SURVEY OF ANTHOXANTHINS—PART I

By K. S. Pankajamani and T. R. Seshadri, F.A.Sc.

(*From the Department of Chemistry, Delhi University, Delhi*)

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Flavonoids occur widely in the plant kingdom, and their study may probably be more freely used than hitherto in genetics, plant classification and physiology. In this respect they should be considered to be as important as anthocyanins, whose analysis in small quantities of plant materials has been carefully worked out by Robinson and collaborators.[1] The use of paper chromatography for the identification of anthocyanins in petal extracts has been investigated by Batesmith,[2] using the linear flow method. In the case of anthoxanthins, the evolution of a satisfactory chemical micromethod has not been possible so far. The more promising method of paper chromatography has been studied by Wender and collaborators,[3] again using the linear migration method. They have recorded the Rf values of a large number of anthoxanthins belonging to different groups, aglycones and glycosides, using various solvent combinations and fluorescence and chromogenic sprays. But its dependability for analysing naturally occurring mixtures has not been investigated in detail. It is the purpose of the present work, to examine convenient and quick methods of analysing plant materials in small quantities and applying them for the survey of anthoxanthins. These occur usually as mixutres which are sometimes complex. Complete analysis has rarely been effected; though by employing the method of fractionation adopted by Seshadri and co-workers,[4] it is possible to get more information than by earlier procedures, still the results could not be taken as complete. Paper chromatography has now been used for diagnostic purposes and the results verified by regular methods of fractionation. They corroborate each other and indicate that the paper chromatography is quite dependable.

Among the methods of paper chromatography the circular one described by Rao and Beri[5] for sugars is found to work quite satisfactorily. Its special virtue is quickness. In the case of flavonols which are chosen in the first instance for study, there is no need to develop the rings by means of chromogenic sprays, since they are sufficiently coloured yellow to indicate their location.

To begin with, authentic samples of pure flavonols have been prepared synthetically, chromatographed and the circular Rf values determined. This choice of substances is based on their occurrence in nature, since the results are intended to be used for the study of natural sources. Each flavonol gives its own zone with a fixed Rf value. There is no difficulty of multiple zones occurring as reported in certain cases by others.[6] Next extracts from plant sources have been examined; there is no difficulty in identifying the components from the rings. For purposes of verification, extraction and separation of flavonoid mixtures have been effected using methods of fractional precipitation and crystallisation and also chromatographic separation using alumina. It may be recorded here that these fractionation methods have not only corroborated the results of filter-paper chromatography, but indicate that with greater precaution, the existence of various entities in plant materials could be independently proved. These tested materials could therefore be used as standards of reference in case pure synthetic samples of the flavonols are not available for comparison. Since the Rf values are liable to vary with the conditions of t xperiment, it is essential that this comparison should be carefully done usin ure synthetic flavonol samples or plant sources whose composition has been definitely established. Our results indicate that flavonoids in general, occur as mixtures of two or more entities, and it is rare to find them single. Further, in the large majority of cases, quercetin is an invariable component.

For the present survey, we have laid particular emphasis on the identification of the aglycones and their separation, rather than on the glycosides in which form they may occur. We feel that this procedure is more dependable and more useful. However, some information regarding the nature of the glycosides present could be obtained even with small quantities by the study of reactions with lead acetate and zinc and concentrated hydrochloric acid (Pew's reaction).[7] The following typical examples will illustrate the general methods of fractionation that have been adopted.

1. Moringa Flowers (*Moringa pterygosperma*)

Various parts of this tree, leaves, flowers and pods have been used as food and as drug, and therefore have been investigated carefully. But so far the existence of flavonoids in the flowers has not been recorded.[8] In the course of the present work two varieties of Moringa trees have been chosen, one the 'sweet' variety and the other the 'bitter'. Both have been found to contain rather low quantities of a flavonoid mixture, circular chromatography indicating the presence of two, a pentahydroxy and a tetrahydroxy flavone, closely agreeing with quercetin and kæmpferol res-

pectively. The separation of the mixtures has been carried out using their full methyl ethers and passing them through a column of alumina. Kæmpferol tetramethyl ether is washed out first and quercetin ether later.

2. Neem Flowers

The flowers of the neem tree were examined by Mitra and Siddiqui[9] and they reported the isolation in poor yield of kæmpferol. In our experiments we find that the fresh flowers contain a high percentage of extractable flavonoids, about 0·5%, which will work out to be about 1·5% on air-dried matter. But there seems to be considerable loss of pigments when the flowers are sun dried and stored. Still about 1% yield can be secured, if the flowers are moistened first with water, allowed to stand for a few hours and then alcohol added for extraction. Chromatography indicated the presence of three compounds, corresponding to hexa-, penta-, and tetra-hydroxy flavones agreeing with myricetin, quercetin and kæmpferol. For effecting the separation, the total pigment in aqueous alcoholic solution, is treated with excess of neutral lead acetate and after filtering off the neutral lead salt, excess of basic lead acetate added. The portion obtained by the decomposition of the neutral lead salt is fractionated by the acetate method. Quercetin is obtained as the main component and myricetin as the minor. In the basic lead salt fraction kæmpferol happens to be the only entity and is readily obtained as the free hydroxy flavone.

3. Indian Podophyllum (*Podophyllum emodi*)

Podwyssotski[10] was the earliest to isolate a yellow colouring matter from the American Podophyllum (*Podophyllum peltatum*). He called this podophylloquercetin. Kursten[11] extending the work of Podwyssotski showed, from an examination of the acetyl, and benzoyl derivatives and also the methyl ether that podophylloquercetin was not identical with quercetin from quercitron bark. Obviously it was a mixture. Later Dunstan and Henry[12] made a comparative study of both Indian podophyllum (*P. emodi*) and *P. peltatum*. They established the presence of quercetin in both these but only after considerable purification they could make sure of its identity. In the recent work of Seshadri and Subramanian[13] quercetin has been obtained more readily pure. In the present analysis using filter-paper chromatography the flavonoid part of this resin yields two distinct zones, one corresponding to a tetra-(kæmpferol) and the other corresponding to a penta-hydroxy flavone (quercetin). Fractionation of the acetates yields in the first crop pure quercetin acetate, then mixtures as the intermediate fractions, and in the later fractions kæmpferol acetate. Thus it is clear that

the podophyllum resin contains a mixture of flavonols, quercetin and kæmpferol. The difficulties of earlier workers in isolating pure quercetin was explained by Seshadri and Subramanian[13] as due to the inefficient removal of podophyllotoxin and podophylloresin. It would now appear that the difficulties could also be attributed to the presence of kæmpferol in varying proportions.

EXPERIMENTAL

Preparation of Synthetic Flavonols

Synthetic samples of the flavonols were obtained by dealkylating in the usual way the methyl or ethyl ethers prepared synthetically. The flavonol ether was dissolved in a small quantity of acetic anhydride and excess of hydriodic acid (sp. gr. 1·7, 10 c.c. for 1 g.) added with cooling. The mixture was then refluxed in an oil-bath at 140° for 2 hours, cooled, poured into an excess of a saturated solution of sodium bisulphite and then extracted with ether. The ether extract on evaporation gave a pure yellow crystalline product of the flavonol. To ensure purity this was further crystallised from dilute alcohol and the melting point taken.

The following flavonols were prepared from their methyl or ethyl ethers: Kæmpferol from its tetramethyl ether,[14] fisetin from 7-hydroxy-3: 3′: 4′-trimethoxy flavone,[15] datiscetin from its 3: 2′-dimethyl ether,[16, 17] morin and resomorin both from their 3: 2′: 4′-trimethyl ethers,[17] robinetin from its pentamethyl ether,[18] quercetin from its 3: 3′: 4′-trimethyl ether,[19] myricetin from 3-ethoxy-5: 7: 3′: 4′: 5′-pentamethoxy flavone,[20] quercetagetin from its hexamethyl ether[21] and gossypetin from its 3: 3′: 4′-trimethyl ether.[22]

Flavonols from natural plant sources could also be used in place of synthetic samples, after sufficiently purifying them through the acetate. This was verified in the case of quercetin and gossypetin. The natural flavonols were fully acetylated and the acetate completely purified by crystallisation from ethyl acetate. The fraction having the correct melting point was then hydrolysed using 1: 1 alcoholic hydrochloric acid, by gently boiling the mixture for 15 minutes. The yellow solution obtained was then diluted with water and extracted with ether. The ether extract on evaporation gave pure yellow crystals of the free flavonol. The purity of the samples was checked by taking their melting points.

Circular (Horizontal Migration) Chromatography: Details and Results

The procedure adopted was essentially the same as that followed by Rao and Beri.[5] Acetone was the solvent chosen to dissolve the flavonol, because of the ease with which it evaporated from the filter-paper, leaving

the pigment spot in the centre. The temperature used was 36–37°. There was no special reason for selecting this temperature, except for the fact that it was the convenient temperature for the double-walled incubator which was used as a thermostat and the room temperature in hot weather at Delhi is not appreciably less. The time of diffusion was only 90 minutes; in this period each flavonol spread itself into a circular zone of convenient size. For preparing a suitable solvent, phenol distilled over zinc dust was shaken up with excess of water and allowed to stand at room temperature (30°), when separation into two layers took place. The lower layer of water in phenol was tapped off and found to be a satisfactory solvent. To obtain correct and reproducible Rf values, certain precautions were necessary. The solvent once prepared was not used for more than a week, since otherwise the zones were not sharp and the Rf values were not consistent. It was also allowed to stand at the temperature of the experiment for at least 48 hours.

The circular Rf values for the important flavonols whose preparation has been given above are listed in the following table:

TABLE I

Flavonol	Rf value $\frac{\text{(Distance travelled by the solute)}}{\text{(Distance travalled by the solvent)}}$	Position of hydroxyl groups
1. Kæmpferol ..	0·78	3:5:7:4′
2. Fisetin ..	0·64	3:7:3′:4′
3. Datiscetin ..	0·78	3:5:7:2′
4. Resomorin ..	0·47	3:7:2′:4′
5. Morin ..	0·41	3:5:7:2′:4′
6. Quercetin ..	0·54	3:5:7:3′:4′
7. Robinetin ..	0·44	3:7:3′:4′:5′
8. Myricetin ..	0·30	3:5:7:3′:4′:5′
9. Quercetagetin ..	0·35	3:5:6:7:3′:4′
10. Gossypetin ..	0·28	3:5:7:8:3′:4′

The Rf values given above support in general the conclusions of Batesmith,[23] that the number of hydroxyl groups has an important influence and that the effect of chelation (especially of the hydroxyl in the 5-position)

brings about an increase in the Rf values. Even other positions are not all equivalent and different arrangements give appreicably different Rf values: *cf.*, numbers 1 to 4, 5 to 7 and 8 to 10, in the table. Thus isomers in many cases seem to be capable of being differentiated by their Rf values. However, it may be mentioned that with this solvent though the compounds can be identified when present alone or with flavonols having less or more number of hydroxyl groups, with mixtures of isomers the rings tend to be broad and separation is not very distinct. Probably other solvents may serve better in these cases.

Extraction of the Plant Materials

Fresh flowers were employed and extracted repeatedly with cold or hot 95% alcohol, the combined extracts concentrated to small bulk and hydrolysed using sulphuric acid (7%). Any alcohol remaining after hydrolysis was removed by evaporation from a porcelain basin, the brownish yellow solution filtered hot, diluted with water and ether extracted. The ether extract on evaporation gave a yellow residue and this was used for chromatography.

Chromatography of Natural Pigments

The flavonol aglycones were chromatographed by the horizontal migration technique and the circular Rf values calculated for the various zones. The sources of the flavonols along with the number of zones and their Rf values are given in the following table:

TABLE II

Source of the flavonol aglycones	Number of zones	Rf_1	Rf_2	Rf_3
Flowers of *Moringa pterygosperma* (both sweet and bitter varieties)	2	..	0·56	0·80
Neem flowers ..	3	0·31	0·54	0·75
Podophyllum emodi (Indian podophyllum) resin	2	..	0·54	0·78

Moringa pterygosperma

Extraction and Isolation of the Colouring Matter

There are two varieties of *M. pterygosperma*, the leaves, flowers and fruits of one variety being bitter while those of the other variety are not. Flowers of both these varieties were extracted and studied separately. 3 kg.

each of the fresh winter flowers were extracted repeatedly in the cold with 95% alcohol, each time the solvent being allowed to stand in contact with the flowers for 2 days. The combined extracts were concentrated to about 200 c.c. by distilling off the excess of alcohol on a boiling water-bath. The concentrate was cooled and allowed to stand for 12 hours, when most of the wax and oil present separated out as a greenish flocculent mass. This was filtered off, through a fluted filter paper, and the precipitate washed with small quantities of 50% alcohol until the test with magnesium and hydrochloric acid was not given. The washings were combined with the original filtrate and the excess of alcohol removed by evaporation in a porcelain basin over a water-bath. The extract which was about 200 c.c. was cooled, and treated with 8 c.c. of concentrated sulphuric acid diluted with 20 c.c. of water, and gently refluxed on a wire-gauze for 2 hours. It was then diluted with twice the volume of water and the whole evaporated in a broad evaporating basin on a water-bath to remove all traces of alcohol. The remaining portion was then filtered hot, the filtrate cooled and extracted repeatedly with ether. The combined ether extracts were washed with water, dried over anhydrous sodium sulphate and distilled to remove solvent. A yellow residue containing some resin was obtained. This was dissolved in the minimum of 50% alcohol, shaken with animal charcoal, heated to boiling and filtered hot. Most of the resins were adsorbed by the animal charcoal and a clear deep yellow filtrate obtained, which on cooling deposited a pure yellow crystalline solid. Yield 0·50 g. from the sweet variety and 0·47 g. from the bitter variety.

In earlier experiments the air-dried flowers were extracted with boiling alcohol, and this involved a number of difficulties. The flowers contain a large quantity of resinous and waxy matter and these were easily extracted by boiling alcohol, and the hot concentrated extract on cooling deposited large quantities of this dark resinous matter, which answered the magnesium-hydrochloric acid test very strongly. Most of the flavonol was thus carried away in the resin and isolation was rather difficult. So in later experiments cold extraction of the fresh flowers was employed as described above, and found to be more convenient since in the cold most of the resin was not extracted. The buds were also used for extraction and the yield of flavonol was found to be the same as in the case of the flowers.

The samples obtained from both the varieties sintered at about 200° and melted over a large range. With magnesium powder and hydrochloric acid a deep red colour was developed in both cases, a colour reaction characteristic of flavonols. With ferric chloride both gave an olive green colour. They did not dissolve in sodium bicarbonate, but with sodium carbonate

they gave a yellow solution. With sodium hydroxide they gave a yellow solution which on shaking with air turned reddish brown. The samples when analysed by the circular paper chromatography, gave 2 rings closely corresponding to kæmpferol and quercetin.

Separation of Methyl Ethers

The flavonol mixture (0·4 g.) was dissolved in pure, dry acetone and treated with excess of pure dry dimethyl sulphate (5 c.c.) and freshly ignited potassium carbonate (5 g.). The mixture was refluxed on a water-bath for 42 hours. Acetone was then distilled off and the residue treated with excess of water, when the methyl ether separated out in the form of an almost colourless solid. This was filtered off, and purified further by crystallising from very dilute alcohol; colourless needles, sintering at 60° and melting at 75–108°, yield 0·27 g.

The methyl ether mixture (0·27 g.) was dissolved in pure dry acetone (20 c.c.) and the solution allowed to pass through a column of activated alumina (Merck) (18 cm. by 1 cm.) under gentle suction. The column was then successively washed with acetone-petroleum ether mixture (1 : 2) using 15 c.c. each time. The fractions were collected separately and the dissolved solid was obtained by evaporating off the solvent and crystallising the residue from the same solvent mixture. Details of analysis are given below:

TABLE III

Serial number	Fraction	Yield in mg.	Melting point
1	Original filtrate	Nil	..
2	Acetone-petroleum ether (1: 2) (15 c.c.)	100	Sintered at 140° and melted at 151–52°
3	do	30	151–52
4	do	50	151–52
5	do	20 (fractions 5–8)	151–52 (fractions 5–8)
6	do		
7	do		
8	Acetone-petroleum ether (2:2)		

Fractions 3 to 8 were found to be identical with quercetin pentamethyl ether. Fraction 2 was recrystallised from acetone-ligroin mixture. The first crop again had an indefinite melting point (152–62°) whereas the second melted sharp at 151–52° and was identical with quercetin penta-

methyl ether. Further crystallisation of the first crop yielded a small quantity of an ether melting at 162° and it did not depress the melting point of a pure sample of kæmpferol tetramethyl ether (165°). Thus the ether mixture consisted mainly of quercetin along with a small amount of kæmpferol.

Fractionation of the Acetates

The acetate was prepared from the sample obtained from the bitter variety. 0·2 g. of the flavonol was dissolved in excess of acetic anhydride (3 c.c.) and freshly fused sodium acetate (0·5 g.) added. The mixture was refluxed on an oil-bath at 138–40° for 2 hours and poured on to crushed ice with constant stirring. The acetate soon separated out as a colourless solid, partly in the form of needles. Yield 0·23 g. It crystallised from absolute ethyl acetate as colourless needles, m.p. 172–78°.

The acetate mixture was dissolved in a liberal volume of hot absolute ethyl acetate and allowed to stand for 12 hours. The first fraction that separated out was filtered off, and was found to melt at 190–91°. The mixed melting point with an authentic sample of quercetin penta-acetate was undepressed. Subsequent fractions also melted at 190°, and the last fraction at 181–84°. The yield of the last fraction was only 30 mg. Mixed melting point of this fraction with pure quercetin penta-acetate was 181–90°. Since no considerable depression in the melting point was shown, it was concluded that the quercetin acetate which formed the main fraction in the acetate mixture was contaminated with a trace of an unisolatable impurity. This impurity may be the acetate of kæmpferol as indicated by paper chromatography.

Separation through the Glycosides

Neutral lead salt fraction (*Quercetin*).—1 kg. of the fresh flowers (bitter variety) were extracted in the cold with 95% alcohol, twice repeatedly. The extracts were combined and the excess of alcohol distilled off on a boiling water-bath, and the concentrated solution cooled, when plenty of chlorophyll and waxy matter separated out. This was filtered off, and the filtrate treated with an excess of a concentrated solution of neutral lead acetate. The lead salt of quercetin glycoside separated out as a bulky yellow precipitate. This was filtered off (filtrate marked F), washed well with water, suspended in alcohol, and a current of hydrogen sulphide passed through for 2 hours, till all the lead was removed in the form of lead sulphide. The black precipitate of lead sulphide was filtered off. This was then ground in a mortar with a small quantity of alcohol to remove traces of the glycoside present along with it, and filtered. The filtrate was combined with the

original filtrate and hydrogen sulphide again passed through. No appreciable precipitation of lead sulphide took place, indicating the complete removal of lead from the lead salt. The alcoholic filtrate containing the glycoside in solution was concentrated. It gave a positive test with zinc and concentrated hydrochloric acid (Pew's reaction)[7] thereby indicating that the sugar molecule is attached to the 3-position. At first a faint orange yellow colour developed which gradually became more and more intense on keeping and assumed a deep orange red colour after 2 days. The yellow colour of the lead salt also indicated the presence of a 3-glycoside.

The concentrated alcoholic solution was subjected to acid hydrolysis in the usual way, and the aglycone extracted with ether, and the ether distilled off, when a yellow residue was obtained (0·25 g.). This gave a deep red colour with magnesium and hydrochloric acid. With zinc and hydrochloric acid no colour was developed, showing that the 3-hydroxyl was free.

The yellow residue when analysed by paper chromatography gave a single yellow zone, having an average Rf value of 0·55 which corresponded with that obtained for pure synthetic quercetin. The identity was confirmed by preparing the acetate and taking the mixed melting point with an authentic sample of quercetin penta-acetate. Yield 0·12 g.

Basic lead salt fraction (*Kæmpferol*).—The filtrate (F) left behind after removal of the neutral lead acetate precipitate was treated with a large excess of basic lead acetate. A lemon yellow flocculent precipitate was obtained. This was filtered off, suspended in alcohol and decomposed with hydrogen sulphide. The alcoholic solution of the glycoside (which gave a positive test for Pew's reaction) was hydrolysed as usual; the aglycone was found to be kæmpferol by means of the acetate which lost water at 118° and melted at 180–85°.

In the above experiments using the separation of the glycosides, it was possible to obtain both the quercetin and the kæmpferol fraction more definitely. This may be attributed not only to the improved technique but also to the effect of seasonal variations on the pigment composition. Kæmpferol seems to be present more in the later flowers.

The aqueous acid solutions left after ether extraction of the flavonol aglycones were separately treated with barium carbonate little by little, till all the sulphuric acid was removed as barium sulphate and the solutions became neutral to litmus. The insoluble barium sulphate was filtered off and the filtrates containing the sugar concentrated to convenient bulk. Both yielded the same osazone which was identified as glucosazone.

Neem Flowers

Isolation of the Colouring Matter

Neutral lead salt fraction (*Quercetin and myricetin*).—200 g. of the air-dried flowers were soaked with water and allowed to stand for 12 hours. Alcohol was then added and the flowers repeatedly extracted thrice, each time refluxing on a water-bath for 6 hours. The combined extracts were concentrated and treated with an excess of neutral lead acetate. The bulky yellow precipitate of the neutral lead salt was filtered off (filtrate marked A), suspended in aqueous alcohol and decomposed by passing hydrogen sulphide. Lead sulphide was filtered off and the glycoside remaining in the filtrate was hydrolysed using sulphuric acid (7%). Alcohol was removed by evaporation from a basin. On cooling a yellow amorphous mass separated out. This was extracted with ether and the ether extract evaporated when a yellow residue was obtained which when analysed by paper chromatography gave two distinct zones corresponding to myricetin and quercetin. It was crystallised from 50% alcohol and pure yellow crystals obtained. Yield 0·42 g. The product gave with aqueous sodium carbonate and sodium hydroxide an emerald green colour which on shaking quickly changed into a purplish violet. This indicated the presence of myricetin in the crystalline product.

0·4 g. of the above product was acetylated using acetic anhydride and pyridine. The acetate mixture came out as colourless needles from ethyl acetate; yield 0·5 g., sintering at 108° and melting between 140–68°. The fractionation of the acetate was done using ethyl acetate as solvent. The first crop of crystals (100 mg.) sintered at 200° and melted at 204°. Recrystallisation of this from the same solvent yielded colourless crystals melting at 212–14°. The mixed melting point with an authentic sample of myricetin hexa-acetate was undepressed. The intermediate fractions did not melt sharp and the later fractions melted correctly at 191–92°, and were identical with quercetin acetate. The mixed melting point with an authentic sample of quercetin acetate was not lowered.

Basic lead salt fraction (*Kæmpferol*).—The filtrate (A) obtained from the neutral lead salt was treated with an excess of basic lead acetate, when a lemon yellow precipitate was formed. This was filtered off, deleaded and the alcoholic solution containing the glycoside hydrolysed using sulphuric acid (7%). Excess alcohol was then removed by evaporation from a porcelain basin and the aglycone extracted with ether. The ether extract on distillation left behind a yellow crystalline solid. This was further purified by crystallising from dilute alcohol. Yield 0·4 g., m.p. 276–78°. It

agreed with pure kæmpferol and the mixed melting point with an authentic sample of kæmpferol was undepressed.

The proportions of the three pigments in the flowers were approximately as follows: Myricetin 3, quercetin 7 and kæmpferol 10 parts.

The Glycosides

The yellow colour of the lead salts indicated that all the three flavonols might be existing as their 3-glycosides. This was further supported by the positive Pew's reaction,[7] given by each of the fractions.

Indian Podophyllum (*Podophyllum emodi*)

Podophyllum resin was supplied by the Drug Research Laboratories, Jammu-Tawi. For the isolation of the colouring matter from this, 40 g. of the resin was taken up in acetone (100 c.c.) and filtered. The filtered solution was then treated with chloroform (800 c.c.) and shaken vigorously. The clear solution was decanted off from the sticky resin that was precipitated and diluted with an equal volume of chloroform. The crude colouring matter separated out as a fine yellow powder (1 g.). This was filtered off and crystallised from dilute alcohol. Yellow crystals, yield 0·8 g.

Fractionation of the Acetate

0·8 g. of the flavonol mixture was converted into the acetate using acetic anhydride and pyridine. Yield, quantitative. The fractionation of the acetate was done using ethyl acetate as solvent. The first crop of crystals melted at 192–93°, corresponding to quercetin acetate. The subsequent fractions did not melt sharply; later fractions sintered at 120° and melted at 140°. These when recrystallised lost water at 120° and melted at 185°. Tail fractions also behaved similarly and were found to be identical with kæmpferol acetate. The mixed melting point with a genuine sample of kæmpferol tetra-acetate was undepressed. Yield, 0·15 g. The proportions of quercetin and kæmpferol were approximately 87% and 13% respectively.

Another sample of *Podophyllum emodi* supplied by Stafford Allen & Sons Ltd., London, was also examined for the colouring matter; 40 g. of the resin gave 2·3 g. of the almost pure colouring matter, the yield being thus thrice that obtained from the Jammu-Tawi sample. Paper chromatography indicated the presence of two flavonols corresponding to quercetin and kæmpferol. Separation of these two was effected through the fractionation of the acetate mixture, and the proportions of quercetin and kæmpferol were found to be roughly 80% and 20% respectively.

SUMMARY

Circular Rf values of important synthetic flavonols have been determined using phenol saturated with water. The results have been used for analysing the flavonol composition of plant extracts, three typical examples being taken; *Moringa pterygosperma* flowers, Neem flowers and Indian Podophyllum (*Podophyllum emodi*) resin. The results have been verified by applying regular methods of fractionation involving lead salt precipitation, fractional crystallisation of the acetates and adsorption chromatography of the methyl ethers. These materials could therefore be used as standards of comparison where synthetic flavonols are not readily available. Some information about the nature of the glycosides have been provided by colour reactions.

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