

THE CONSTITUTIONAL FEATURES OF ANTHOXANTHINS IN RELATION TO THE MORIN REACTION IN ANALYTICAL CHEMISTRY

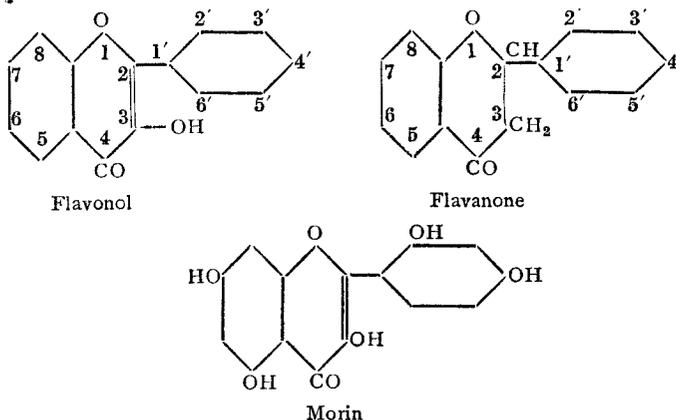
Part I. Some Naturally Occurring Hydroxy-flavonols and Flavanones

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MORIN (5:7:2':4'-tetrahydroxyflavonol) was first introduced by Goppelsroeder¹ in 1868 as a reagent for the detection of small amounts of aluminium with which it yields in weakly acid solutions, a very prominent green or blue-green fluorescence ordinarily visible in daylight. This reaction did not receive much attention until Schantl² in 1924 showed that it is a very sensitive reaction, as little as 10^{-8} mg. of the element in 10 c.c. of the solution being detected with the aid of Tswett's luminoscope. Since then it has received considerable attention, as a result of which it is now known not to be specific for aluminium. A similar yellowish green fluorescence has also been obtained with beryllium,² and a large number of other metals, and the acid radicals, arsenite and arsenate.^{3, 4, 5} In spite of this defect, this reaction has been utilised for the detection, as well as determination of aluminium and beryllium, and the fluoride ion indirectly.^{3, 6, 9} However, no attempt has been made till now to study the influence of constitutional factors in morin over the appearance of fluorescence with aluminium or any of the other metals.



The correlation of constitution with fluorescence is a problem of considerable difficulty. In the group of hydroxyflavonols of which morin is one, a considerable amount of investigation has been done regarding the relation between number and position of hydroxyls and the tinctorial properties of these compounds,¹⁰ but not much work has yet been done regarding the fluorescence which some of them exhibit under certain conditions. It is only recently that Rangaswami and Seshadri¹¹ examined this problem with reference to the closely allied hydroxy-flavones.

Several of the naturally occurring as well as the synthetic flavonols exhibit fluorescence in daylight, when dissolved in concentrated sulphuric acid, and a few in alkaline solution. Fisetin and the isomeric 6:3':4'-trihydroxy-flavonol are exceptional in that they exhibit fluorescence in alcoholic solutions. Morin itself yields a bluish green fluorescence in concentrated sulphuric acid, but none in alkaline solutions. It is, however, a unique case of a hydroxy-flavonol which yields fluorescence with metals in alkaline or acid solutions and so far employed in analytical chemistry. As is well known, the hydroxy-flavonols have marked dyeing properties and yield coloured lakes with metals such as aluminium, beryllium, etc. Morin behaves similarly but surprisingly enough it yields with the same metals in dilute solutions very prominent fluorescence in daylight itself. This fluorescence has been ascribed to a neutral, colloiddally dispersed aluminium salt of morin having the formula $\text{Al}(\text{C}_{15}\text{H}_9\text{O}_7)_3^2$ but it seems probable that the paraquinonoid structure which these lakes possess¹⁰ also plays an important part. The fact that a wide variety of metals yields similar fluorescence seems to support this view.

As a result of the investigations on the anthoxanthin pigments of flowers which have been in progress in these laboratories for several years, a number of compounds which have certain similarities and certain differences when compared with morin were available and, therefore, it was considered desirable to examine them in order to throw more light on the problem of the fluorescence obtained in the morin reactions. The examination was, however, limited to the reaction of morin with aluminium and beryllium as in practice this reaction is mostly used for the detection and determination of these metals.

Experimental

For the following experiments pure samples of the flavanones, naringenin, butin and hesperitin, and the flavonols, k ampferol, quercetin, herbacetin, gossypetin and quercetagenin which were obtained from natural sources were used.

Preparation of Solutions

Reagents.—Saturated solutions of all the pigments, except kæmpferol and herbacetin, in methyl alcohol were employed. The available amount of kæmpferol and herbacetin being small only dilute solutions were used.

Test Solutions: (1) *Aluminium.*—A dilute solution of potash alum was prepared using A.R. quality sample and standardised by precipitating the aluminium with 8-oxyquinoline. It was then diluted to contain 1 mg. of aluminium per c.c.

(2) *Beryllium.*—A dilute solution of beryllium nitrate was prepared using a "Pure" sample and the absence of aluminium was assured by testing with "Oxine". It was standardised by precipitating the beryllium as hydroxide and igniting to the oxide, and then diluted to contain 1 mg. of beryllium per c.c.

(3) *Potash.*—A dilute (2 N) solution of potassium hydroxide was prepared and tested for the absence of aluminium by the "Oxine" method.

Procedure.—Tests were carried out in alkaline, as well as in solutions acidified with acetic acid, with aluminium and beryllium provided the reagent itself did not undergo atmospheric oxidation in alkaline solution.

One c.c. of the test solution was placed in a quartz test-tube and treated with dilute potash until precipitation occurred. Thereafter, the precipitate was either dissolved in acetic acid or in an excess of the alkali, the solution diluted to 10 c.c. with water and treated with one or more drops of the reagent. Observations were made first in daylight and then under the "Cenco Black Light Source" which consists of an argon-mixed gas glow lamp provided with a U.V. filter which allowed light down to 3100 Å to pass through.

Results

Blank tests were carried out in all cases on the reagents themselves under identical conditions; the results were negative in all except hesperitin, 4'-methoxy-3': 5: 7-trihydroxyflavanone (*Citrus aurantium* peels) which gave in alkaline solution under the lamp a bright blue fluorescence which was destroyed by acetic acid. Similar results were obtained with hesperitin in alkaline as well as in acetic acid solutions both with aluminium and beryllium. For comparison the reactions were carried out with a saturated solution of morin in methyl alcohol. With the reagents recorded in the table below, however, no fluorescence was observed in daylight and in no case was it so very prominent as with morin under identical conditions. The results obtained under the lamp are recorded in a tabular form below:

Reagent	Sources	Aluminium	Beryllium	Remarks
1. Naringenin. (5:7:4'-trihydroxy-flavanone).	Flowers of <i>Citrus decumana</i> .	Faint bluish fluorescence in acetic acid; alkaline solution yellow in colour but no fluorescence.	Faint bluish fluorescence in acetic acid; alkaline solution deep yellow with a bluish fluorescence.	
2. Kæmpferol. (5:7:4'-trihydroxy-flavonol)	Senna leaves.	Alkaline solution yellow in colour with no fluorescence. In faintly alkaline or almost neutral solution, a bluish green fluorescence, moderately strong; on adding acetic acid the fluorescence is practically unchanged.	Bright yellow solution in alkalies with a bright greenish yellow fluorescence. None in acetic acid.	
3. Herbacetin. (5:7:8:4'-tetrahydroxy flavonol)	Flowers of <i>Gossypium herbaceum</i> .	Bright yellow solution with no fluorescence in acetic acid.	Bright yellow solution, with no fluorescence in acetic acid.	The reagent undergoes oxidation in alkaline solutions.
4. Morin. (5:7:2':4'-tetrahydroxy-flavonol)	Schering-Kahlbaum, Berlin.	In acetic acid extraordinarily brilliant green fluorescence; in alkaline solutions deep yellow without any fluorescence.	In acetic acid no fluorescence; in alkaline solution chromate yellow with deep yellowish green fluorescence.	Identical results were obtained even in daylight in both cases.
5. Quercetin. (5:7:3':4'-tetrahydroxy-flavonol)	Flowers of <i>Gossypium herbaceum</i>	Bright yellow solution with yellowish green fluorescence in acetic acid; deep yellow solution with no fluorescence in alkalies.	Bright yellow solution with no fluorescence in acetic acid; deep yellow solution with a very feeble greenish fluorescence in alkalies.	
6. Gossypetin. (5:7:8:3':4'-penta-hydroxy-flavonol)	Do.	Yellow solution with no fluorescence in acetic acid.	Pale yellow solution with no fluorescence in acetic acid.	Undergoes oxidation in alkaline solutions.
7. Quercetagenin. (5:6:7:3':4'-penta-hydroxy-flavonol)	Flowers of <i>Tagetes erecta</i> .	Yellow solution with no fluorescence in acetic acid.	Yellow solution without any fluorescence in acetic acid.	Undergoes oxidation in alkaline solutions.
8. Butin. (7:3':4'-trihydroxy-flavanone).	Flowers of <i>Butea frondosa</i> .	In acetic acid solution pale yellow with no fluorescence; in alkaline solution, only red with no fluorescence.	In acetic acid pale yellow solution with no fluorescence; in alkalies a red solution with no fluorescence.	In alkaline solution the compound is converted rapidly into the corresponding chalcone, butein.

Discussion

The results obtained show that among the group of compounds examined morin is exceptional in giving a very prominent fluorescence; none of the others gives any fluorescence with aluminium or beryllium in daylight and the fluorescence observed under the lamp is not at all so intense. A comparison of these results, however, throws light on the relation between the constitution of these compounds and the fluorescence. The only difference between morin and quercetin is in the 2'- instead of 3'- position of one hydroxyl in the side phenyl group and the difference in behaviour may, therefore, be due to the 2'-position of one hydroxyl. The results obtained with k ampferol and morin show the intensifying effect of a hydroxyl in the 2'-position on the fluorescence given by a 4'-hydroxy compound. A comparison of k ampferol with herbacetin reveals the inhibitory effect of the 8-hydroxyl. This conclusion is further supported by the comparison of quercetin with gossypetin. Further the available data shows the inhibitory effect of 6-hydroxyl as in quercetagenin. The exhibition of fluorescence both by k ampferol and naringenin shows that the 3-hydroxyl is not absolutely necessary for the appearance of fluorescence with metals. It thus appears probable that a hydroxyl in 2'-position alone or in conjunction with a 4'-hydroxyl is responsible for the brilliant fluorescence obtained with morin and metals. This conclusion seems to be supported by the fact that morin unlike other flavonols, yields an anhydrosulphate ($C_{15}H_8O_6, H_2SO_4$) when treated with concentrated sulphuric acid, and this behaviour is closely related to the presence of a hydroxyl in the 2'-position.¹² If this conclusion were correct, similarly constituted compounds such as datiscetin (5:7:2'-trihydroxyflavonol), resomorin (7:2':4'-trihydroxyflavonol), and 5:7:2':4'-tetrahydroxyflavone synthesised by Robinson and Venkata Raman,¹² should yield similar results with metals. Further work is in progress along these lines.

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