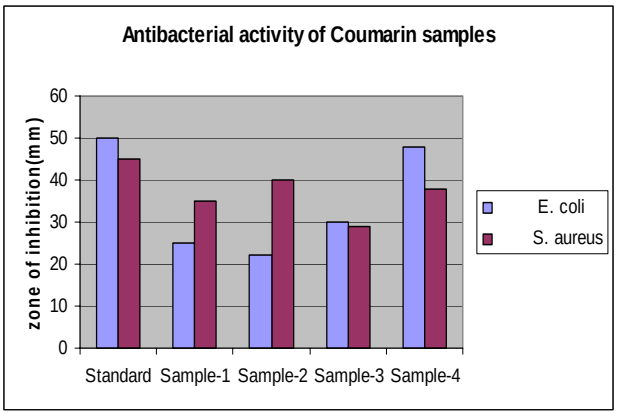

Fig. 4: Antibacterial activity of Coumarin samples.

Sample-4 had shown the best antibacterial activity against both of the bacterial strains as compared to the remaining three coumarin samples in comparison with standard reference. In addition, sample-4 was found more active against *S.aureus* than *E.Coli*. Zone of inhibitions formed by sample-4 are shown in the fig. 5 and fig. 6.

Fig. 5: Antibacterial activity of Coumarin sample-4 against *E. coli.*

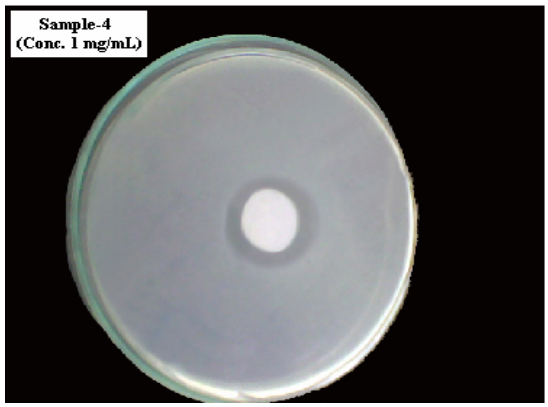

Fig. 6: Antibacterial activity of Coumarin sample-4 against *S. aureus*.

DISCUSSION

Perkin reaction provides a useful method for the synthesis of coumarin providing significant yield as himself done by Perkin in1868 by the reaction of salicylaldehyde, sodium acetate and acetic anhydride (Majumder and Suman, 2007). Our results of coumarin samples regarding age yield (%) were not in accordance with the previous work on Coumarin synthesis by William Henry Perkin, the discoverer of Coumarin, who had reported 50% yield. As we had substitute acetic acid for acetic anhydrirde in the Perkin's protocol, this caused reduced yield suggesting that acetic acid had not worked efficiently as an acetylating agent in Perkin reation (Majumder and Suman, 2007).

The yields of all the Coumarin sample obtained were low as compared to the yield reported by Kar, (2004). Through similar studies he had reported 53% coumarin yield, leading to the rejection of our proposed hypothesis that acetic acid could be used as an acetylating agent in place of acetic anhydride in Perkin reaction for coumarin synthesis.

Our yield was again not in accordance with the 39% yield obtained by using iodine as a catalyst in the same protocol of Perkin reaction (Ahluwalia and Aggarwal, 2007). As many of the steps involved in the synthesis of fine chemicals and pharmaceuticals make use of large quantities of chemicals, some of the protocols may lead to the less yield of the desired product and wastage of reactants in the form of side products. This wastage must be overcome to full fill the demand of high yielding protocols (Ramani *et al.,* 1999). Therefore, as a consequence of all above, acetic acid is not recommended to employ on commercial scale as an acetylating agent in place of acetic anhydride due to the high wastage of reactants and very low yield as given in the table 2. However, low sample yields are somewhat in accordance with the previous work on coumarin synthesis suffering from low chemical yield (Updhyay and Kumar, 2008).
In addition, low products could also be attributed little to lack of purity in starting material such as salicylaldehyde. Slightly less solubility of different samples was attributed to a large number of impurities presences as compared to complete solubility of pure coumarin crystals obtained after removal of impurities (Kirk and Othmer, 1954).

Regarding the antibacterial activity obtained from Coumarin samples, against *E. coli* and *S.aureus,* our results are in accordance with the previous observations, describing antibacterial, antifungal and anti-inflammatory properties of different coumarins (Al-Haiza *et al.*, 2005).

From the comparison of coumarin activity against *Escherichia coli* and *Staphylococus aureus*, it is cleared that coumarin has shown greater antibacterial activity against *Staphylococus aureus* than against *Escherichia coli* which is in co-ordination with the previous observations, in which coumarin is more active against *Staphylococus aureus* than *Escherichia coli* (Vyas *et al*., 2009). These values also cope with the results given by Simone, representing more activity against gram positive bacteria (*Staphylococus aureus*) (Simone *et al*., 2005). The reason for different potential and sensitivity between Gram-positive and Gram-negetive bacteria could be ascribed to morphological differences between these micro-organisms, Gram-negetive bacteria having another phospholipidic membrane (Nikadio and Vaara, 1985). Antibacterial activities against *S.aureus* obtained in our study work are in agreement with the *previous observations* describing coumarin derived carboxylate ligands and their silver(I) complexes showing potent activity against the clinically important methicillin resistant *S.aureus* bacterium.(MIC 80=0.631M) (Creaven *et al*., 2006).

In addition, our results revealed that antibacterial activity of Coumarin samples is also stronger than antibacterial activity of Chalcone, prepared from 2-hydroxy-1-acetonapthone and 3-acetylcoumarin (Prasad *et al*., 2006).

CONCLUSION

As a consequence, the present study showed that the yields of all the four coumarin samples are very low and this had proved that acetic acid had not done the acetylation efficiently as compared to acetic anhydride. Therefore, acetic acid cannot be suggested as a substitute for acetic anhydride in Perkin reaction for coumarin synthesis. So, there is need for some other acetylating agent to obtain sufficiently high yield of coumarin because the preferred requirement to be employed on commercial scale is low reactants wastage and considerable high yield. However, among the prepared samples, highest yield, best characteristics and antibacterial activity of sample-4 is proposing a trend that equal proportions of reactants could lead to the better yield than with different proportions as described in table-1. Furthermore, it was also inferred that coumarin is more active against *S.aureus* as an antibacterial agent than against *E.Coli* as shown in table-3 and figs. 4,5 and 6. At last it is concluded that although, the yields of Coumarin samples are very low but their remarkable antibacterial activity proved that Coumarin must be used as an antibacterial agent such as in beauty soaps, bathing gels and in other applications, requiring strong antibacterial agent.

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