Synthesis of nitrocoumarin derivative and separation of its isomers

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الخلاصة

الكومارينات تعمل بشكل مضاد لتأثيرات فيتامين K . خلال تكوين عوامل تخثر الدم (II, VII, IX وكذلك X) في الكبد، تتحول الكومارينات إلى ناتج ايضي غير فعال حيوياً، وبعد ذلك يختزل ويصبح فيتامين K فعالاً ويكون ذلك بتأثير انزيم (epoxide reductase). يعتقد بأن الكومارينات (والتي تتشابه مع فيتامين K من حيث التركيب) تتداخل وتمنع عمل الانزيم المشار إليه بشكل تضاد تنافسي، والذي يؤدي إلى منع تكوين فيتامينK الفعال حيوياً. يتم توصيف الكومارينات (وارفارين) في بعض الامراض: كتخثر الدم في الأوردة ... الخ . الغرض من هذه الدراسة هو تحضير وفصل مركب النتروكومارين (والذي من المحتمل ان يزيد ذلك من فعاليتها). تم تحضير مشتق النتروكومارين وذلك بمفاعلة (7–hydroxy, 4–methylcoumarin) مع خليط من حامض الكبريتيك المركز وحامض النتريك المركز في 0°C. تم تحديد النتائج بواسطة الاجهزة الحديثة المتوفرة .

ABSTRACT

Coumarins antgonise the effects of vitamine K
During the formation of clotting factors II, VII, IX and X in the liver, vitamine K is converted into a biologically inactive metabolite which then reduced back to the active vitamine by the enzyme epoxide reductase.
Coumarins which are structurally similar to vitamine K, are believed to act as competitive inhibitors of this enzyme and thus limit the availability of the active form of the vitamine to form clotting factors. Coumarins, e.g. (warfarin) is indicated in deep–vein thrombosis and in pulmonary embolism …etc.
The aim of this study is to synthesize and isolate a nitrocoumarin derivative with a possible high intrinsic activity.
The nitrocoumarin derivative was synthesized by the reaction of 7–hydroxy, 4–methylcoumarin with mixture of concentrated sulfuric acid and concentrated nitric acid at 0°C.
The identity of the prepared compound had been confirmed using UV–Vissibile spectroscopy, I.R spectroscopy, elementary analysis, (sodium fusion test) and HPLC.

INTRODUCTION:

Coumarin (anhydride of o–coumaric acid) is oxygen containing hetrocyclic organic compound. (Fig. 1).

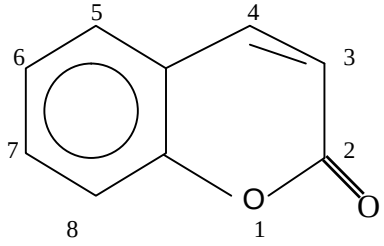

FIG 1 . COUMARIN.

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Coumarin [1,2–benzopyron or 5,6–benzno–(alpha)–pyronel], is a white crystalline solid characteristic odour of new–mownhay, M.P. 69–73°C stable under ordinary conditions and it is slightly soluble in water[1].
Coumarin is a natural product obtainable from several plants such as tonkabean, lavender, sweat clover, grass, strawberries and cinnamon[2].
Coumarin was first reported by vogel[3]. He discovered long colorless crystals on slicing open tonka beans, and which crystallized as glistening needles from aqueous alcohol, or produced synthetically from the amino acid phenylalnine W.H. Perkin[4], the prepared coumarin by the reaction of sodium salt of ortho–hydroxy benzaldehyde (salicylaldehyde) with acetic anhydride and the synthetic coumarin obtained is identical with that isolated from tonka bean.

Neumerous coumarin derivative were synthesized among these:

1- poly (cinnnamate) derivatives as photoalignment mount layer[5].
2- 3–(4–coumarinyl/methylene) phthalides and 2–, or 3–(4–coumarinyl/ethenyl) 4–chromones were prepared by condensation reaction. Beneficial effect of microwave irradiation on length of reaction time was investigated[6].
3- Nowadays these is new path via synthesis of K–trap–coumarin dye[7].

On the light of the introductory remarks mentioned before, we decided to nitrate coumarin derivative in the hope of elevating its intrinsic activity.
Further more it is hoped that the nitrated coumarin derivative has the ability to be a precursor for producing azo–compounds, which represent a wide range intermediate compounds for synthesizing of numerous organic reactants. Posterior to these steps we try to use HPLC which offers the advantage of high speed separation and automated operation more than other chromatographic method.

EXPERIMENTAL:

MATERIALS:

All chemicals were used of analyrical reagent grade, and deionized distilled water was used through out the HPLC separation work.
Resorcinol, BDH, Purity 99.9%. Ethylacetoacetate. Fluka, pure. Conc. Sulfuric acid. BDH. Conc. Hydrochloric acid. BDH. Conc. Nitric acid. BDH. Absolute Ethanol. Lab–scan. Pure Methanol 98%. Lab–scan. Sodium. Dihydrogen phosphate. Fluka. Disodium hydrogen phosphate. Fluka. Sodium metal. BDH. Iron (II) sulfate. BDH. Iron (III) sulfate. BDH. Hydroxylamine. BDH.

GENERAL METHODS:

Melting points were measured using an electrothermal melting point apparatus and are uncorrected.
Infra–red spectra were recorded on Pye–Unicam SP3–300 spectrophotometer, Germany.
Ultraviolet spectra were recorded on Pye–Unicam Ultraviolet spectrophotometer SP8 –O100 Germany.
HPLC–separation were carried out on Lambda–Max Model 481, LC (waters). Spectrophotometer.

Coumarin derivatives:

Synthesis of 7–hydroxy, 4–methylcoumarin (Fig.2)

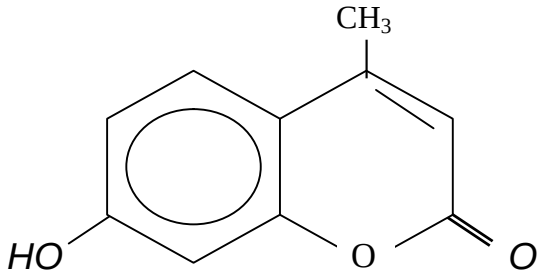


FIG 2 . 7–HYDROXY, 4–METHYLCOUMARIN.

Powdered resorcinol (3.7gm , 0.0336mole) was added to (4.4ml , 0.0346mole) of ethylacetoacetate and stirred till complete solution is obtained and then it was added to conc. H_2SO_4 15ml. and stirred at about (5–10)°C for 30 minutes, later the mixture was poured into crushed ice (about 100gm) the solid 7–hydroxy, 4–methyl coumarin was separated and filtered off then reacted with 10% aqueous NaOH and reprececipitated using 0.5N HCl (80ml).
Then recrystallized from ethanol using charcoal. Yield obtained was (3gm 50.67%). (M.P. 187°–189°C)[8].

Classification test for (Esters, lactones)[8]:

To a few drops of 7–hydroxy, 4–methylcoumarin solution. 0.2gm of hydroxylamine hydrochloride was added followed by the addition of 15ml. of 10% NaOH solution and the mixture gently boiled for 1–2 minutes, then cooled and acidified with dilute HCl, a few drops of ferric chloride solution was added, a deep reddish brown colour developed.
The structure of the compound obtained was confirmed using UV and IR spectroscopy.

Synthesis of nitrocoumarin derivatives (Fig.3):

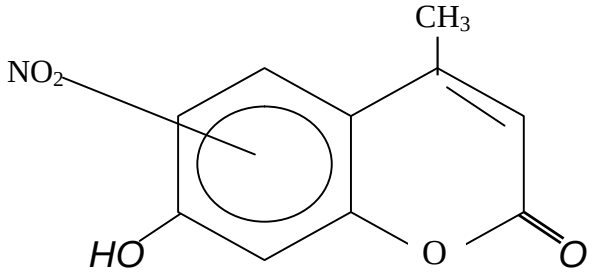


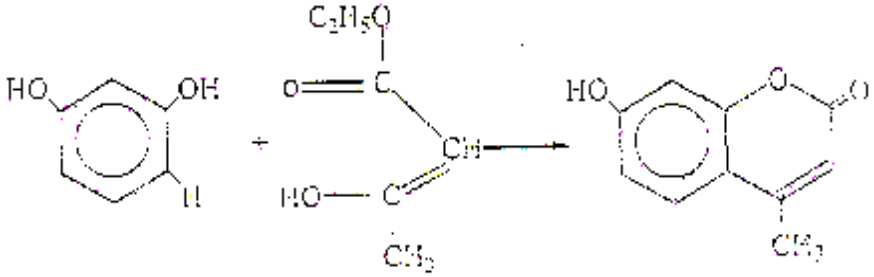
FIG 3 . NITROCOUMARIN DERIVATIVE.

7–hydroxy, 4–methylcoumarin (3gm,0.017mole) was dissolved carefully lowly in cold solution at 0°C of concentration sulfuric acid (1.5ml,0.015mole) and concentration nitric acid (0.75 ml,0.012 mole), stirring was continued at this temperature for 45 minutes.
Nitrocoumarin was recrystallized from ethanol, a yellowish crystalline powder were obtained M.P. (166 –171)°C yield was 1.9 gm ≈ 50%.

Sodium fusion method were used for detection of nitrogen in the nitrocoumarin derivatives[9].
IR spectra of this compound showed characteristic absorption frequencies Fig (3).
High performance liquid chromatography (HPLC) is used to separate and detect the products using ultra violet detector.
Stationary phase is packed in column C8 (Lichrosorb RP–8):4mm in diameter and 25cm long.
Mobile phase is methanol –phosphate buffer pH 7. (30:70).
The inlet pressure necessary to pump the eluant at a flow rate 1ml/min is often in excess of 1000 psi and can reach as high as 5000 psi, sample size is 0.5ml.
Three samples are prepared for detection and separation by HPLC.
Sample (1): Coumarin in methanol (concentration ppm =1).
Sample (2): Coumarin derivative (7–hydroxy,4–methylcoumarin). in methanol (concentration ppm =1).
Sample (3): Nitrocoumarin derivative in methanol (concentration ppm =1).
The chromatograms of these samples shown in Fig. (7).

RESULTS AND DISCUSSIONS :

Synthesis of 7–hydroxy, 4–methylcomarin was carried according to[10].



The UV spectrum of the compound (0.1mg/ml methanol) shows λ max, at 322 nm. (Fig. 4).
IR spectrum (Fig. 5) revealed the following absorption frequencies, cm^{-1} in KBr disc: 3498.13 (O–H) str.: 3111.36 (C–H) str. aromatic and alkene: 2818 (C–H) str. of CH_3 group; 1670.72 (C=O) str.: 1606.69 (C=C) str.

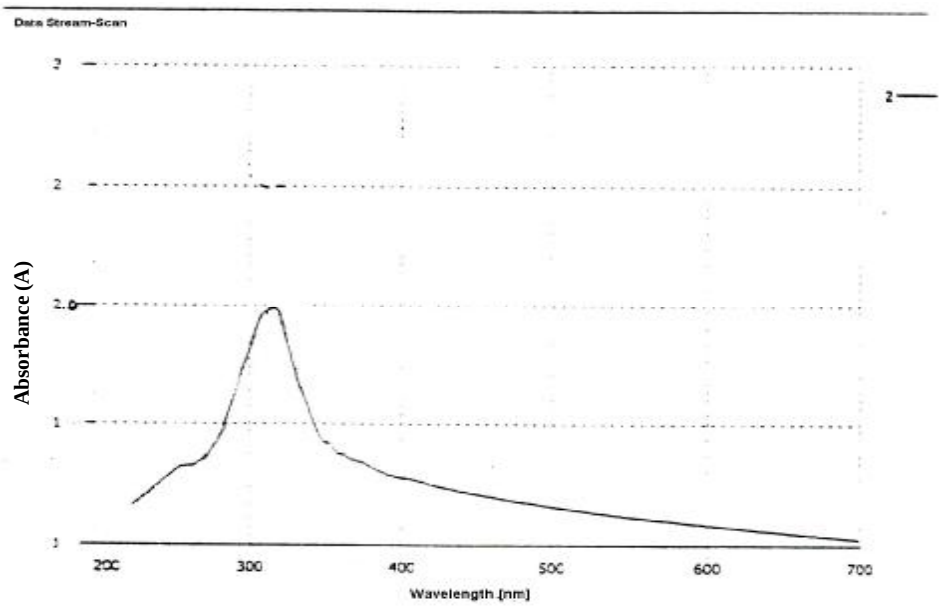


FIG 4 . UV SPECTRUM OF 7–HYDROXY, 4–METHYLCOMARIN.

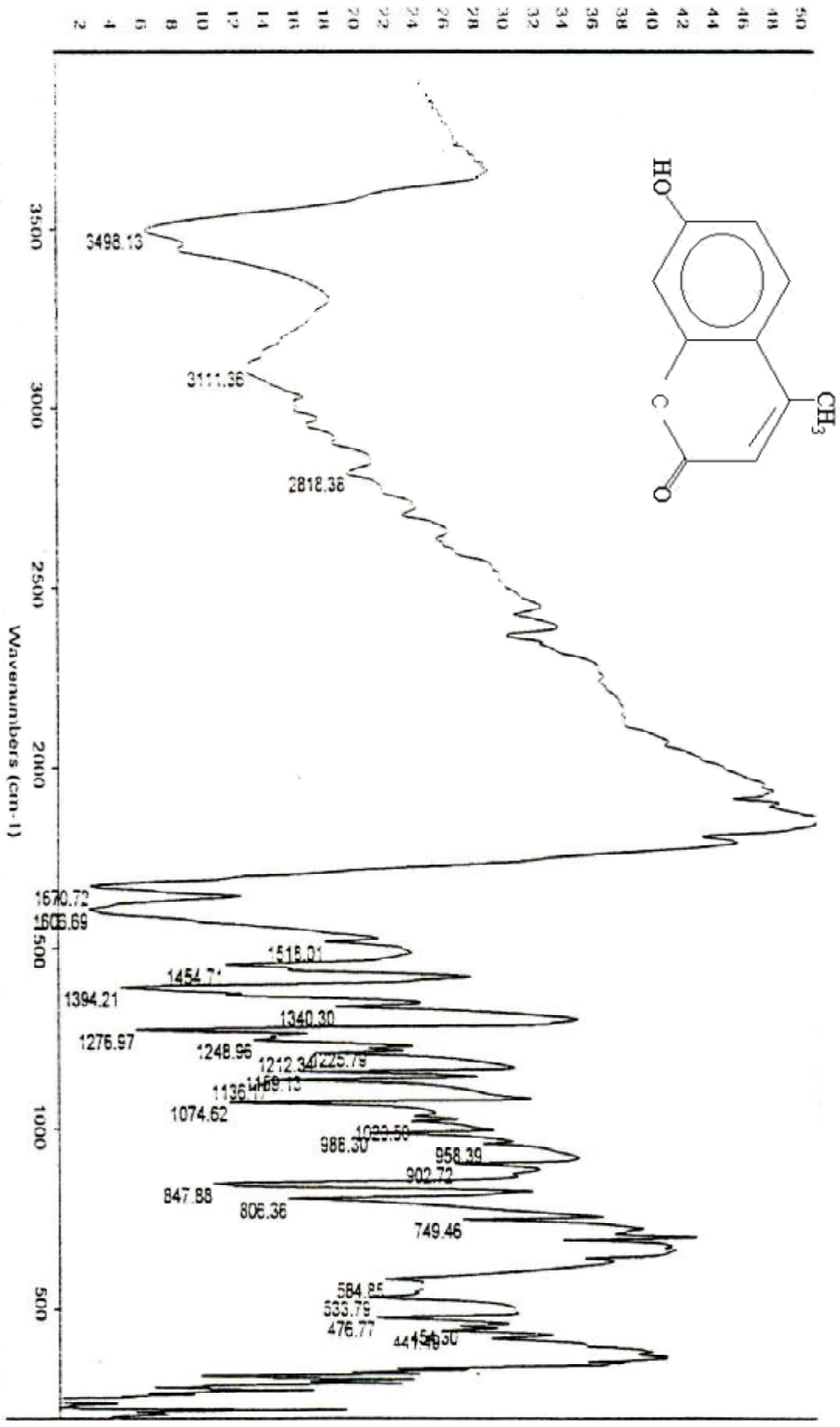


FIG 5 . IR SPECTRUM OF 7–HYDROXY, 4-METHYLCOMARIN.

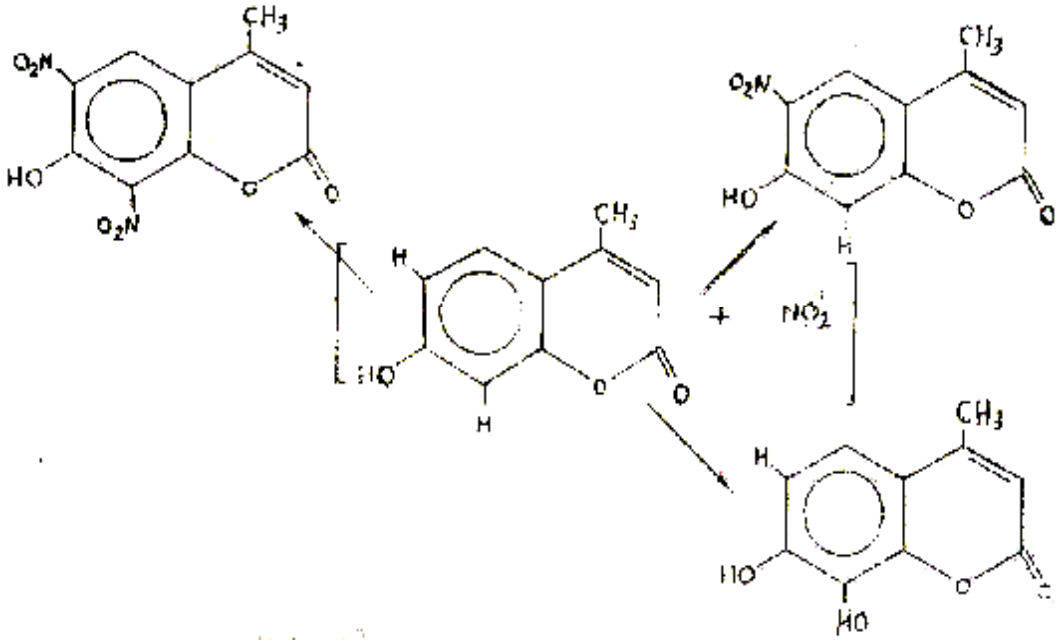
The synthesized coumarin derivatives 7–hydroxy, 4–methylcomarin undergoes substitution reaction was carried out to obtain a good yield it is believed that the nitrating species in such mixture is the nitronium ion NO_2^+.

$$HNO_3 + 2H_2SO_4 \xleftrightarrow{\text{slow}} NO_2^+ + H_3O^+ + 2HSO_4^-$$

The mechanism of this reaction involves protonation of nitric acid by sulfuric acid followed by loss of water molecules to form a nitronium ion. The water is subsequently protonated by the sulfuric acid :

$$HO - NO_2 + H_2SO_4 \xrightarrow{-HSO_4} H - \underset{\displaystyle H}{\overset{\displaystyle |}{O^+}} - NO_2 \xrightarrow{-H_2O} NO_2^+$$

Here the weaker nitric acid functions as a base in the highly acidic sulfuric acid medium, releasing the nitronium ion NO_2, which then undergoes substitution reaction in the ring. The resulted nitronium ion is electrophilic and can attack the polarized benzene ring as follows:



The hydroxyl group is electron releasing and considered strong benzene ring activating group lead to a faster nitrations, it orients the nitronium ion (NO_2^+) toward both ortho–positions with respect to it i.e. to (6 and 8) positions.
This indicate that positions 6 and 8 are most vulnerable to electrophilic attack by nitronium ion because benzene ring is electron rich species, it has six electrons while postion 3 is less vulnerable because it has only one electron further more it is with partial positive charge due to the inductive effect since the carbonyl oxygen of the lactone ring is a potent electrone attracting group.

It has been well established in the field of organic chemistry, that the substitution reaction in benzene ring requires less calories than that in straight hydrocarbon chains [10, 11]. Why? Confirmation of the molecular structure of the derivative was provided by sodium fusion test. IR spectrum (Fig. 6) revealed the following absorption frequencies, cm^{-1} in KBr disc.: 3265.86 (O–H) str.: 3080 (C–H) str. of aromatic and alkene. 2926.00 (C–H) str. aliphatic; 1732.66 (C=O) str.; 1621.17 (C=C) str. 1534.56 ($–NO_2$) asym str.; 1301.20 ($–NO_2$) asym str.

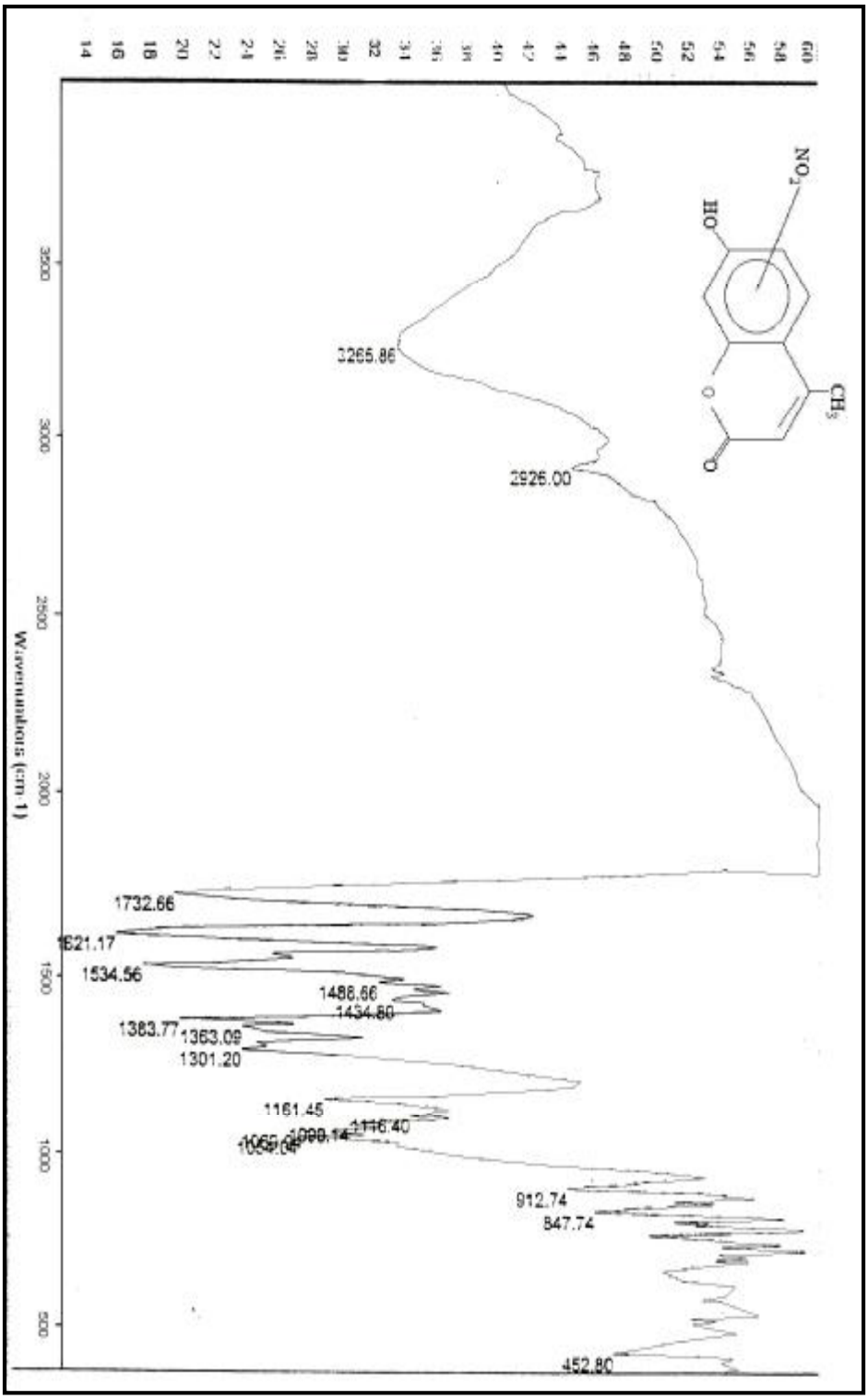


FIG 6 . IR SPECTRUM FOR NITROCOUMARIN DERIVATIVE

The compounds also are comfirmed by HPLC (Fig. 7).
Sample (1): Coumarin in methanol (conc. ppm =1)
t_R = 6.25 min. (Fig. 7) peak (A).
Sample (2): Coumarin derivative (7–hydroxy, 4–methylcomarin) in methanol (conc. ppm =1)
t_R = 4.39 min. (Fig. 7) peak (B).
Sample (3): Nitrocoumarin derivative in methanol (conc. ppm =1)
t_R = 4.19 min. related to 6–nitrocoumarin–derivative and 8–nitrocoumarin–derivative (Fig. 7) peak ($C_{(1)}$).
t_R = 24.24 min. related to 6.8–dinitrocoumarin–derivative (Fig. 7) peak ($C_{(2)}$).

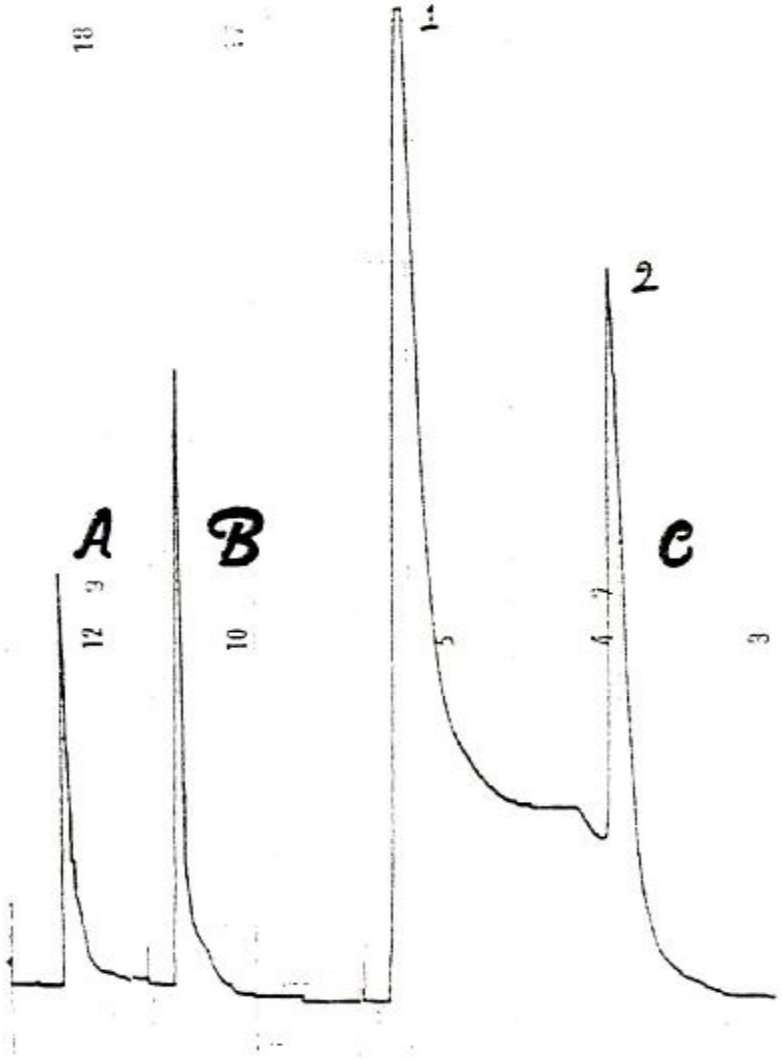


FIG 7 . HPLC CHROMATOGRAMS OF:

A. *COUMARIN.*
B. *7–HYDROXY, 4–METHYLCOMARIN.*
C. *NITROCOUMARIN DERIVATIVE FOR DETERMINATION OF (t_R).*

Nitration of coumarin derivative yielded two compound, the 7–hydroxy, 4–methyl, 6.8–dinitrocoumarin and isomers compound consist of equal parts of 8 –nitrocoumarin and 6–nitrocoumarin.
HPLC technique was applied for detection and separation of these isomers. The single peaks in Fig. (7) part A and B represents a pure coumarin and 7–hydroxy, 4–methylcoumarin respectively, but the nitrocoumarin Fig. (7) part C shows two peaks with different retention times, the first peak (shows interferences with other peak) represents the two isomers (6–nitrocoumarin–derivative and 8–nitrocoumarin–derivative) while the second peak represents (6.8–dinitrocoumarin–derivative) and it is indicated by UV spectrum in Fig. (8).

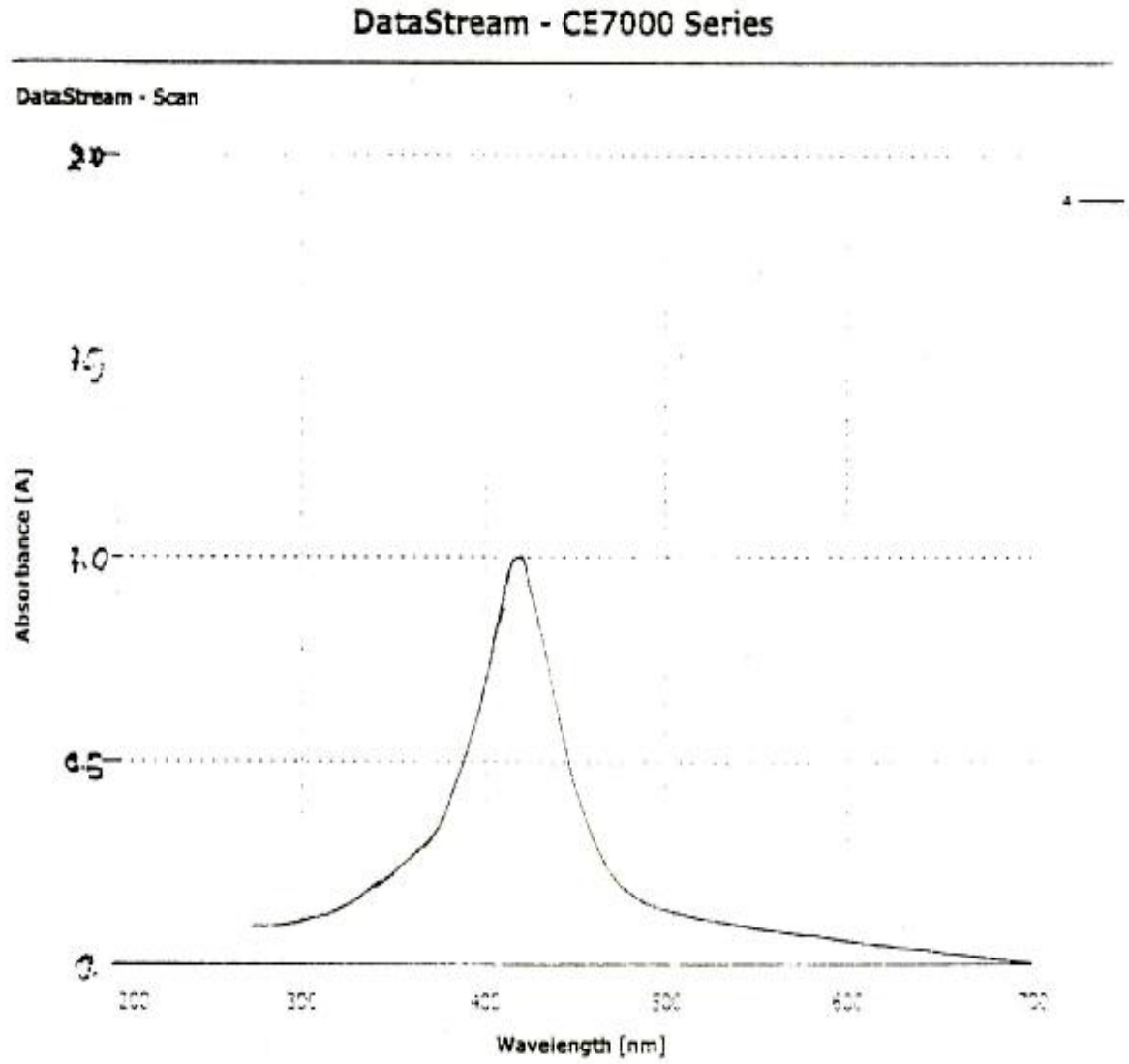


FIG 8 . UV SPECTRUM FOR NITROCOUMARIN DERIVATIVE PART C_2 .

The UV spectrum of compound two [t_{Rf} = 24.24 min shows λ max at 425 nm. in (Fig. 8), when it is collected for about 6 runs between 17 –30 min intervals after the injection of samples collected from the outlet of HPLC mobile phase.
The first beak represents the isomers in (Fig. 7) part C, that are eluted more rapidly under the influence of the polar mobile phase while (6.8–dinitrocoumarin–derivative), the higher molecular weight compound with resonance is eluted later.

IR spectrum (Fig. 9) sample in KBr disc. Revealed the following absorption frequencies in cm^{-1} : 3220 (O–H str.): 3030 (aromatic and alkene C–H str.); 2950 (aliphatic C–H str.): 1740 (C=O str.): 1635 (C=C str.); 1540 (asym $–NO_2$ str.); 1360 (asym $–NO_2$ str.).

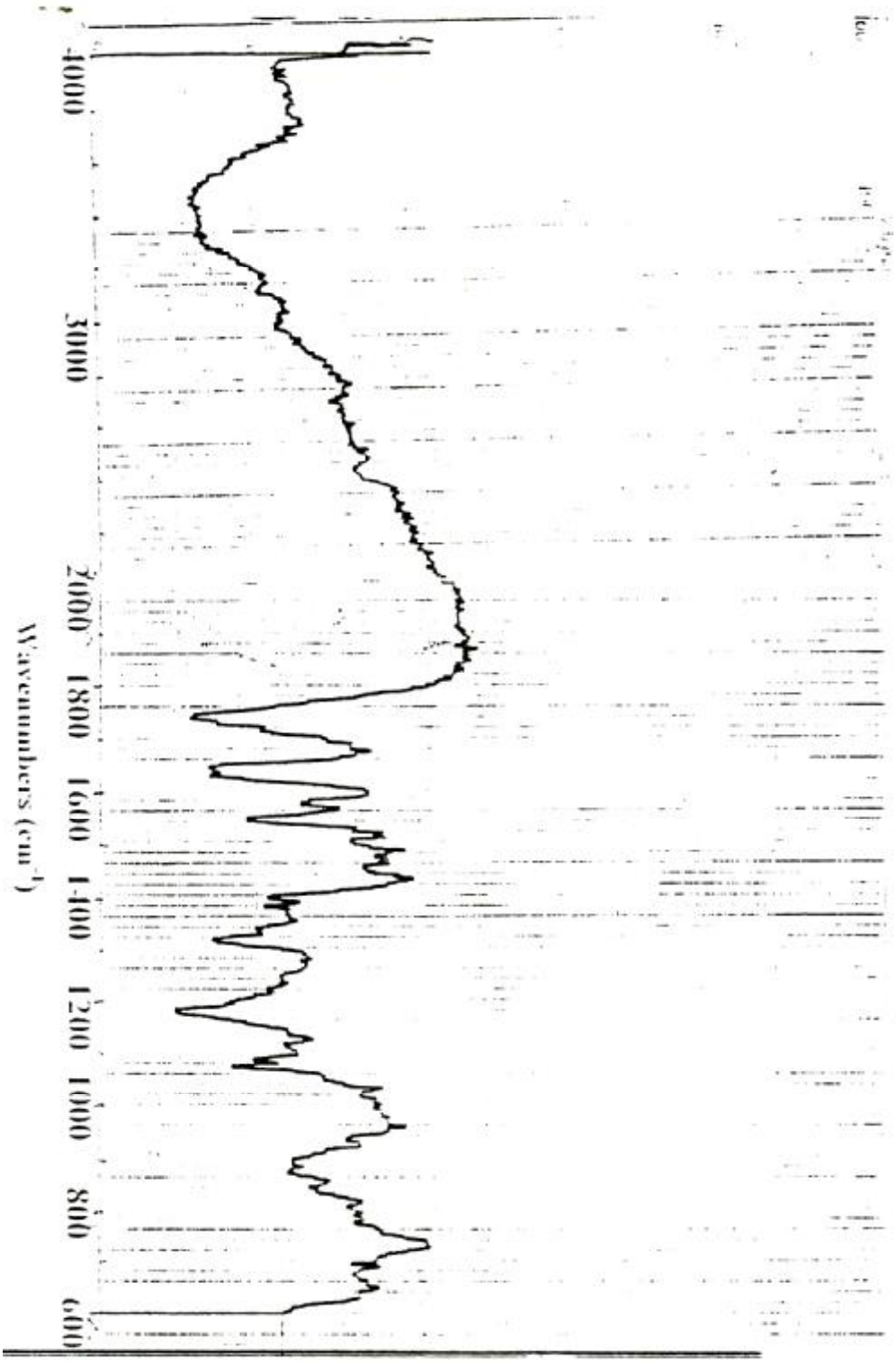


FIG 9 . IR SPECTRUM FOR (6.8-DINITROCOUMARIN) PART C_2.

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