VISIBLE FLUORESCENCE AND CHEMICAL CONSTITUTION OF COMPOUNDS OF THE BENZO-PYRONE GROUP

Part III. Further Study of Structural Influences in Coumarins

By V. Balaiah, T. R. Seshadri and V. Venkateswarlu

(From the Department of Chemistry, Andhra University, Waltair)

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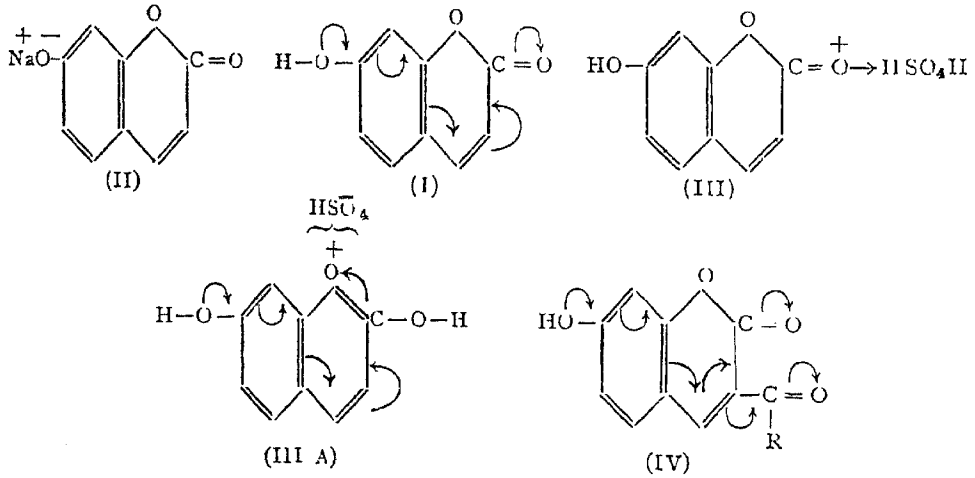
In two previous communications[1,2] which may be considered as Parts I and II of this series certain aspects of the subject have been discussed. In Part I a general study of hydroxy coumarins and hydroxy chromones was made and comparison effected. The subsequent part dealt with the remarkable influence of carbonyl groups in the 3-position of 7-hydroxy coumarins in enhancing fluorescence. In this connection it was pointed out that emission of fluorescence is accompanied by increasing capacity of the molecule to exhibit resonance. Formulæ I, II and III may represent the changes taking place in umbelliferone when dissolved in aqueous alkali and in concentrated sulphuric acid. The normal polarisation in umbelliferone (I) is increased by the formation of the negative ion in alkaline solutions and of the hydrogen bond in sulphuric acid. The formation of an oxonium salt (III A) is also possible and will produce a similar effect. The introduction of a carbonyl in the 3-position intensifies resonance as shown in IV and consequently the substances fluoresce strong even in neutral alcoholic solution.



A number of possibilities of verifying the validity of the above ideas and of amplifying existing knowledge suggest themselves. Several coumarin

compounds having the desirable structures have therefore been prepared and their fluorescence examined. The results are presented in the form of a table and based on them the following special points may be mentioned.

A. Effect of pyrone double bond

If the above explanations are valid the reduction of the pyrone double bond in umbelliferone should mean complete absence of fluorescence in the product as there should be no further possibility of resonance initiated by the carbonyl, reaching the hydroxyl across the molecule as in (I). Dihydro-umbelliferone and seven derivatives (V, R = H or C_6H_5, CH_2CO_2H, etc. and R′ = H or CH_3) have been prepared and studied. All of them exhibit no fluorescence in solutions in alcohol, in dilute alkali or in concentrated sulphuric acid. This forms an interesting support of the explanations given above.

B. Effect of cyano and phenyl groups in position 3

In continuation of the work relating to the influence of the carbonyl group in the 3-position, the effect of groups which are similar to it such as the cyano and the phenyl have now been studied. 3-cyano-umbelliferone (VI) and a few 3-phenyl-7-hydroxy-coumarins (VII) have been prepared and examined. Observations regarding their fluorescence are given in the Table and from them it appears that the cyano and phenyl groups are somewhat more powerful than the C = O in enhancing fluorescence.

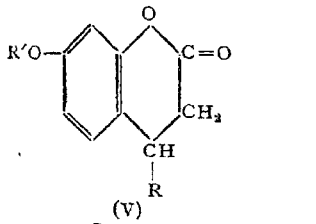
C. Effect of substitution in the 3 and 4 positions

Having observed and explained the remarkable influence of certain electron-attracting groups such as the carbonyl, cyano and phenyl in enhancing fluorescence, the question of the position of these groups comes in for consideration. When present in position 4 their effect on the resonance of the molecule should be very different from what has been described to take place in position 3. This has been tested in regard to two compounds, umbelliferone-4-carboxylic acid (VIII) and 4-phenylumbelliferone (IX). Comparison of their properties with those of the corresponding 3-substituted isomers shows the existence of large differences. The 4-substituted derivatives exhibit comparatively feeble fluorescence.

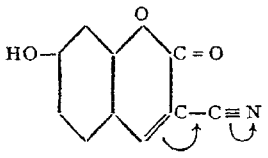
The reason for this marked difference between the two isomers seems to be as follows: When these groups are in position 3, conjugation (alternative single and double bonds) between them and the benzene ring is kept up and they reinforce the original resonance of umbelliferone. On the other hand when in position 4 they are not in conjugation with the benzene ring and may even work in antagonism to some extent as shown in VIII and IX.

D. Comparison of 3-carboxylic and 3-acetic acids

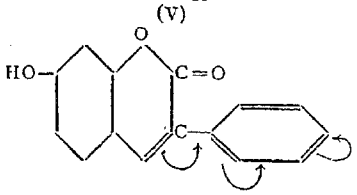
Another aspect of the influence of conjugation can be studied by comparing umbelliferone-3-carboxylic (IV, R = OH) and 3-acetic acids (X, R = OH). In the former, conjugation of the C = O group with the benzene ring exists whereas in the latter it is absent due to the intervention of a methylene group. Consequently, the 3-acetic acids should behave very similar to umbelliferone itself. This expectation is amply justified by the results presented in the table when observations relating to 7-hydroxycoumarin-3-acetic acid and its derivatives are compared with closely allied derivatives of 7-hydroxycoumarin-3-carboxylic acid. The presence of a methyl group in the 4-position in some of them is not expected to make any great difference.



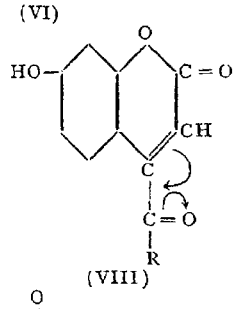
(V)



(VI)



(VII)



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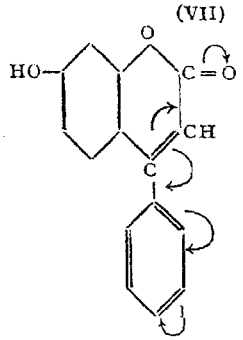
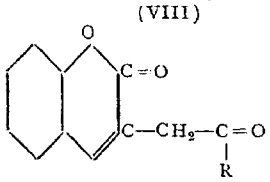


(IX)



(X)

E. Electron attracting groups in the benzene ring

When these groups are present in the benzene ring the normal movement of electrons towards the pyrone ring will be hampered and

consequently fluorescence should be diminished or lost. In Part I[1] the behaviour of a number of substituted umbelliferones containing nitro, acetyl and formyl groups were recorded and the observations are according to the above expectations.

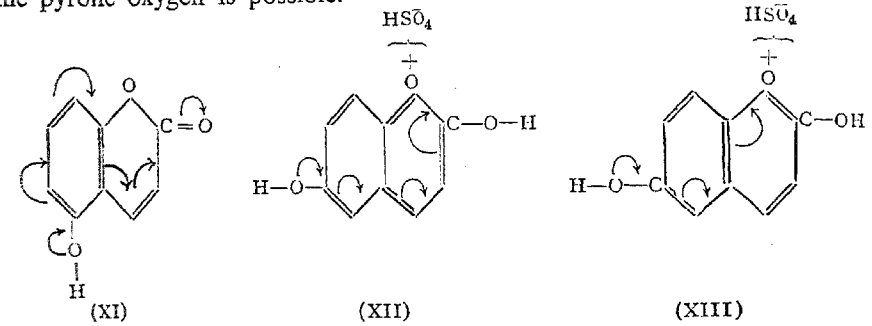
F. The effect of the position and number of hydroxyl groups on fluorescence

From the evidence already available it is clear that 7-hydroxy-coumarins emit fluorescence. Coumarin compounds containing only one phenolic hydroxyl group in position 5 have not so far been noticed to give fluorescence. The examples given in the Table confirm this observation. In general they give yellow solutions devoid of fluorescence. The behaviour of 6-hydroxy-coumarins is slightly different since they emit fluorescence in concentrated sulphuric acid solutions though not in others. 8-hydroxy-coumarin also does not give any fluorescence in spite of the fact that its solutions are not markedly coloured. The observation recorded by Bizzarri[3] that 8-hydroxy coumarin dissolves in alkali with a red colour and a blue fluorescence seems to be wrong. Obviously he was not dealing with the correct substance. Recently Dey and Kutti[4] have shown that his method of preparation employing catechol and malic acid is not satisfactory and they have obtained the correct compound by demethylating 8-methoxy coumarin. This substance does not exhibit any fluorescence. The same behaviour is noticed in the methyl ether and in 8-methoxy-coumarin-3-carboxylic acid. The lack of fluorescence in the last compound in spite of the favourable influence of the 3-carboxyl group is noteworthy. It may therefore be concluded that the position 7 is the most favourable one for a phenolic hydroxyl to enable the coumarin molecule to emit fluorescence.

In attempting an explanation of the above observations the behaviour of 7-hydroxy-coumarins is easily accounted for. The location of the hydroxyl group happens to be *para* to the position of branching of the unsaturated carbon system of the α-pyrone ring. Consequently resonance is easily favoured. A similar effect should be expected from the 5-hydroxyl (XI) which is in the ortho position. However in this case owing to the larger resonance possible, deeper absorption has occurred and fluorescence lost probably by the shift of the fluorescent band into the infra-red. It is interesting that 5-methoxy coumarins exhibit some fluorescence in neutral alcoholic solutions or in sulphuric acid. This seems to be due to a reduction of resonance and reduction of absorption so that flourescence band falls in the visible region. Even in the ethers, alkaline solutions do

not fluoresce. This may be attributed to the adverse effect of the hydroxyl ions on the C = O centre.

The 6 and 8-positions are meta and hence hydroxyls in these positions are not active. The feeble fluorescence of 6-hydroxy-coumarin in sulphuric acid may be attributed to the formation of oxonium salts of the following constitutions (XII, XIII) in which conjugation between the hydroxyl and the pyrone oxygen is possible.



(XI) (XII) (XIII)

With a view to study the influence of additional hydroxyl groups on the property of 7-hydroxy-coumarins a number of known compounds and new ones with the desired constitution have been prepared and examined very carefully. From the observations recorded in the Table it could be concluded that a second hydroxyl in position 6 diminishes the fluorescence. The inhibiting effect is greater when position 5 is involved and the emission is completely lost in 7 : 8-dihydroxy-compounds. It may be mentioned here that 5 : 7-dihydroxy coumarins have not been observed to emit fluorescence before. This may be due to the deep yellow solutions which they give in alkali. It is necessary to use very dilute solutions and employ very little alkali in order to observe correctly the feeble fluorescence that is emitted. A few compounds which are methyl ethers of 5 : 6 : 7-trihydroxy coumarin have been examined. They do not fluoresce thereby suggesting that the increase of the number of hydroxyls or methoxyls to more than two is not favourable to the development of fluorescence.

G. Effect of methylation

In the previous communications it has already been mentioned that the methylation of the hydroxyl groups brings about marked changes in fluorescence. The methyl ether of umbelliferone is indifferent to the addition of alkali whereas umbelliferone fluoresces brightly blue under these conditions. The ether exhibits a feeble fluorescence in concentrated sulphuric acid and the emission is more on the violet side. This has been attributed to the inability of the methoxyl to produce ions in alkali. Though

5-hydroxy-coumarin and its derivatives in which the hydroxyl group is free do not exhibit any fluorescence under any conditions, when the hydroxyl group is methylated or benzylated feeble fluorescence is exhibited in alcohol and sulphuric acid solutions. Similarly 6-methoxy coumarins fluoresce better than the corresponding hydroxy compounds. In these cases methylation is favourable to fluorescence. On the other hand methylation has no effect on 8-hydroxy-coumarin and both the hydroxy compound and its ether are non-fluorescent. There is thus some parallelism between the hydroxylic compounds and the methoxy derivatives in regard to the influence of position.

In Part I it was pointed out that C-methyl groups have a small influence though it is not very considerable. In a number of umbelliferone derivatives with a methyl group in the 8th position a tendency towards the production of green fluorescence in concentrated sulphuric acid is noticed whereas without it the emission is more towards blue and violet.

H. Fluorescence of some solids

Organic compounds which exhibit fluorescence in the solid state have been rare. Even certain dyes which emit strong fluorescence in solution do not show any in the solid state. This has been attributed to association and close packing of the molecules. During the course of the present investigation a number of compounds have been found to fluoresce in the crystalline condition. In general they give violet fluorescence. 7-acetoxy-3-phenyl-coumarin, 7-methoxy-3-phenyl coumarin, 7-methoxy-3-carbethoxy-coumarin, 7-methoxy-3-carboxy-coumarin and 7-methoxy-8-methyl-3-carbethoxy-coumarin give comparatively bright violet fluorescence. 7-methoxy-4-methyl-coumarin-3-ethylacetate, 7-methoxy-4-methyl-coumarin-3-acetic acid, 5-methoxy-4 : 7-dimethyl-coumarin-3-acetic acid, 5 : 7- dimethoxy-4-methyl-coumarin-3-ethylacetate and 5 : 7-dimethoxy-4-methyl coumarin-3-acetic acid give only weak violet fluorescence. It may be noted that the first four emit very strong fluorescence even in solution and the others yield solutions of comparatively feeble fluorescence. Most of the compounds are umbelliferone derivatives. Though many other similar substances may be expected to exhibit this property the occurrence of colour usually yellow makes the detection impossible. For the detection of solid flourescence it is necessary to purify the compounds thoroughly and it is especially necessary to remove impurities which may confer colour on the crystals. Though the first 4 compounds which are strongly fluorescent solids have been prepared before, this special property has not so far been detected mainly due to the fact that solid fluorescence is rather uncommon and fairly close observation is necessary to detect it. The best procedure is

to obtain the solid in the form of fine crystals and shake it in a clean test-tube so that it spreads over the sides to some extent.

Results of Observations

The following dihydroumbelliferone derivatives were examined. They did not exhibit any fluorescence under any condition. 7-hydroxy-3 : 4-dihydro-coumarin[5], 7-hydroxy-4-phenyl-3 : 4-dihydro-coumarin[6], 7-hydroxy-3 : 4-dihydro-coumarin-4-cyano-acetamide[7], 7-hydroxy-3 : 4-dihydro-coumarin-4-acetic acid[7], 7-methoxy-4-phenyl-3 : 4-dihydro-coumarin, 7-methoxy-3 : 4-dihydro-coumarin-4-cyano-acetamide[7], 7-methoxy-3 : 4-dihydro-coumarin-4-cyano-acetic acid[7], and 7-methoxy-3 : 4-dihydro-coumarin-4-acetic acid[7].

The following hydroxy and methoxy compounds did not exhibit any fluorescence under any condition : 5-hydroxy-4-methyl-coumarin[8], 5-hydroxy-4 : 7-dimethyl-coumarin[9], 5-hydroxy-4 : 7-dimethyl-coumarin-3-ethyl-acetate[10], 5-hydroxy-4 : 7-dimethyl-coumarin-3-acetic acid, 8-hydroxy-coumarin[4], 7 : 8-dihydroxy-4-methyl-coumarin[11], 7 : 8-dihydroxy-4-methyl-coumarin-3-ethyl-acetate[10], 7 : 8-dihydroxy-4-methyl-coumarin-3-acetic acid, 8-methoxy-coumarin[12], ethyl-8-methoxy-coumarin-3-carboxylate[13], 8-methoxy-coumarin-3-carboxylic acid[13], 5 : 7-dimethoxy-6-hydroxy-4-methyl-coumarin[14], 5 : 6 : 7-trimethoxy-4-methyl-coumarin[14], 7 : 8-dimethoxy-4-methyl-coumarin-3-ethyl acetate, 7 : 8-dimethoxy-4-methyl-coumarin-3-acetic acid, and 7 : 8-dimethoxy-4-methyl-coumarin.

Colour of visible fluorescence of Hydroxy and Methoxy compounds

Compound	In concentrated sulphuric acid	In alcohol	In dilute alkali
7-Hydroxy-3-acetyl coumarin	Pale blue	Violet blue; solution pale yellow	Solution yellow ; bright pale blue
7-Hydroxy-3-cyano coumarin	Deep yellow solution; blue fluorescence	Bright blue	Solution yellow ; bright blue fluorescence
7-Hydroxy-3-phenyl coumarin[15]	Deep blue fluore - cence	Deep blue with violet tinge	Solution pale yellow ; exhibits bright blue fluorescence with a green tinge
7-Hydroxy-5-methyl-3-phenyl coumarin	Solution pale yellow; bright greenish blue	Bright-blue	Solution pale yellow ; exhibits bright greenish blue fluorescence
7-Hydroxy coumarin-4-carboxylic acid[16]	Feeble blue	Negligible (blue)	Solution pale yellow ; very feeble blue
7-Hydroxy-4-phenyl coumarin[11]	Blue	,,	Solution pale yellow ; very feeble green

Compound	In concentrated sulphuric acid	In alcohol	In dilute alkali
7-Hydroxy-4-methyl coumarin-3-ethyl acetate[17]	Violet blue	Pale blue	Solution colourless ; deep blue fluorescence
7-Hydroxy-4-methyl coumarin-3-acetic acid[17]	,,	,,	,,
6-Hydroxy coumarin[18]	Solution colourless; feeble blue fluorescence with a green tinge	Nil	Solution yellow ; no fluorescence
6-Hydroxy-4-methyl coumarin[16, 27]	,,	,,	,,
5 : 7-Dihydroxy-3-phenyl coumarin[19]	Solution yellow ; bluish green fluorescence	Weak blue	Solution yellow ; exhibits weak blue fluorescence
5 : 7-Dihydroxy-4-methyl coumarin-3-acetic acid	Solution pale yellow; feeble blue	Feeble blue	Solution yellow ; pale blue fluorescence stronger than in neutral alcohol
5 : 7-Dihydroxy-4-methyl coumarin-3-ethyl acetate	Feeble blue	,,	Solution yellow ; feeble blue fluorescence
5 : 7-Dihydroxy-4-methyl coumarin[20]	,,	,,	,,
6 : 7-Dihydroxy-4-methyl coumarin[21]	Violet blue	,,	Solution pale yellow ; feeble blue fluorescence
7-Hydroxy-8-methyl coumarin[22]	Bluish green	Very pale blue	Bright blue ; intensity increased
7-Hydroxy-8-methyl-3-acetyl coumarin	Solution yellow; pale green	Solution pale yellow; bright blue	Solution yellow ; strong blue
Ethyl-7-hydroxy-8-methyl coumarin-3-carboxylate	Solution colourless ; blue	Solution colourless ; bright blue	Solution very pale yellow ; strong blue
7-Hydroxy-8-methyl coumarin-3-carboxylic acid	,,	,,	,,
7-Hydroxy-8-methyl-4-phenyl coumarin	Colourless solution ; very feeble blue fluorescence	Nil	Nil
7-Acetoxy-3-phenyl coumarin[15]	Bright blue	Weak violet blue	Bright blue fluorescence
7-Methoxy-3-phenyl coumarin[23]	,,	Bluish violet	Bluish violet
7-Methoxy-5-methyl-3-phenyl coumarin	Solution yellow ; bright greenish blue	Violet	Violet

Compound	In concentrated sulphuric acid	In alcohol	In dilute alkali
7-Methoxy-4-methyl coumarin-3-ethyl acetate[17]	Bluish violet	Feeble violet	Feeble violet
7-Methoxy-4-methyl coumarin-3-acetic acid	,,	,,	,,
5-Methoxy-4-methyl coumarin[8]	Bluish green	Very weak blue	Very feeble violet
5-Methoxy-4 : 7-dimethyl coumarin[9]	,,	,,	,,
5-Methoxy-4 : 7-dimethyl coumarin-3-ethyl acetate	Very feeble blue	Weak blue	Feeble blue
5-Methoxy-4 : 7-dimethyl coumarin-3-acetic acid	,,	Feeble blue	,,
5-Benzyloxy-4 : 7-dimethyl coumarin	Feeble blue	,,	,,
6-Methoxy-4-methyl coumarin[27]	,,	,,	,,
5 : 7-Dimethoxy-3-phenyl coumarin	Bright green	Bright violet blue	Bright blue
5 : 7-Dimethoxy-4-methyl coumarin-3-ethyl acetate	Bluish green	Feeble bluish violet	Bluish violet
5 : 7-Dimethoxy-4-methyl coumarin-3-acetic acid	Bluish green	Feeble bluish viotet	Bluish violet
5 : 7-Dimethoxy-4-methyl coumarin[20]	,,	,,	,,
Ethyl-5 : 7-dimethoxy-6-hydroxy coumarin-3-carboxylate	Green	Pale yellow solution; no fluorescence	Pale yellow solution ; no fluorescence
7-Methoxy-8-methyl coumarin[22]	Bluish green	Very pale violet	Very pale blue
8-methyl-7-methoxy-3-acetyl coumarin	Solution yellow ; green fluorescence	Violet	Solution yellow ; blue fluorescence on dilution with water
Ethyl-8-methyl-7-methoxy coumarin-3-carboxylate[22]	Green	,,	Fluorescence lost ; addition of acid or large dilution restores fluorescence
8-Methyl-7-methoxy coumarin-3-carboxylic acid[22]	Bright green	,,	Flourescence lost ; restored on dilution or addition of acid

Note.—In the case of compounds which do not dissolve in dilute aqueous alkali, alcoholic solutions were employed and a few drops of aqueous alkali added before observing fluorescence.

Experimental

Preparation of the necessary compounds

7-*Hydroxy*-3-*acetyl-coumarin.*—This was originally prepared by Weiss and Merksammer[24] using resorcinol and ethoxymethylene-ethyl-aceto-acetate. It is more easily prepared by condensing β-resorcylic aldehyde (1·0 g.) with ethyl aceto-acetate (1·5 g.) employing piperidine as the condensing agent. The product is recrystallised from alcohol when it is obtained in the form of light yellow rectangular crystals melting at 236-37° ; yield 1·3 g. (Found : C, 68·5 ; H, 4·1 ; $C_{11}H_8O_4$ requires C, 68·6 ; H, 3·9%.)

7-*Hydroxy*-3-*cyano-coumarin.*—A mixture of cyanoacetic ester (3·0 g.) and β-resorcylic aldehyde (2·0 g.) was treated with 4 drops of piperidine and left overnight. It was then digested with cold water and filtered ; the solid residue was washed with ether and dried. The crude product thus obtained was recrystallised from absolute alcohol when yellow rectangular plates melting at 262° were obtained. (Found : N, 7·7 ; $C_{10}H_5O_3N$ requires N, 7·5%.) It is easily soluble in acetone and sparingly in ether and chloroform.

7-*Hydroxy*-5-*methyl*-3-*phenyl-coumarin.*—It was previously prepared by Badhwar *et al.*[25] by condensing orcinol with benzoyloxymethylenephenyl-aceto-nitrile and subsequently hydrolysing the resulting compound. The following procedure has now been found to be more covenient : Orcylic aldehyde (1·5 g.), dry sodium phenyl acetate (3 g.) and acetic anhydride (20 c.c.) were thoroughly mixed in a 100 c.c. conical flask and heated in an oil-bath at 170-80° for 4 hours. The product was then poured into water and let stand for several hours. The separated crystals of 7-acetoxy-5-methyl-3-phenyl-coumarin were filtered, washed with much water and then with a little ether. The substance was then deacetylated by dissolving in alcohol, adding 20 c.c. of 10% caustic soda and heating on a boiling water-bath for 15 minutes. 7-hydroxy-5-methyl-3-phenyl-coumarin obtained on acidification, crystallised in long light yellow needles from alcohol and melted at 233°.

7-*Hydroxy*-8-*methyl*-3-*acetyl-coumarin.*—This was prepared by the condensation of 3-methylresorcylaldehyde (0·5 g.) with ethyl aceto-acetate (0·8 g.) using piperidine as the condensing agent. When crystallised from alcohol it came out as long light yellow rectangular plates which melted at 256--57°. Yield 0·8 g. (Found : C, 61·O ; H, 5·1 ; $C_{12}H_{10}O_4$, H_2O requires C, 61·0 ; H, 5·1%.)

Ethyl-7-*hydroxy*-8-*methyl-c o u m a r i n*-3-*carboxylate.*—3-methylresorcyl-aldehyde (0·5 g.) was condensed with diethyl malonate (0·6 g.) using

piperidine as the condensing agent. The product crystallised from alcohol as long pale yellow rectangular plates and melted at 250-51°. Yield 0·7 g. (Found : C, 63·2 ; H, 5·2 ; $C_{13}H_{12}O_5$ requires C, 62·9 ; H, 4·8%.)

7-Hydroxy-8-methyl-coumarin-3-carboxylic acid resulted from the hydrolysis of the above ester using 20% methyl alcoholic potash in the cold. It crystallised from alcohol as yellow rectangular plates and melted at 277-78°.

7-Hydroxy-8-methyl-4-phenyl-coumarin.—Concentrated sulphuric acid (1·2 g.) was added to an intimate mixture of 2-methyl resorcinol (0·5 g.) and ethyl benzoylacetate (0·5 g.) and the solution was kept overnight. It was then poured into ice water next morning. The solid product was filtered, washed with ether and recrystallised from alcohol when it came out in lustrous rhombohedral tablets and melted at 279-80°. Yield 0·9 g. (Found : C, 76·4 ; H, 4·8 ; $C_{16}H_{12}O_3$ requires C, 76·2 ; H, 4·8%.)

5-Hydroxy-4 : 7-dimethyl-coumarin-3-acetic acid.—The ester (1 g.) prepared according to the method of Chakravarti[10] was dissolved in 10% sodium hydroxide solution (15 c.c.) and kept in a water-bath at 60-70° for about 15 minutes. The solution was then cooled and acidified with dilute hydrochloric acid. The acid separated out gradually as a colourless solid. On recrystallisation from alcohol, it came out in the form of shining plates and melted at 271°. (Found : C, 62·5 ; H, 4·7 ; $C_{13}H_{12}O_4$ requires C, 62·9; H, 4·8%.) It is soluble in acetone and insoluble in ether, benzene and chloroform.

5 : 7-Dihydroxy-4-methyl-coumarin-3-ethyl-acetate.—To a mixture of phloroglucinol (4 g.) and ethyl acetosuccinate (6 g.), 10 c.c. of concentrated sulphuric acid was added in small quantities with much shaking. It was kept for one hour and poured into water. The separated solid was filtered, washed with water and crystallised from alcohol when it came out in clusters of colourless needles melting at 240°. (Found : C, 60·7 ; H, 4·8 ; $C_{14}H_{14}O_6$ requires C, 60·4 ; H, 5·0%.)

5 : 7-Dihydroxy-4-methyl-coumarin-3-acetic acid.—It was obtained by hydrolysing the above ester with alkali as in the previous case. It formed colourless needles with rounded edges melting at 264°, when recrystallised from alcohol. (Found : C, 57·5 ; H, 4·2 ; $C_{12}H_{10}O_6$ requires C, 57·6 ; H, 4·0%.) It is soluble in acetone and insoluble in ether and chloroform.

7 : 8-Dihydroxy-4-methyl-coumarin-3-acetic acid.—7 : 8-dihydroxy-4-methyl-coumarin-3-ethyl acetate (1·0 g.) prepared according to the method of Chakravarti[10] was hydrolysed to the acid by the usual method using sodium hydroxide as described previously. The acid on crystallisation from aqueous alcohol came out in colourless rectangular plates melting

t 277°. (Found: C, 57·6; H, 3·9; $C_{12}H_{10}O_6$ requires C, 57·6 ; H, 4·0%.) The substance is soluble in acetone and chloroform and sparingly soluble in ther.

7-methoxy-3-acetyl-8-methyl-coumarin.—2-hydroxy-4-methoxy-3-methyl-enzaldehyde[22] (0·5 g.) was mixed with ethyl aceto-acetate (0.8 g.) and iperidine (10-15 drops) was added slowly while cooling in ice. It was llowed to stand overnight. The solid was treated with excess of dilute ydrochloric acid ; after filtration the residue was recrystallised from lcohol when it came out in long colourless rectangular plates, melting t 191-92°. (Found : C, 66·9 ; H, 5·1 ; $C_{13}H_{12}O_4$ requires C, 67·2 ; H, 5·2%.)

5 : 7-*Dimethoxy-6-hydroxy-3-carbethoxy-c o u m a r i n.*—3 : 5-dimethoxy-uinolaldehyde (1·0 g.) prepared according to the method of Späth nd Jezmanowska-Siekiewiczowa[25] was intimately mixed with ethyl nalonate (1·5 g.) and piperidine (2 drops) was added. The mixture was lightly warmed on a water-bath until a clear solution resulted and it was eft overnight. Next morning, it was poured into water and the solid btained was recrystallised from alcohol, when it came out in long yellow eedles melting at 190°. (Found : C, 57·5 ; H, 4·5 ; $C_{14}H_{14}O_7$ requires , 57·2; H, 4·8%.) It is easily soluble in ether, acetone and chloroform.

5 : 7-*Dimethoxy-6-hydroxy-coumarin-3-carboxylic acid.*—This was ob-ained from the above ester by hydrolysis using 10% sodium hydroxide olution. It recrystallised in colourless rectangular prisms from dilute alco-ol and melted at 252°. (Found: C, 54·3, H, 4·0 ; $C_{12}H_{10}O_7$ requires , 54·1 ; H, 3·8%.) It is easily soluble in acetone and chloroform and noderately in ether.

The following are methoxy compounds obtained by the methylation f the appropriate hydroxy compounds, by means of excess of methyl iodide nd anhydrous potassium carbonate in dry acetone solution. The general rocedure will be clear from the following example.

7-Methoxy-4-p h e n y l-3 : 4-dihydro-coumarin.—7-hydroxy-4-phenyl-3 : 4-lihydro-coumarin (1 g.) prepared according to the method of Liebermann nd Hartmann[6] from resorcinol and cinnamic acid, was dissolved in dry cetone (20 c.c.). The solution was treated with anhydrous potassium arbonate (3·0 g.) and methyl iodide (4 g.) and the mixture kept gently oiling for about 6 hours. The solution was then filtered, the solvent re-noved by evaporation and the residue crystallised from alcohol. The methyl ther came out as colourless long rectangular plates and melted at 43-44°. Found: C, 72·8; H, 6·0 ; $C_{16}H_{14}O_3$, $\frac{1}{2}H_2O$ requires C, 73·0; H, 5·7%.)

7-Methoxy-5-methyl-3-phenyl-coumarin.—This was obtained methylation of 7-hydroxy-5-methyl-3-phenyl-coumarin. On lisation from alcohol, it came out as stout golden yellow rectangu and melted at 155°. (Found: C, 76·9; H, 5·5; $C_{17}H_{14}O_3$ requires H, 5·3%.) It is moderately soluble in acetone and sparingly in chloroform.

7-Methoxy-4-methyl-coumarin-3-ethyl-acetate is easily prepar above method from the corresponding 7-hydroxy compound.[1] originally prepared by a different method by Banerjee.[17]

5-Methoxy-4:7-dimethyl-coumarin-3-ethyl-acetate.—This resulte methylation of the corresponding 5-hydroxy compound which was according to the procedure of Chakravarti[10]. The methyl ether stallised from alcohol came out in long colourless needles melting (Found: C, 66·3 ; H, 6.1 ; $C_{16}H_{18}O_5$ requires C, 66·2 ; H, 6·2 substance is soluble in acetone, chloroform and ether.

5-Methoxy-4:7-dimethyl-coumarin-3-acetic acid.—The above e dissolved in aqueous alcoholic potash and heated just to boiling. cooled and the acid obtained on acidification was recrystallised from when it came out in long colourless needles melting at 225°. (Found: H, 5·6; $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·3%.) It is easily so acetone and chloroform but with difficulty in ether.

5:7-Dimethoxy-3-phenyl-coumarin was prepared by the met of 5:7-dihydroxy-3-phenyl-coumarin[13]. On crystallisation from it came out in colourless rectangular plates melting at 179-80°. (C, 68·4; H, 5·3; $C_{17}H_{14}O_4$, H_2O requires C, 68·0; H, 5·3%.) It is in acetone and chloroform and less so in ether.

5:7-Dimethoxy-4-methyl-coumarin-3-ethyl-acetate.—This was p from the corresponding dihydroxy compound described in this It cyrstallised from aqueous alcohol as colourless shining plates, and at 134°. (Found: C, 62·4; H, 5·7; $C_{16}H_{18}O_6$ requires C, 62·8 ; H, It is soluble in acetone and ether and moderately so in chlorofor

By the hydrolysis of the above ester using aqueous alcoholic the corresponding acid, *5:7-dimethoxy-4-methyl-coumarin-3-acetic ac* obtained. It crystallised from alcohol as colourless rectangular plates ed at the edges melting at 218-20°. (Found: C, 60·6, H, 5·0; C_1 requires C, 60·4 ; H, 5·0%.) It is sparingly soluble in chloroform and

7 : 8-Dimethoxy-4-methyl-coumarin-3-ethyl acetate was obtained the corresponding dihydroxy compound[19]. The substance cryst

in long colourless needles from dilute alcohol and melted at 87°. (Found : C, 63·2 ; H, 5·7 ; $C_{16}H_{18}O_6$ requires C, 62·8 ; H, 5·9%.) It is soluble in ether, acetone and chloroform.

The corresponding 3-*acetic acid* was prepared by the hydrolysis of the above ester using aqueous alcoholic potash. It crystallised from alcohol as colourless rectangular prisms melting at 194°. (Found : C, 60·5 ; H, 5·4 ; $C_{14}H_{14}O_6$ requires C, 60·4 ; H, 11·5%.) It is easily soluble in acetone and chloroform but moderately in ether.

5-Benzyloxy-4 : 7-dimethyl-coumarin.—5-Hydroxy-4 : 7-dimethyl-coumarin (1 g.) was dissolved in acetone (25 c.c.) and to the solution was added benzyl chloride (1·5 g.) and anhydrous potassium carbonate (3 g.). The mixture was refluxed on a water-bath for about 12 hours. The solvent was then distilled off, and water was added to the residue and kept overnight. Crystals of 5-benzyloxy-4 : 7-dimethyl-coumarin and a little unchanged benzyl chloride gradually separated out. The substance was filtered and washed with a little ether to remove the benzyl chloride. On recrystallisation from dilute alcohol, stout colourless prisms melting at 150° were obtained. (Found: C, 77·4 ; H, 6·0 ; $C_{18}H_{16}O_3$ requires C, 77·1; H, 5·7%.) It is easily soluble in acetone and moderately in ether.

Observation of fluorescence.—There is no difficulty in observing fairly strong fluorescence but when it is weak, it may be missed unless careful observation is made. Bright sunlight helps in such cases and if a concentrated beam of light could be obtained using a lens the emission of fluorescence becomes clearer. As the most satisfactory method of observing fluorescence using diffused day light, the following has been employed in this investigation. A round flat-bottomed flask filled with water and commonly used as a wash-bottle is kept on a table with a dark surface in the path of diffused day light from a door or window. When the test solution contained in a test-tube is placed in the patch of light thus focussed, weak fluorescences are readily visible. With a view to eliminate discrepancies, comparison is made with the particular solvent contained in the same test-tube. Solutions having colour were examined after progressive dilution in order to see if fluorescence appeared at any stage.

Summary

From the data presented in the paper, the following points have been established: (1) Dihydro-umbelliferone and its derivatives, in which the pyrone double bond does not exist, do not give any fluorescence. (2) Similar to the carbonyl group cyano and phenyl groups enhance fluorescence emission markedly when present in the 3 position of umbelliferone. (3) No

such effect is noticed when these groups occupy position 4. (4) A similar lack of influence is noticed in umbelliferone-3-acetic acid and its derivatives in which a methylene group breaks the conjugation. (5) Amongst monohydroxy coumarins, the presence of the hydroxy group in position 7 is most effective ; in position 6, a feeble effect exists ; in positions 5 and 8 no fluorescence is noticed. (6) Amongst dihydroxy-coumarins 6 : 7, 5 : 7, 8 : 7, represent a decreasing series, the last giving no fluorescence. Derivatives of higher polyhydroxy-coumarins do not exhibit fluorescence. (7) Effect of methylation of the hydroxyl groups introduce marked changes in the capacity of the compounds to fluoresce and the colour of the fluorescence. 5-Methoxy-coumarin emits a feeble fluorescence whereas 8-methoxy compounds do not. (8) A few compounds exhibit fluorescence in the solid state. The above observations are explained from the effect of the structural features on the resonance of the molecule.

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