A NOTE ON CERTAIN CONSTITUTIONAL FACTORS CONTROLLING VISIBLE FLUORESCENCE IN COMPOUNDS OF THE BENZO-PYRONE GROUP

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Certain groups of organic compounds are noted for their capacity to exhibit fluorescence and this has been frequently found to be of help for purposes of identification. The benzo-pyrone group is one such and several compounds coming under the category of coumarins, chromones, flavones and *iso*-flavones have been known to exhibit marked fluorescence. However, no systematic study seems to have been attempted till now on the influence of constitutional factors in controlling this property.

As a result of synthetic work in progress in these laboratories a large number of coumarin derivatives have become available. They have now been studied with reference to their capacity to exhibit fluorescence. The observations were made in ordinary sunlight with carefully purified materials in very dilute solutions; in almost all cases the solutions were colourless or pale yellow when fluorescence was exhibited. Coumarin, 7-methyl-coumarin, 4:7-dimethyl- and 3:4:7-trimethyl-coumarins, 6-nitro- and 6-amino-coumarins and coumarino-7:8-α-pyrone did not exhibit fluorescence either in concentrated sulphuric acid or in alkaline solution. The results obtained with other compounds are summarised in the accompanying table.

Name of compound	Nature of the fluorescence in ordinary sunlight	
	In aqueous sodium hydroxide	In conc. sulphuric acid
[illegible]	Blue (disappears in about 10 minutes)	Violet blue
[illegible]	Nil	Bluish violet
[illegible]	Pale blue turning bright blue (slowly disappears)	Do.
[illegible]	Strong blue (disappears after about 1 hour)	Do.
[illegible]	Blue	Nil
[illegible]	Do.	Nil
[illegible]	Nil	Bluish violet

Name of compound	Nature of the fluorescence in ordinary sunlight	
	In aqueous sodium hydroxide	In conc. sulphuric acid
7-Acetoxy-4-methylcoumarin	Very pale violet fast turning bright blue (slowly disappears)	Violet
7-Hydroxy-4: 8-dimethylcoumarin	Pale blue (persists even after 5–6 hours)	Very feeble blue
7-Acetoxy-4: 8-dimethylcoumarin	At first nil; but slowly develops pale blue due to hydrolysis	Feeble blue
7-Allyloxy-4: 8-dimethylcoumarin	Nil	Blue
7-Hydroxy-5-methylcoumarin	Intense blue (disappears in 20–30 minutes)	Intense blue
7-Hydroxy-3: 4-dimethylcoumarin	Blue	Violet blue
7-Hydroxy-3: 4-dimethyl-8-nitrocoumarin ..	Nil	Nil
7-Hydroxy-6-allyl-4: 8-dimethylcoumarin ..	Blue (disappears after about 2 hours)	Blue
7-Hydroxy-8-formylcoumarin	Nil	Nil
7-Hydroxy-8-acetyl-4-methylcoumarin ..	Nil	Nil
7-Hydroxy-6-acetyl-4: 8-dimethylcoumarin ..	Nil	Nil
7-Hydroxy-3: 6-dibromo-4: 8-dimethylcoumarin	Nil	Nil
7-Hydroxy-5-methyl-3-carboxycoumarin ..	Intense blue (disappears in ½–1 hour)	Pale blue
7-Hydroxy-5-methyl-3-carbethoxycoumarin ..	Intense blue (persists even after 5–6 hours)	Pale blue
5-Hydroxy-7-methylcoumarin	Nil	Nil
5-Hydroxy-4: 7-dimethylcoumarin	Nil	Nil
5: 7-Dihydroxycoumarin	Nil	Nil
5-Hydroxy-4: 7-dimethyl-6-carbethoxycoumarin	Nil	Nil
5-Hydroxy-4: 7-dimethyl-6-carboxycoumarin..	Nil	Nil
5-Hydroxy-7-methyl-3: 8-dicarbethoxycoumarin	Nil	Nil
5-Hydroxy-7-methyl-8-carboxycoumarin ..	Nil	Nil
Coumaric acid	Very pale green	Nil
Mercurated coumaric acid	Bright green	Nil
Umbellic acid	Nil	Nil
Mercurated umbellic acid	Nil	Nil
5-Nitrocoumaric acid	Nil	Nil

From the data given above and those found scattered in the literature the essential requirement for the production of fluorescence by the coumarins seems to be the existence of a hydroxyl group in the 7-position. In neutral solution 7-hydroxycoumarin itself is non-fluorescent; the fluorescence is developed in alkaline (*e.g.*, aqueous sodium hydroxide) and strong acid (sulphuric acid) media, the colour of the fluorescence being the same or slightly different in the two solutions. It is obvious that the fluorescence found in these cases is the property of the ions. The fluorescence exhibited in strong sulphuric acid solution is lost on pouring it into water; this is due to the fact that the oxonium ions to which the fluorescence in the acid solution is due are very unstable in the presence of a large volume of water. The intensity of the fluorescence is markedly greater in the alkaline solutions than in the solutions in sulphuric acid. The gradual disappearance of the fluorescence in alkaline solutions of umbelliferone and its derivatives can be attributed to the opening of the pyrone ring leading to the formation of the corresponding coumarinic acids. This opening of the ring is known to take place with lesser speed when substituents are present in the pyrone ring and the greater persistence of the fluorescence in such cases agrees with expectations. It may, however, be mentioned that the rapidity of the change is dependent on the amount of alkali added. In our observations we have employed sufficient sodium hydroxide to make the strength of the solution about one per cent.

When the hydroxyl is converted into a methoxyl or allyloxyl it is significant that there is no fluorescence in alkaline solution whereas there is fluorescence in sulphuric acid. No ions can be produced in alkaline solution in these cases. 7-Acetoxycoumarin, however, exhibits a pale blue fluorescence even in alkaline medium, probably because of the ease with which it is hydrolysed to umbelliferone. The fluorescence which is at first practically nil becomes more and more intense on standing for some time as a result of progressive hydrolysis; the resulting solution thereafter behaves just like an alkaline solution of umbelliferone ultimately becoming non-fluorescent. A similar behaviour is noticed with the 4-methyl homologue of 7-acetoxycoumarin.

Regarding the influence of other substituent groups on the fluorescence exhibited by 7-hydroxycoumarin it may be said that alkyl groups in the 4- and 5- positions enhance and in the 8-position reduce considerably the intensity. Formyl, acetyl and nitro-groups in the *ortho*-position (8-position) to the hydroxyl completely inhibit fluorescence and the introduction of bromine atoms reduces the intensity markedly and in some cases removes all fluorescence. It has been further noted that carbethoxy and carboxy groups in the 3-position enhance greatly the fluorescence and make it become

intense blue; it is noteworthy that these compounds exhibit strong fluorescence (blue) even in neutral alcoholic solutions.

When the hydroxyl group is present not in the 7- but in the 5-position a deep yellow coloured solution is produced without any fluorescence. This is a characteristic difference highly useful for the allocation of hydroxyl groups to the various nuclear positions in the aromatic ring. The existence of a 5-hydroxyl further counteracts the original property of the 7-hydroxy group of producing fluorescence and hence 5 : 7-dihydroxy-coumarins are devoid of the property of exhibiting fluorescence. A similar lack of fluorescence has been recorded in the case of 7 : 8-dihydroxycoumarin. But 6 : 7-dihydroxy-coumarin (aesculetin) is said to give a weak blue fluorescence.

Unlike coumarin, coumaric acid exhibits a feeble fluorescence in alkaline solutions but not in sulphuric acid medium. This is considerably intensified by the existence of even traces of mercury in the compound. It has been noted during the preparation of coumaric acid by the simple method described by Seshadri and Rao[1] that if the procedure has been carried out correctly and no mercuration has taken place the coumaric acid solution is simply yellow with only a feeble fluorescence. On the other hand any presence of mercury is indicated by a brilliant green fluorescence; this combined mercury can be removed by passing hydrogen sulphide into the solution. 4-Methyl-coumaric acid obtained from 7-methylcoumarin behaves in a similar fashion. The presence of a hydroxyl substituent, however, makes a great difference; umbelliferone loses its fluorescence in alkaline solution as has already been pointed out owing to the opening out of the pyrone ring with the formation of the hydroxy-coumarinic acid. The addition of mercuric oxide accelerates the loss of fluorescence since umbellic acid (4-hydroxy-coumaric acid) and its mercuration products that may be formed do not exhibit any fluorescence in alkaline or acid solutions.

From a study of past literature relating to the incidence of fluorescence in the flavone, *iso*-flavone and chromone series the first point that strikes one is that it is very widely met with in concentrated sulphuric acid medium but almost absent in alkaline solutions. 2-Methyl-3-methoxy-7-hydroxy-chromone and 2-methyl-7-hydroxy-*iso*-flavone seem to be the only two compounds that exhibit fluorescence in alkaline solutions. In this respect the γ-pyrone derivatives differ fundamentally from the coumarins wherein fluorescence is much more prominent in alkaline than in sulphuric acid solutions. Further in contrast to the coumarin series the flavones do not seem to depend on the presence of a hydroxy group for exhibiting fluorescence. It has been recorded that the colourless solution of simple flavone itself in strong sulphuric acid exhibits a strong violet blue fluorescence. Nor does

the position occupied by a hydroxyl group in the different rings seem to have any specific influence on the incidence of fluorescence, since 3-, 6-, 7- and 4′-hydroxy-flavones are all said to exhibit fluorescence. The only reasonable generalisation seems to be that all the simple hydroxy derivatives of chromones, flavones and *iso*-flavones are fluorescent. The presence of a large number of hydroxyl groups however has an adverse effect. Thus kæmpferol (3 : 5 : 7 : 3′-tetrahydroxyflavone) is fluorescent (blue) but not quercetin (3 : 5 : 7 : 3′ : 4′ :-pentahydroxyflavone). It may however be noted that the inhibitory influence of the large number of hydroxyl groups is considerably removed when some of them are methylated; rhamnetin (7-methyl ether of quercetin) and *iso*-rhamnetin (3′-methyl ether of quercetin) are fluorescent (greenish blue and green respectively) and so also is 3 : 3′ : 4′-trimethylquercetin.

Regarding the influence of alkyl and acyl groups situated in different positions in the chromone and flavone ring-systems no data seem to have been collected till now. Hence a few substances which are derivatives of 7-hydroxy-chromone and flavone and available in our laboratories have now been examined and as the observations are likely to be informative and useful they are given in the following table:

Name of compound	Nature of the fluorescence in conc. sulphuric acid
7-Hydroxyflavone	Deep blue
7-Acetoxyflavone	Blue deepening with time
7-Methoxyflavone	Blue
7-Allyloxyflavone	Strong blue
7-Hydroxy-8-methylflavone	Nil
7-Hydroxy-8-allylflavone	Green
7-Hydroxy-6 : 8-diallylflavone	Do.
7-Hydroxy-3-methoxyflavone	Very pale blue
7-Hydroxy-3-methoxy-8-methylflavone	Nil
7-Hydroxy-3-benzoylflavone	Nil
7-Hydroxy-8-formyl-3-methoxyflavone	Nil
7-Hydroxy-3-methoxy-2-methylchromone	Blue
7-Allyloxy-3-methoxy-2-methylchromone	Do.

Name of compound	Nature of the fluorescence in conc. sulphuric acid
7-Hydroxy-8-allyl-3-methoxy-2-methylchromone	Pale green
7-Hydroxy-3-methoxy-2-styrylchromone	Green
7-Hydroxy-3-acetyl-2-methylchromone	Nil
7-Hydroxy-8-formyl-3-methoxy-2-methylchromone	Nil

Regarding the flavones conversion of the 7-hydroxyl group into the acetoxyl, methoxyl and allyloxyl does not produce any marked change. A methyl group situated in the 8-position which is *ortho* to the hydroxyl removes the capacity to fluoresce; whereas one or two allyl groups similarly located only modify the colour of the fluorescence and convert it to green. An acyl group present in the 3- or in the 8- positions inhibits the fluorescence. The influence of substituent groups on the fluorescence exhibited by the chromones seems to be on the same lines as above though there are small differences particularly of intensity.

REFERENCE

1. Seshadri and Rao .. *Proc. Ind. Acad. Sci.*, (A), 1936, 3 294.