RESEARCH REVIEW JOURNALS
Volume-04
Issue-01
January-2019

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com[UGC Listed Journal]

Synthesis of Simple Coumarins: Mixed Solvent Recrystallization Approach and Modification

[1]NagaretnamPrahadeesh&[*2]Maheswaran Sithambaresan

[1]Demonstrator, Department of Chemistry, Eastern University Sri Lanka, Chenkalady, Sri Lanka
*[*2]Senior Lecturer, Department of Chemistry, Eastern University Sri Lanka, Chenkalady, Sri Lanka*

ARTICLE DETAILS

Article History
Published Online: 10January 2019

Keywords
Simple coumarins, Coumarin, Umbelliferone,Perkin condensation, Pechmann condensation, Mixed solvent recrystallization

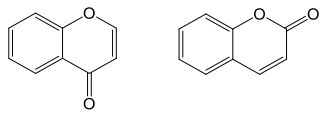
***Corresponding Author**
Email:msithambaresan[at]gmail.com

ABSTRACT

Coumarin compounds have great interest for their wide range of medicinal and pharmacological properties as well as for their fluorescent properties. Simple coumarins are being the major precursor for the synthesis of most of the coumarin derivatives. Simple coumarin (C_1) was synthesized by using Perkin condensation reaction whereas 7-hydroxy coumarin (C_2) and 7-hydroxy-4-methyl coumarin (C_3) were synthesized by using Pechmann condensation reaction. Solvent combination which gives higher recovery percentage on recrystallization was found out. The synthesized simple coumarins were characterized by using spectroscopic analysis (UV, IR, ^{1}H and ^{13}C NMR). The solvent combination which gave higher recovery percentage for C_1 and C_3 were 40 % aqueous methanol and 34 % aqueous ethanol respectively. Two mixed solvents of 33 % aqueous ethanol and 25 % aqueous acetic acid were used to obtain pure crystal of 7-hydroxy coumarin (C_2) for the higher recovery percentage.

1. Introduction

Benzopyrones are a class heterocyclic compounds, have immense interest among many researchers nowadays. Fusion of a pyrone ring with a benzene nucleus gives rise to this class of compounds. There are two distinct types of benzopyrones are recognized, they are benzo-α- pyrones, and benzo-γ-pyrones (Figure 1). Coumarins are belonging to benzo-α-pyrones and flavonoids are the principal members of benzo-γ-pyrones.



benzo-γ-pyrone benzo-α-pyrone

Figure 1: Major types of benzopyrones

Coumarin is considered to be as the parent compound of the benzo-α-pyrone group. It was first isolated from Tonka bean (*DipteryxodorataWilld*) in 1820 by Vogel of Munich[1]. The English chemist William Henry Perkin first chemically synthesized the coumarin in 1868[2]. The coumarin scaffold has large electron conjugated system with electron rich and charge transport properties. The distinctive and versatile skeleton of coumarin scaffolds make it for the applications like fluorescent probes, biological stains, pathologic probe, organic dye lasers, artificial ion receptors, monitoring enzyme activity etc[3]. Coumarin compounds are being an attractive highlight for a long time because of their various pharmacological properties such as anticoagulant, vasorelaxant, lipid lowering, antioxidant, antidepressant, anticonvulsant, antihistaminic, anticancer, antiviral, antiprotozoal, antimicrobial, anti-inflammatory, analgesic, antinociceptive, anti-tumor, antiasthmatic, anti-HIV, and antituberculosis[4,5,6].Coumarins are widely dispersed in the plant kingdom as natural product especially in higher plants. They are highly occurred in Rutaceae, Apiaceae and Umbelliferae plant families as well as in some microbes (*Streptomyces*sp and *Aspergillus*sp) too[6,7,8].

Coumarins are mainly classified into Simple coumarins, Furanocoumarins, Pyranocoumarins and Pyrone substituted coumarins. Simple coumarins are the hydroxylated, alkoxylated and alkylated derivatives of the parent compound - coumarin.Severalcoumarins are synthesized for various applications rather than the pharmacological applications, they are industrial additives, perfumes, aroma enhancer in cosmatics and odor neutralizer in rubber, plastics and paints[9,10]. Simple coumarins are being as the major precursor for the synthesis of most of the coumarin derivatives. Some key organic reactions which are highly used for the synthesis of coumarins, are Perkin condensation reaction[11], Pechmann condensation reaction (Figure 2)[12], Knoevenagel condensation reaction (Figure 3)[13], Friedel-Crafts reaction[14], Claisen rearrangement and Wittig reaction[15,16]. For the various organic synthesis different solvents are used. Solvents can influence on chemical reactivity, solubility and reaction rates. The suitable solvent allows the kinetic and thermodynamic control of the reaction. However, solvent free synthesis of coumarins through Knovenagel and Pechmann condensation reactions has also been reported[17].

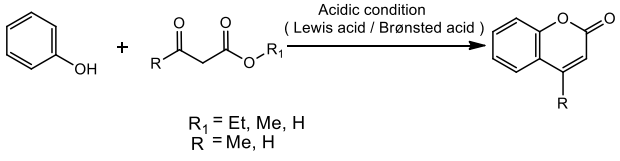


Figure 2: A general reaction scheme for Pechmann reaction

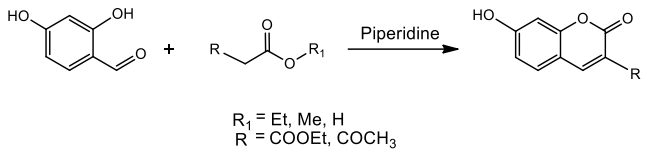


Figure 3: A general reaction scheme for Knoevenagel synthesis of Coumarins

Recrystallization is a purifying technique that removes impurities from organic solid substance by forming the crystals of the desired organic compound from suitable solvent. In this technique, selection of suitable solvent/solvents plays a major role. The target organic compound should be soluble in the hot suitable solvent and insoluble or nearly insoluble in the cold solvent; preferably at room temperature. Conversely, impurities should be insoluble in the solvent at all temperatures or well soluble in the cold solvent thus must remain in the solvent[18]. Suitable solvent can be single solvent or mixed solvent. In case of mixed solvent mostly two solvents are involved; one is "soluble solvent" in which the desired organic compound is soluble where as in another solvent which is the "poor soluble solvent" in which the desired compound is nearly insoluble. During the mixed solvent recrystallization, initially the crude product is dissolved completely in the soluble solvent with heating which is the dissolution step of recrystallizationprocess. Then the poor soluble solvent is added drop wisely with continuous heating until the solution becomes just cloudy. Then again soluble solvent is added until it gives a clear solution. The addition of poor soluble solvent induces the crystallization by getting the solution into super saturated point. However the amount of poor soluble to be added is frequently managed by the self-judgment of visible cloudiness formation in the solution, therefore it may vary in a range but the amount is very important to avoid amorphous solid formation of the desired compound as well to get higher recovery percentage of the desired compound as pure crystals. This volume of poor soluble solvent depends on the crude weight and the volume of the used soluble solvent. This common mixed solvent recrystallization method is often suffered with a disadvantage that unduly large volumes of the poor soluble solvent may be required to reach the cloudiness and thus it is time consuming. It is interested, if the volume percentage of the soluble solvent in final mixed solvent (super saturated stage) is known already for the particular weighed compound which is easy to carry out the mixed solvent recrystallization process by doing the dissolution step with the known appropriate mixed solvent initially. This will help to save the time and reduce the steps of common mixed solvent recrystallization process and make it easier as single solvent recrystallization approach. Furthermore, it will reduce the risk of adding hot poor soluble solvent into the hot soluble solvent which contains the dissolved crude with stirring while heating[19,20].

Seeding process is a method of adding the seed crystal; a small piece of crystal to super saturated solution in order to start crystal growth. Preferentially, the seed crystal is the same material to be grown in the super saturated solution[21]. However, seed crystals are not available for all compounds in all laboratories for all time. In such case, there is a need for recrystallization without seeding. This study deals with the recrystallization process without involving the seeding process.

2. Materials and Methodology

2.1. Materials and Instruments

Standard chemicals (Sigma-Aldrich) were obtained from chemical laboratory, Department of Chemistry, EUSL. The chemicals used were purified prior to its use. Reactions were monitored by using precoated TLC plates; the spots were visualized in iodine bath. Melting points were measured using Gallenhamp melting point apparatus. Infrared spectra were recorded on ATR– Thermo scientific Nicolet IS10 spectrometer. The ^{1}H spectra and ^{13}C spectra were recorded on Bruker NMR spectrometer (400 MHz, Methanol-d_4 as solvent). UV-Visible absorption spectra were recorded using UV-Vis Spectrophotometer: BK-D580, in the range of 190-700 nm.

2.2. Methodology

2.2.1. Synthesis of Coumarin

Salicylaldehyde (8.0 g), fused sodium acetate (10 g) and acetic anhydride (20.0 ml (0.208 mol)) were placed in three necked flasks equipped with a thermometer, small reflux condenser and a calcium chloride drying tube. The mixture was refluxed gently for 6 hours at 180 °C and was TLC monitored (20% of ethyl acetate in hexane as eluent). Then the mixture was steam distilled and the obtained residue was rendered to basic with solid $NaHCO_3$. Then cooled, filtered and washed with cold water. The obtained crude coumarin was boiled in water (800 ml) with activated charcoal (0.8g), filtered and concentrated[22]. Then cooled product was collected and subjected for the mixed solvent recrystallization. The reaction scheme for this synthesis is shown in Figure 4.

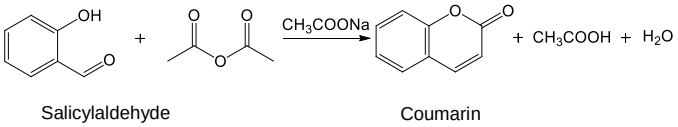


Figure 4: Reaction scheme for the synthesis of coumarin

2.2.2. Synthesis of 7-hydroxy coumarin

Resorcinol (8.8 g) and malic acid (11.896 g) were added into concentrated H_2SO_4 (21.6 ml) with stirring. The reaction mixture was maintained at about 120 °C till the effervescence ceased. Then the hot solution was poured into crushed ice with vigorous stirring. Then the mixture was allowed to stand for 24 hours and filtered[23]. The obtained crude product was subjected for the mixed solvent recrystallization. The reaction scheme for this synthesis is shown in Figure 5.

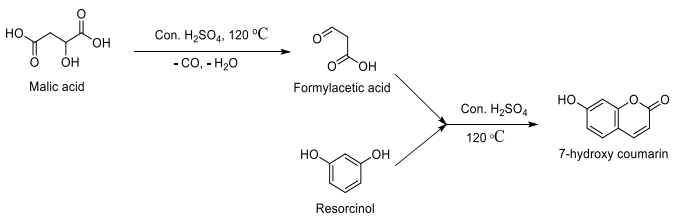


Figure 5: Reaction scheme for the synthesis of 7-hydroxy coumarin

2.2.3. Synthesis of 7-Hydroxy-4-methyl coumarin

A solution of resorcinol (6.0 g) in redistilled ethyl acetoacetate (7.83 ml, 0.0618 mol) was added drop wisely with stirring into concentrated H_2SO_4 (60 ml) and maintained between 5-10 °C. The reaction mixture was kept at room temperature for about 18 hours and TLC monitored (a combined solvent of 30% of ethyl acetate in hexane as eluent). Then it was poured with vigorous stirring into a mixture of crushed ice and water. The obtained precipitate was filtered and washed with cold water. Then the precipitate was dissolved in 5 % NaOH solution and filtered. To the filtrate diluted H_2SO_4 (2M) was added with vigorous stirring until the solution become acid to litmus. The crude product was filtered and washed with cold water[24]. Then it was subjected for the mixed solvent recrystallization. The reaction scheme for this synthesis is shown in Figure 6.

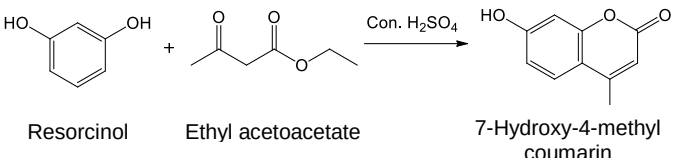


Figure 6: Reaction scheme for the Synthesis of 7-hydroxy-4-methyl coumarin.

2.2.4. Mixed solvent recrystallization

Dried crude sample of each synthesized simple coumarin was tested with various solvents (ethanol, methanol, IMS and water) to find out the "soluble solvent" and "poor soluble solvent". One soluble solvent and one poor soluble solvent were chosen for the mixed solvent recrystallization. Crude sample (1.5 g) was dissolved in the soluble solvent (initial volume- 6 ml) with heating in a specially graduated Erlenmeyer flask. When necessary additional volume of soluble solvent was added for the complete dissolution of the crude product and the final total volume was marked just before adding poor soluble solvent. A measured volume of poor soluble solvent was added drop wisely till the cloudiness first appeared. Then again a measured volume of a small portion of soluble solvent was added drop wisely until get a homogeneous mixture. Then it was continued for further common steps of recrystallization (hot filtration and cooling).

The volume percentage of soluble solvent in the homogeneous mixture at the end was calculated. A series of mixed solvent was prepared in various volume percentage of soluble solvent decreasing 5% deviation from the volume percentage of soluble solvent in the initial homogeneous mixture. The whole recrystallization process was repeated with the series of mixed solvents as in single solvent recrystallization process; by doing the dissolution of the crude product in a series of mixed solvent at first. Finally, dry weight of the crystals- obtained from each solvent mixture at the end of 24 hours was measured and the recovery percentage of the crystal of the desired product was calculated by using the following equation,

Recovery percentage = [Obtained weight of crystal / Weight of crude] ×100%

3. Results and Discussion

3.1. Coumarin analysis(C_1):

Colour of the crystal	: White
Melting point of the crystal	: 69 ^{0}C
Yield percentage of Crude product	: 40.5 %

UV-Visible (nm): 310 and 271 (n→π* of carbonyl chromophore and π→π* aromatic compound).

The UV-Visible absorption spectrum of C_1showed characteristic bands at 310 nm and 271 nm, could be assigned for the n→π * of carbonyl chromophore and π→π* aromatic compound. The obtained peaks havebeen attributed to the carbonyl group of pyrone ring and the fused benzene ring.

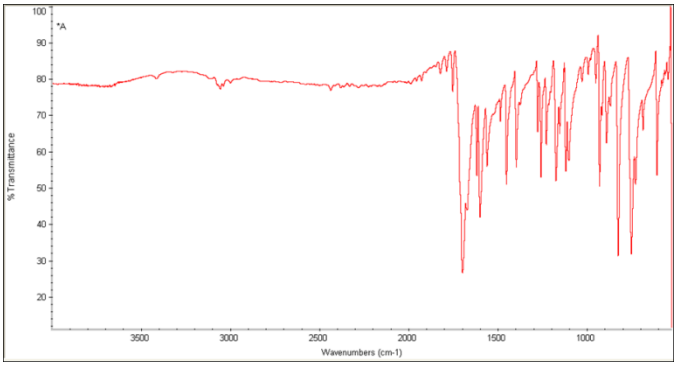
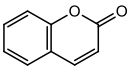


Figure 7: IR spectrum of C_1

IR (cm^{-1}): 3056 (C-H aromatic), 1703 (C=O), 1617 (C=C), 1602 and 1563 (C=C aromatic), 1276 and 1258 (C-O lactone's ester).

The IR spectrum of C_1 (Figure 7) has shown characteristic bands at 1703 cm^{-1} (sharp band) and 3056 cm^{-1}, could be due to the conjugated C=O stretching of the lactone ring and C-H stretching of the aromatic benzene ring respectively. The observed bands at 1276 cm^{-1} and 1258 cm^{-1} could be assigned for the C-O stretching of the lactone's ester bond. The observed bands at 1602 cm^{-1} and 1563 cm^{-1} could be due to the C=C stretching of the compound.

Structure: 

Coumarin can be synthesized by using Perkin condensation reaction. The synthesis involves Aldol condensation of aldehyde group of salicylaldehyde and acid anhydride in the presence of alkali salt of the acid (NaOAc) which acts as a base catalyst to produce an intermediate, o-hydroxycinnamic acid derivative which passes spontaneously into a lactone and produces coumarin. There are possibilities for the formation of *trans* isomer and *cis* isomer of the intermediate, o-hydroxycinnamic acid. The *trans* isomer cannot be lactonised under the conditions of the reaction whereas the *cis* isomer lactonises very readily. However, the formation of *cis* isomer intermediate in Perkin reaction is small thus the reaction produces low yield (40.5 %) of crude Coumarin.

For the best solvent mixture analysis for all three simple coumarins, a series of solution with decreasing volume percentage of soluble solvent from the volume percentage at saturated point (homogenous mixture) were used because the further addition of poor soluble solvent into the mixture reduces the volume percentage of soluble solvent in the mixture but this addition makes the solution into supersaturated stage and induces the crystal formation. If the poor soluble solvent is added excessively this is represented by the solvent with lower volume percentage of soluble solvent that leads to the amorphous formation. Amorphous solids are non-crystalline solids thus they often trap other compounds or atoms which are the impurities whereas crystals have regular 3D-molecular lattice structure thus they are in highly pure form.

Table 1: Recovery percentage of crystals of C_1 in a series ofaqueous methanol mixed solvent

Solvent used for recrystallization	Recovery % of crystal
30 % aqueous methanol	0 %
35 % aqueous methanol	0 %
40 % aqueous methanol	86.4 %
45 % aqueous methanol	65.2 %
50 % aqueous methanol	25.0 %

Coumarin was soluble in methanol whereas poor soluble in water. These two solvents were chosen for the mixed solvent recrystallization. First cloudiness was appeared when the mixture was nearly 50 % of methanol. However, after making the homogeneous mixture by adding more soluble solvent with measured volume, the volume percentage of soluble solvent in the mixture was 50 %. The recovery percentage of coumarin in various mixture of aqueous methanol has been shown in Table 1. The highest recovery percentage (86.4 %) of Coumarin has been obtained in 40 % of aqueous methanol. There is a loss of around 14 % of the coumarin in this recovery. This is due to the loss of impurities and also the desired product (Coumarin) remained in the solvent. It is a common disadvantage associated with the recrystallization process that a smaller amount of desired product remains in the solution as this recrystallization technique mainly involves the solubility difference of the desired compound with temperature. The 45 % aqueous methanol solvent has given 65.2 % of Coumarin recovery whereas 50 % aqueous methanol has given 25 % of Coumarin recovery. These results support the above statement too. The reason for low recovery of Coumarin in these two solvents (45% and 50% aqueous methanol) is due to the higher solubility of Coumarin in methanol. When increase the methanol concentration, certain amount of Coumarin is retained in the solvent mixture. Thus, these two solvents (45% and 50% aqueous methanol) have given lower recovery than 40 % of aqueous methanol and also the recovery percentage of 45 % aqueous methanol is higher than 50% aqueous methanol. However, 30 % and 35 % aqueous methanol showed 0 % of recovery of Coumarin crystals since the amount of poor soluble solvent (water) is increased highly in the solvent mixture thus it produced amorphous solid of Coumarin. If the amount of poor soluble solvent is higher than a certain amount then it will affect orderly arrangement of the compound for the formation of crystals and hence it has produced the amorphous solid.

3.2. 7-Hydroxy coumarin analysis (C_2):

Colour of the crystal : Yellowish orange
Melting point of the crystal : 228 ^{0}C
Yield percentage of Crude product : 41 %

IR spectral analysis of C_2:

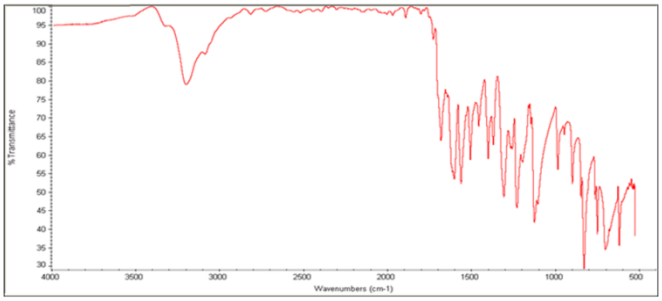


Figure 8: IR spectrum of C_2.

IR (v, cm^{-1}): 3200 (O-H), 1696(C=O), 1229 and 1256(C-O lactone's ester), 1601 (C=C), 1563 and 1510 (C=C aromatic).

The IR spectrum of C_2 (Figure 8) has shown characteristic bands, observed at 3200cm^{-1} (broad band) and 1696 cm^{-1} (sharp band) could be responsible for O-H stretching and the conjugated C=O stretching of the pyrone ring respectively. The observed bands at 1229 cm^{-1}and 1256 cm^{-1} could be assigned for the C-O stretching of the lactone's ester bond. The bands observed at 1601 cm^{-1}, 1563 cm^{-1} and 1510 cm^{-1}may be due to C=C stretching of the compound.

^{1}H- NMR spectral analysis of C_2

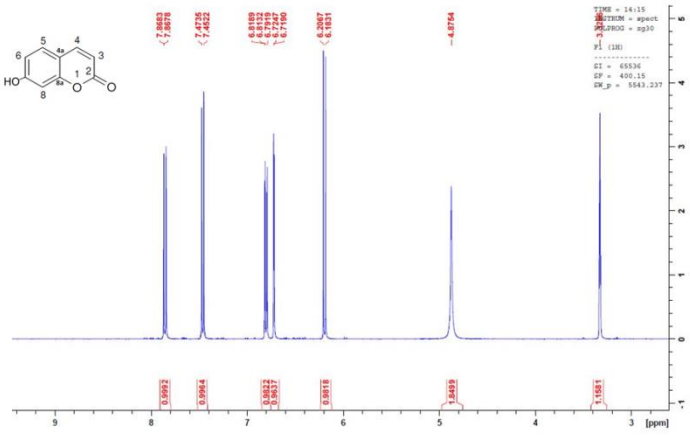


Figure 9: ^{1}H NMR spectrum of C_2

^{1}H-NMR (CD_3OD, 400MHz) (δ, ppm):6.19 (doublet, ^1j=9.3 Hz, ^{1}H integral, 3-H), 6.72(doublet, ^2j= 1.5 Hz, 1H integral, 8-H),

6.81(double doublet, ^1j= 8.4 Hz, ^2j= 1.5Hz, 1H integral, 6-H), 7.46 (doublet, ^1j= 8.4 Hz, 1H integral, 5-H), 7.86 (doublet,^1j= 9.3 Hz, 1H integral, 4-H).

The proton NMR spectrum of C_2 (Figure 9) has shown signals centered at δ=6.72 ppm, δ =6.81 ppm could be assigned for proton 8-H and 6-H. These two hydrogens have coupled with each other with the coupling constant value of 1.5 Hz.The observed signals centered at δ=7.86 ppm and the signals centered at δ=6.19 ppm could be assigned for the protons, 4-H and 3-H respectively. They both have undergone vicinal coupling with the coupling constant of *j*= 9.3 Hz. The observed signals centered at δ= 7.46 ppm could be assigned for the proton 5-H which is ortho coupled with 6-H with coupling constant of *j*= 8.4 Hz.Signal for the proton of hydroxyl group hasn't obtained. The reason for not obtaining any peak for Hydroxyl proton may be due to the fast hydrogen-deuterium exchange between the hydroxyl group of the compound (solvent used was CD_3OD and its corresponding peaks are at δ= 4.8869 and 3.3286 ppm).

^{13}C- NMR spectral analysis of C_2

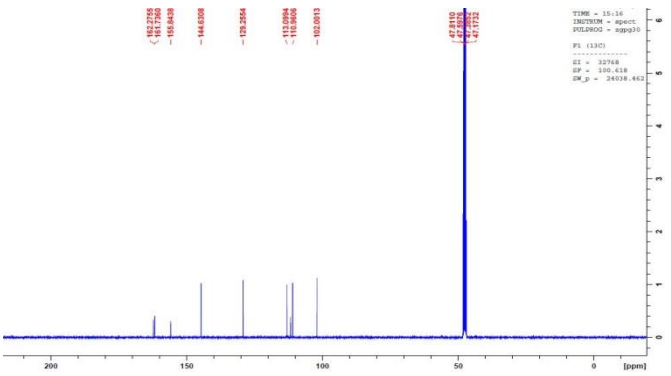
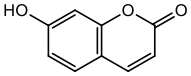


Figure 10: ^{13}C- NMRspectrumof C_2

^{13}C NMR (CD_3OD, 400MHz) (δ, ppm): 2 signals at 110.96 (4a-C and 3-C), 102.00 (8-C), 113.09 (6-C), 129.25 (5-C), 144.63 (4-C), 155.84 (8a-C), 161.73 (2-C), 162.27 (7-C).

^{13}C NMR spectra of C_2 (Figure 10) has shown signals downshifted at δ = 162.27, 161.73 and 155.84 ppm could be assigned for the carbons bonded with electron withdrawing functional group/atoms (ester group and Oxygen atom in hydroxyl group). The observed signals between at δ=144.63 ppm, 129.25 ppm, 113.09 ppm, 102.00 ppm could be assigned for carbons, 4-C, 5-C, 6-C and 8-C respectively. Two signals have been appeared at about δ=110.96 ppm could be assigned for carbons, 4a-C and 3-C. An observed complex signal centered at δ=47.5 ppm could be due to the used solvent (CD_3OD).



The synthesis involves Pechmann condensation reaction which involves the condensation of beta keto acid and phenols in the presence of acidic condition. Here, the malic acid forms formyl acetic acid *in-situ* by giving off $CO_{(g)}$ in the presence of concentric sulphuric acid. Formyl acetic acid is a beta keto carboxylic acid that undergoes condensation with resorcinol in the presence of acid catalyst (con. H_2SO_4) and forms 7-hydroxy coumarin.

Table 2: Recovery percentage of crystals of C_2 in a series of aqueous ethanol mixed solvent

Solvent used for recrystallization	Recovery % of crystal
28 % aqueous ethanol	0 %
33 % aqueous ethanol	82.2 %
38 % aqueous ethanol	68.9 %
43 % aqueous ethanol	32.5 %
48 % aqueous ethanol	0 %

7-hydroxy Coumarin (C_2) was well soluble in ethanol whereas poor soluble in water. These two solvents were chosen for the mixed solvent recrystallization. First cloudiness was appeared when the mixture has nearly 48 % of ethanol. However, after making the initial homogeneous mixture by adding more soluble solvent with measured volume, the volume percentage of soluble solvent in mixture was 48 %.The recovery percentage of coumarin in various mixture of aqueous ethanol has been shown in Table 2. The highest recovery percentage of Coumarin has been obtained in 33 % aqueous ethanol; the recovery percentage was 82.2 %. Around 18% of loss was observed which represents the desired product C_2 and impurities. The 48 % aqueous methanol solvent has given 0 % of Coumarin recovery whereas 43 % and 39 % aqueous ethanol produced 32.5 % and 68.9 % of crystal recovery respectively. This lower amount of recovery percentage is due to the higher solubility of C_2 in ethanol. Certain amount of the C_2 has been retained in the solvent. However, 28 % aqueous ethanol did not produce any crystals but it produced amorphous solid of C_2.

Table 3 : Recovery percentage of crystals of C_2 in a series of aqueous acetic acid mixed solvent

Solvent used for recrystallization	Recovery % of crystal
15 % aqueous acetic acid	0
20 % aqueous acetic acid	84.0 %
25 % aqueous acetic acid	85.6 %
30% aqueous acetic acid	62.5 %
35 % aqueous acetic acid	0

The product obtained in the recrystallization from aqueous ethanol was again subjected for the recrystallization in aqueous acetic acid in order to get extra pure 7-ydroxy Coumarin crystals. The product was soluble in acetic acid whereas poor soluble in water. These two solvents were chosen for the mixed solvent recrystallization again. First cloudiness was appeared when the mixture has nearly 35 % of acetic acid. When the mixture made into a homogeneous mixture, the volume percentage of acetic acid in mixture was 35 %. However this 35 % aqueous acetic acid mixture didn't produce any crystal but 20 %, 25% and 30% of aqueous acetic acid produced 84.0 %, 85.6 % and 62.5 % of crystal of 7-hydroxy Coumarin (Table 03). The highest recovery of C_2 was obtained in 25 % aqueous acetic acid. 20 % aqueous acetic acid mixture also gave quite

higher value of recovery percentage. These two solvent mixtures are adorable for the recrystallization of C_2.

3.3. 7-Hydroxy4-methyl coumarin analysis(C_3):

Colour of the crystal : Colourless crystal
Melting point of the crystal : 186 ^{0}C
Yield percentage of Crude product : 84 %

UV-Visible (nm):322 and 296 (n→π* of carbonyl chromophore and π→π* aromatic compound).

The UV-Visible absorption spectrum of C_3showed characteristic bands at 322 nm and 296 nm, which could be responsible for the electronic transition of n→π * of carbonyl chromophore and the π→π* absorption of the aromatic compound. Hence, the obtained peaks have been attributed to the carbonyl group of pyrone ring and the fused benzene ring.

IR spectral analysis of C_3**:**

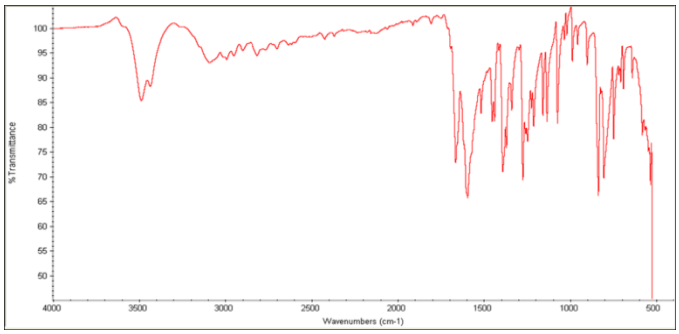
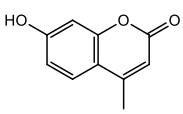


Figure 11: IR spectrum of C_3

IR (v, cm^{-1}): 3490 (O-H), 1674(C=O), 3096(C-H aromatic), 2818 (4-C-CH_3), 1275 and 1247 (C-O lactone's ester), 1599 (C=C), 1521 (C=C aromatic).

The IR spectrum of C_3 (Figure 11) has shown characteristic bands, observed at 3490 cm^{-1} (broad band), 1674 cm^{-1} (sharp band), 3096 cm^{-1} and 2818 cm^{-1}are responsible for O-H stretching, conjugated C=O stretching of the lactone, C-H stretching of the aromatic benzene and C-H stretching of aliphatic group (methyl group) respectively. The observed bands at 1275 cm^{-1} and 1247 cm^{-1} could be attributed to the C-O stretching of the lactone's ester bond. The bands observed at 1599 cm^{-1} and 1521 cm^{-1}may be assigned for C=C stretching.



The synthesis involves Pechmann condensation reaction of resorcinol with a beta keto ester (ethyl acetoacetate) in the presence of the sulphuric acid as catalyst to form 7-Hydroxy-4-methyl coumarin.

Table 4 : Recovery percentage of crystals of C_3 in a series of aqueous ethanol mixed solvent

Solvent used for recrystallization	Recovery % of crystal
24 %aqueous ethanol	0%
29 % aqueous ethanol	82.7%
34 % aqueous ethanol	83.0
39 % aqueous ethanol	60.6%
44 % aqueous ethanol	0 %
49 % aqueous ethanol	0 %
95 % ethanol	56.5 %

Table 4 shows the recovery percentage of 7-hydroxy-4-methyl coumarin (C_3). Crude product of C_3 was soluble in ethanol whereas poor soluble in water.These two solvents were chosen for the mixed solvent recrystallization.First cloudiness was appeared when the mixture has nearly 49% of ethanol and the homogeneous mixture had 49 % ethanol. Highest recovery was obtained from 34 % aqueous ethanol mixture but 29 % aqueous ethanol mixture also produced higher recovery percentage of C_3. 44% and 49% aqueous ethanol mixture didn't produce crystal whereas 24% aqueous ethanol mixture formed amorphous solid.

The crude product of C_3 additionally tested with 95% ethanol too as suggested by A.I. Vogel. However, the crystal formation rate was very slow in 95% ethanol and which produced 56.5 % of the crystal recovery at the end of 24 hours of time but when it was allowed for solvent evaporation on its own time (about 2 days) without seeding which produced 97.2% of the crystal recovery. However, the formed crystals in this manner were contaminated with reddish patches of impurities since the solvent evaporated. When the crystals were washed with cold ethanol, the desired compound too washed off a little. As this recrystallization from 95 % ethanol suffered with extended time for crystal formation and impure crystal formation, it is better to prefer the mixed solvent recrystallization from 34 % aqueous ethanol for ease. Since the addition of poor soluble solvent induces the crystal growth in mixed solvent recrystallization, the seeding process is also not necessary.

4. Conclusion

Three simple coumarins (Coumarin, 7- hydroxyl coumarin and 7-hydroxy-4-methyl coumarin) were synthesized and characterized by using spectroscopic analysis. The compounds were obtained as highly pure crystals from mixed solvent crystallization. The difficulties of using common mixed solvent recrystallization method was overcome by adding the proper mixed solvent initially for the dissolution step instead of dissolving the crude in soluble solvent first and then adding the poor soluble solvent drop wisely to get the super saturation point. The proper mixed solvent for the recrystallization process of coumarin to get higher yield is 40 % aqueous methanol whereas for 7-hydroxy-4-methyl coumarin, the proper mixed solvent is 34 % aqueous ethanol. Pure crystals of 7-hydroxy coumarin were obtained using 33 % aqueous ethanol and 25 % aqueous acetic acid as two mixed solvents.

References

1. Bruneton, J. Pharmacognosy, Phytochemistry, Medicinal Plants 2nd edition. Hampshire UK, Intercept Ltd. 1999: 263-277.
2. Perkin WH. On the artificial production of coumarin and formation of its homologues. Journal of chemical society. 1868; 21: 53–63. doi:10.1039/js8682100053.
3. Wagner BD. The use of coumarins as environmentally-sensitive fluorescent probes of heterogeneous inclusion systems. Molecules. 2009; 14: 210-237.
4. Venugopala KN, Rashmi V, Odhav B. Review on Natural Coumarin Lead Compounds for their Pharmacological Activity. BioMed Research International. 2013: 1-14. doi:10.1155/2013/963248.
5. Yasameen AM, Ahmed AA, Abdul AK, Abu BM. Antioxidant Activity of Coumarins. Systematic Reviews in Pharmacy. 2017; 8(1): 24-30.
6. Matos MJ, Lourdes S, Eugenio U, Orlando A. Coumarins — An Important Class of Phytochemicals. Intech. 2015: 113-140. doi:10.5772/59982.
7. Cooke D. Studies on the Mode of Action of Coumarins (Coumarin, 6-hydroxycoumarin, 7-hydroxycoumarin and Esculetin) at a Cellular Level. PhD Thesis, Dublin City University, Dublin, Ireland. DCU online research access service; 1999. (Accessed 2017.08.17)
8. Li TX, Meng DD, Wang Y, An JL, BaiJia F, et al. Antioxidant coumarin and pyrone derivatives from the insect-associated fungus *AspergillusVersicolor*. Natural Product Research. 2018: 1-6. doi:10.1080/14786419.2018.1509334.
9. Cohen SM, Ellwein LB. Genetic errors, cell proliferation, and carcinogenesis. Cancer Res. 1991; 51: 6493-6505.
10. Fentem JH, Fry JR. Species differences in the metabolism and hepatotoxicity of coumarin, Comp Biochem Physiol. 1993; 104: 1-8.
11. Vijayan KK. One step synthesis of 3-phenylcoumarins and benzylidene coumaran-2-ones. Ind J Heterocyc chem.1998; 8: 1-4.
12. Pechmann H. New mode of education of coumarins-Synthesis of daphnetin. I. Chem Rep. 1884; 17: 929-933.
13. Knoevenagel E. Condensations between malonic esters and aldehydes under the influence of ammonia and organic amines. Chem Report. 1898; 31: 2585-2588.
14. Bert L. General method for the synthetic preparation of coumarines. Compt. Rend. 1942: 214-230.
15. Cairns N, Harwood LM, Astles DP. Thermal Claisen–Cope rearrangements of coumarate derivatives. Total syntheses of the naturally occurring coumarins: suberosin, demethylsuberosin, ostruthin, balsamiferone and gravelliferone, Perkin Transactions. J Chem Soc. 1942; 1: 3101-3104.
16. Yavari I, Hekmat R, Zonouzi A. A new and efficient route to 4-carboxymethylcoumarins mediated by vinyltriphenylphosphonium salt. TetrahedLett. 1998; 39: 2391-2396.
17. Sugino T, Tanaka K. Solvent-Free Coumarin Synthesis. Chemistry Lett. 2001; 30(2): 110-111.
18. Laurence MH, Christopher JM. Experimental organic chemistry: Principles and Practice. Oxford: Blackwell Scientific Publications.1989: 127–132. ISBN 0-632-02017-2.
19. The Organic Chem Lab survival manual. 9th edition James.W.Zubrick. pp 105-106, pp 115-117. ISBN 978-1-118-08339-0
20. Techniques in Organic chemistry. 3rd edition. Jerry.R.Mohrig. ISBN : 13: 978-1429219563 pp 183-197
21. Mullin JW. Crystallization. 4th edition ISBN 0 7506 483 3. pp 199-201
22. Kar A. Advanced Practical Medicinal Chemistry. 1st edition. New Age International Publishers. 2004; 184-205.
23. Pechmann HV, and Duisberg D. Synthesis of coumarins by condensation of phenols with beta keto ester in the presence of Lewis acid catalyst. Chem Report. 1893; 16: 2119-2121.
24. Furniss BS, Hannaford AJ, Smith, PWG. Vogel's text book of practical organic chemistry, 5th edition. Longman scientific & Technical. 1989; 1193-1195.

