ANTISEPTICS AND ANTHELMINTHICS.

Part III. Pharmacology of Certain Flavones with Special Reference to their Anthelminthic Action.

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The study of the chemistry of the flavones, the great group of naturally occurring colouring matters, was originated by Piccard\(^1\) who isolated a yellow pigment, chrysin, from the leaf buds of the poplar (\textit{Populus pyramidalis} Salisb., \textit{P. nigra} Linn., \textit{P. monilifera} Ait.), which contained chrysin to the extent of 0.5 per cent. On the basis of degradative and synthetic experiments Kostanecki\(^2\) assigned to chrysin the constitution of a 2-phenyl-5:7-dihydroxybenzo-\(\gamma\)-pyrone (I). 2-Phenyl benzo-\(\gamma\)-pyrone or flavone, the parent member of the series, has been found in nature as the meal or farina which occurs on the leaves, stalks and seed capsules of many varieties of \textit{Primula}, e.g., \textit{P. pulverulenta}, \textit{P. Japonica} (Muller\(^3\)); its synthesis had already been accomplished by Feuerstein and Kostanecki.\(^4\) Since 1873 numerous derivatives of flavone, having different numbers of hydroxyls or methoxyls or both in different positions of the molecule and in the form of glykosides, have been isolated from the roots, stems, barks, fruits, flowers and leaves of various medicinal and other plants; to cite a few baikalein (II) from the roots of \textit{Scutellaria baikalensis} Georgi, quercetagetin (III) from the flowers of \textit{Tagetes patula}, tricin (IV) from ‘\textit{Khabli}’ wheat and rhamnetin (V) from the husks of \textit{Persian berries}. Due to the wide

\[ \text{HO} \quad \text{O} \quad \text{CO} \quad \text{HO} \quad \text{CO} \quad \text{HO} \quad \text{CO} \]

\[ \text{(I)} \quad \text{(II)} \quad \text{(III)} \]

\(^1\) Piccard, \textit{Ber.}, 1873, 6, 884; 1874, 7, 888; 1877, 10, 176.

\(^2\) Kostanecki, \textit{Ibid.}, 1893, 26, 2901.

\(^3\) Muller, \textit{J. Chem. Soc.}, 1915, 107, 875.

\(^4\) Feuerstein and Kostanecki, \textit{Ber.}, 1898, 31, 1757.
occurrence of the flavones in nature several methods for their synthesis have been developed by various workers, the more important being by Kostanecki and others, Robinson and his pupils, Baker, Mahal and Venkataraman, Algar and Flynn and Mahal, Rai and Venkataraman.

Although so much is known about the chemistry of the flavone group and in spite of their wide occurrence in medicinal plants, e.g., apigenin (VI) and genkwanin (VII) in the Chinese drug 'Yuen-hua' (Nakao and Tseng), which is reputed to have diuretic and anthelminthic properties and armarbelin in the seeds of *Cuscuta reflexa* Roxb. (Agarwal) also reputed to have anthelminthic properties, very little has been mentioned in literature about the pharmacology of this extensive series of natural and synthetic colouring matters. Koike appears to have been the first to study the pharmacology of the flavones; he observed that they showed diuresis in normal rabbits and the diuretic action of the polyhydroxyflavones increased with increasing number of hydroxyls. Fukuda, a little later, also confirmed the previous observation of Koike and found further that flavonols and their glykosides are cardiac stimulants, vaso constrictors, and increased the blood pressure.

Ratnagiriswaran, Sehra and Venkataraman isolated a flavone, calycopterin, from the leaves of *Calycopteris floribunda* reputed to have laxative and anthelminthic properties (Nadkarni). On the experimental results of Dr. K. Venkatachalam, Research Officer, Medical College, Madras, who found that calycopterin is toxic to round worms (*Ascaris lumbricoides*) suspended in a mixture of bile with aqueous sodium carbonate approximating to the composition of the intestinal fluid in which the worms reside, they

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suggested that calycopterin is the anthelminthic constituent of the leaves of *Calycopteris floribunda*. The present author and Venkataraman\textsuperscript{17} having further shown that calycopterin is 6:4'-dihydroxy-3:5:7:8-tetramethoxy flavone (VIII), the study of the pharmacology, particularly the anthelminthic properties, of flavones has been undertaken.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{flavones.png}
\caption{Structures of flavones studied in the present work.}
\end{figure}

The flavones that have been studied in the present work are (a) chrysin (I) and genkwanin (VII) which are both phloroglucinol derivatives and are therefore of special interest as it is well known that filix derivatives, \textit{e.g.}, male fern, kamala and kausso, which have long been used as specific anthelmintics are phloroglucinol derivatives; (b) 7-hydroxyflavone (IX) and 6-hexyl-7-hydroxyflavone (X), derivatives respectively of resorcinol and hexylresorcinol, which are known antiseptics. Along with the study of the pharmacology of benzo-\(\gamma\)-pyrones or flavones, 1-methylumbelliferone (XI), a member of the closely related group of benzo-\(\alpha\)-pyrones or coumarins, has also been examined.

The anthelminthic action of the compounds has been studied \textit{in vitro} by (A) directly immersing round-worms (*Ascaris lumbricoides*), tape-worms (*Taenia serrata*) and leeches (*Hirudo medicinalis*) in different concentrations of the solution of the substances, (B) by the use of Dale and Laidlaw's apparatus.\textsuperscript{18} The results obtained in the experiments (A) and (B) show that the compounds do not have any anthelminthic properties; calycopterin itself has also been tried again and has likewise given negative results.


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Although these tests in vitro cannot be taken as a conclusive proof that the substances have no anthelminthic action, yet it is a fair indication against their being good anthelminthics. Owing to the very high cost of production of these compounds in sufficient quantities their tests in vivo have not been tried.

Other physiological actions of these compounds have also been studied and it has been observed that they inhibit the movements of the isolated rabbit's gut and uterus in concentrations of about 1–140,000 (Plate XIII, Figs. 5, 6 and 7); cause depression in the blood pressure, increase the movements of respiration and cause contraction in the uterus of dogs in doses varying from 20–40 mg. per kg. weight of the body of the animals (Plates XIV and XV, Figs. 8, 9, 10, 11 and 12) and in concentrations varying from 1–100,000–500,000 inhibit the beats of an isolated frog's heart (Plate XVI, Figs. 13, 14 and 15).

The antiseptic properties of chrysin, genkwanin and 4-methyl-umbelliferone have been studied on the growth of B. coli and it has been observed that chrysin and genkwanin have no effect on the growth of the bacilli, but 4-methylumbelliferone, however, inhibits the growth of the bacilli in 1–1,000 concentration while in concentration 1–10,000 only a slight inhibition has been observed. Germicidal properties of the compounds have been tested by the Rideal Walker's phenol coefficient test and all the compounds are found to have phenol coefficients less than one.

Experimental.

Anthelminthic action of 7-hydroxyflavone (IX), 6-hexyl-7-hydroxyflavone (X), chrysin (I), genkwanin (VII), calycopterin (VIII) and 4-methylumbelliferone (XI)—Method (A).—The compounds being insoluble in water, they were dissolved in minimum amounts of cold N/10 aqueous caustic soda and the stock solutions so obtained were further diluted to the required concentrations by adding the requisite amounts of water, saline or Locke's solution depending upon whether the compounds were to be tried in water, saline or Locke's solution. 6-Hexyl-7-hydroxyflavone being insoluble even in caustic soda solution was used in the form of an emulsion in gum tragacanth. 10 c.c. of each solution of different concentrations was placed in different watch glasses and 2–5 segments of tape-worms previously washed with saline were placed in each. Their activity was constantly watched and when they had completely stopped showing any physical movements they were removed from the watch glasses, washed with saline and put into fresh Locke's solution to see if they could recover their original activity. Leeches and round-worms, for the tests, were dipped into the solution in test-tubes,
plugged loosely with cotton, so that they could not come out of the solution. The results so obtained in a series of experiments with different compounds were as follows:

**Table I.**

7-Hydroxyflavone, 7-hydroxy-6-hexylflavone, chrysin, genkwanin, calycopterin and 4-methylumbelliferone.

<table>
<thead>
<tr>
<th>Conc. . .</th>
<th>1-1,000</th>
<th>1-5,000</th>
<th>1-10,000</th>
<th>1-50,000</th>
<th>1-100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leeches . .</td>
<td>+, +, +</td>
<td>+, +, +, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Round-worms . .</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
</tbody>
</table>

Control in distilled water

Quite active even after 24 hours

**Table II.**

Effect of the compounds on tape-worms.

<table>
<thead>
<tr>
<th>Conc. . .</th>
<th>1-1,000</th>
<th>1-5,000</th>
<th>1-10,000</th>
<th>1-50,000</th>
<th>1-100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Hydroxyflavone . .</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>7-Hydroxy-6-hexylflavone . .</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Chrysin . .</td>
<td>3, 5, 4, 4</td>
<td>7, 8, 10, +</td>
<td>...</td>
<td>13, 15, 17, +</td>
<td>+, +, +, +</td>
</tr>
<tr>
<td>Genkwanin . .</td>
<td>5, +, +, +</td>
<td>10, 12, +, +</td>
<td>...</td>
<td>20, 25, +, +</td>
<td>+, +, +, +</td>
</tr>
<tr>
<td>Calycopterin . .</td>
<td>+, +, +, +</td>
<td>+, +, +, +</td>
<td>...</td>
<td>+, +, +, +</td>
<td>+, +, +, +</td>
</tr>
<tr>
<td>4-Methylumbelliferone . .</td>
<td>8*, 3*, +, +</td>
<td>8*, 11*, +, +</td>
<td>10*, +, +, 13*</td>
<td>15*, 15*, +, +</td>
<td>20*, +, +, 20*</td>
</tr>
</tbody>
</table>

Control in distilled water

Active for 45–60 mts.

Do.

Positive signs (+) in the above tables show that the parasites were as active as in the control experiment; figures in the respective columns show the number of minutes taken by the parasites to die in the particular solution; figures marked(*) show that the parasites stopped their activity during that time and recovered again within five minutes when placed in fresh Locke’s solution.

The results given above in Tables I and II were obtained when the solutions of the compounds were prepared in water but when the solutions were prepared in saline or Locke’s solution the parasites were as active in the test solutions as in the control experiments.

Method (B).—The action of the drugs was studied on tape-worms in Dale and Laidlaw’s apparatus. In a number of experiments that were carried out most inconsistent results were obtained. It was observed that in some experiments the inhibitory action of chrysin solution of 1–20,000
dilution was slower than that of 1–40,000 and the worms also regained their original movements when fresh Locke's solution was replaced in the bath where the worm was kept (Plate XII, Figs. 2 and 4); in others chrysin solution of 1–20,000 dilution on the contrary activated the worm and 1–5,000 was necessary to stop the movements (Plate XII, Fig. 3), while in still others even 1–5,000 concentration of the drug produced only a slight contraction of the worm (Plate XII, Fig. 1).

Similarly, genkwanin, 7-hydroxyflavone, 6-hexyl-7-hydroxyflavone and 4-methylumbelliferone were also tried but showed no effect on the movements of the worms.

**Effect of 7-hydroxyflavone, genkwanin and 4-methylumbelliferone on rabbit's isolated gut and uterine movements.**—Dale and Laidlaw's apparatus was used to study the action of these drugs on the intestinal and uterine movements. The rabbit was killed by cutting its throat and allowing it to bleed to death. The small intestines were removed quickly, the intestinal contents washed with tap water and the intestine cut in small bits about 3–4 cm. long. The segments were immersed in Locke's solution and kept in the refrigerator when not in use. One of the segments was fixed in the inner bath of the apparatus with one of its end tied to the air inlet tube and the other to the recording lever by means of fine threads. Solutions of known amounts of each of the substance were then added at a time to the bath containing the segment. The effect of 7-hydroxyflavone and genkwanin on rabbit's gut and of 4-methylumbelliferone is seen in Plate XII, Figs. 5, 6 and 7. The substances stopped the gut and uterine movements in concentrations of 1–140,000, while lesser concentrations produced correspondingly less effects on their movements.

**Effect of 7-hydroxyflavone and genkwanin on blood pressure and uterine movements of a dog.**—A female pregnant dog (wt. 10 kg.) was anaesthetised with morphia-urethane. The left carotid artery was connected to a mercury manometer through a cannula to record the blood pressure and the right femoral vein was attached to a burette containing normal saline through another cannula. At the same time abdomen of the dog was opened and its uterus was connected by a fine thread to a lever arrangement to record its movements side by side with the blood pressure experiment. Then a concentrated solution of genkwanin dissolved in minimum quantity of 10 per cent. aqueous caustic soda was injected into the animal through the right femoral vein. It was noticed that 100 mg. of the drug clearly produced depression in the blood pressure and contraction in the uterus (Plate XIV, Fig. 8) and the effect was proportional to the amount of the drug injected. Similar results were recorded with 7-hydroxyflavone (Plate XIV, Fig. 9).
Effect of chrysin, genkwanin and 4-methylumbelliferone on blood pressure and respiration of a dog.—The dog was prepared as in the previous experiment but the trachea in this case was connected to a tambour through a cannula to record the movements of respiration. Different amounts of the drugs were injected at a time and their effect recorded. It was found that 200 mg. of each chrysin and 4-methylumbelliferone produced depression in the blood pressure and increased the movements of respiration (Plate XV, Figs. 10 and 12) but in the latter case certain amount of depression in the respiration was also noticed which soon recovered to normal; while the injection of 100 mg. of genkwanin raised the blood pressure for a very short time, again coming to its normal, and an increase in the respiration movements was clearly noticeable (Plate XV, Fig. 11).

Effect of 7-hydroxyflavone, chrysin and genkwanin on the beats of an isolated frog’s heart.—A frog was pithed and its chest exposed; two cannulae were next inserted one in the aorta and the other in the inferior vena cava. All the other blood vessels leading from, and coming to, the heart were ligatured and the heart isolated from the body. A measured quantity of Ringer’s solution was then put into the cannula in the inferior vena cava and the aorta cannula was led into the former by means of a small piece of rubber tubing. The whole preparation was fixed on a stand in position, a pinch clip was attached to the apex of the heart and was tied to a heart lever by means of a fine thread and the heart beats were thus recorded on a smoked paper. The Ringer’s solution from the circulation was pipetted out as far as possible and the solutions of the substances of different concentrations in Ringer’s solution were then added at a time instead. It was observed that 7-hydroxyflavone, chrysin and genkwanin in concentrations 1–200,000, 1–100,000 and 1–500,000 respectively inhibited the action of the heart (Plate XVI, Figs. 13, 14 and 15). A control experiment was also carried out with the maximum amount of caustic soda solution used to dissolve the substance in the above experiments (Plate XVI, Fig. 16) and showed practically no effect on the beats of the heart.

Antiseptic properties of chrysin, genkwanin and 4-methylumbelliferone.—To three different sets of 4 test-tubes each, containing broth culture, was added chrysin, genkwanin and 4-methylumbelliferone so that each one of these compounds had a concentration of 1–1,000, 1–10,000, 1–100,000 and 1–1,000,000 in the four different test-tubes of each set. The tubes were inoculated with a few days’ old B. coli culture and incubated at 37°C. for 48 hours; a control was carried out along with it by inoculating B. coli to pure broth culture. It was found that chrysin and genkwanin had no action on the bacilli, the growth in all the tubes being as profuse as in the control
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In the case of 4-methylumbelliferone, however, there was no growth in 1-1,000 concentration and a slight inhibition of growth in 1-10,000 concentration.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>1-1,000</th>
<th>1-10,000</th>
<th>1-100,000</th>
<th>1-1,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Genkwanin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4-Methylumbelliferone</td>
<td>−−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = growth; − = no growth; † = partial growth.

A control experiment was also carried side by side with these experiments and showed profuse growth of the bacilli.

Germicidal properties of chrysin, genkwanin and 4-methylumbelliferone.—To determine the germicidal properties of the compounds, their Rideal Walker’s phenol coefficient was determined in the usual manner. The test was carried out at 18°C. with about 24 hours’ old broth culture of B. typhosus; the results thus obtained are tabulated below:

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>3</th>
<th>5½</th>
<th>8</th>
<th>10½</th>
<th>13</th>
<th>15½</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>1-105</td>
<td>+</td>
<td>†</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Chrysin</td>
<td>1-105</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Genkwanin</td>
<td>1-105</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4-Methylumbelliferone</td>
<td>1-105</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4-Methylumbelliferone</td>
<td>1-525</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Control = profuse growth of the bacilli.
+ = profuse growth as in the control.
† = partial growth.
− = no growth.

It was therefore evident from the above results that the phenol coefficient of the compounds is less than one.

Summary.

The anthelminthic action of certain flavones has been examined in vitro, negative results being obtained. The compounds do not possess any antiseptic or germicidal properties; they inhibit the movements of isolated
gut and uterus; intravenous injection of these compounds in dogs causes depression of the blood pressure, contraction of the uterus and increases the movements of respiration; they inhibit the beats of an isolated frog's heart.

The author wishes to thank Dr. B. B. Dikshit for his guidance in carrying out the present investigation and Dr. K. Venkataraman, Dr. T. S. Wheeler and Lieut.-Col. S. S. Sokhey for their keen interest in the work. The author also wishes to thank the Trustees of the Lady Tata Memorial Fund for the award of a Scholarship to enable him to carry out this investigation.
Note the effect of 1-5,000 genkwanin and of chrysin on the movements of tape-worm Fig. 1, of 1-20,000 chrysin Fig. 2, of 1-20,000 and of 1-5,000 chrysin Fig. 3 and of 1-40,000 chrysin Fig. 4.
Note the complete inhibition of the movements of an isolated rabbit's gut caused by 1-140,000 chrysin Fig. 5 and 1-140,000 genkwanin Fig. 6; the effect of 1-14,000 4-methylumbelliferone on the movements of an isolated rabbit's uterus is seen in Fig. 7.
Fig. 8.

♀ Pregnant dog, 10 kg., urethane-morphia anaesthesia. Tracings from above downwards—Uterine movements, Blood pressure, line marker at 10 seconds interval and base line. Note the effect of intravenous injection of 100 mg. of genkwanin Fig. 8 and of 100 mg. of 7-hydroxyflavone Fig. 9 on the uterine movements and blood pressure.
♂ Dog, 15 kg., urethane-morphia anesthesia. Tracings from above downwards—Respiratory movements, Blood pressure, line marker at 10 seconds interval and base line. Note the effect of the intravenous injection of 200 mg. of chrysin Fig. 10, of 100 mg. of genkwain Fig. 11 and of 200 mg. of 4-methylumbelliferone Fig. 12 on the respiratory movements and blood pressure.
Mark the effect of 1-200,000 7-hydroxyflavone Fig. 13, 1-100,000 chrysin Fig. 14, and of 1-500,000 genkwanin Fig. 15 on isolated frog's heart beat; Fig. 16 shows the effect of 1-1,000,000 NaOH on isolated frog's heart beat.