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Preparation and preliminary pharmacological assay of a water soluble derivative of rutin

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PREPARATION

and

PRELIMINARY PHARMACOLOGICAL ASSAY

of a

WATER SOLUBLE DERIVATIVE OF RUTIN

by

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Gratitude is extended to Parke Davis and Co. and to the Eastern Regional Laboratory for providing a sample of rutin.

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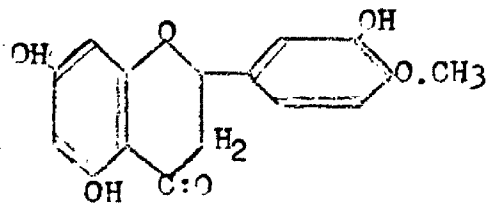
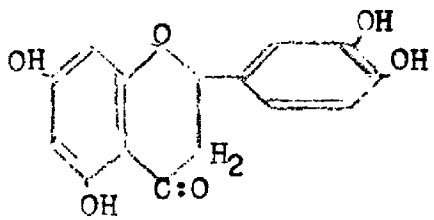
INTRODUCTION

Extensive work has been done on many substances which possess the property of increasing capillary resistance. It is known with certainty that the structure of this substance or substances usually designated as vitamin P is related to the hydroxy-flavones.

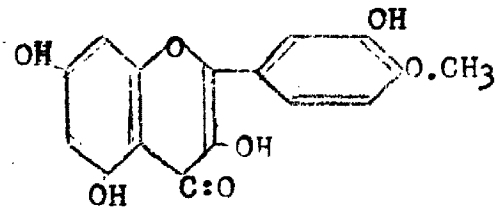
The important work done in this field by Javiller and Lavollay[1] brings out new ideas pertaining to this problem so that their conclusions are worthy of summarization. With the use of the suction cup method, these workers have tested many different substances for their vitamin P properties. Of the hydroxy-flavones or flavanones, hesperetol or its glycoside as well as eriodictyol and quercetol were shown to be active. The structurally related cyanidols and their glycosides as well as one of the catechins were likewise found to possess activity.



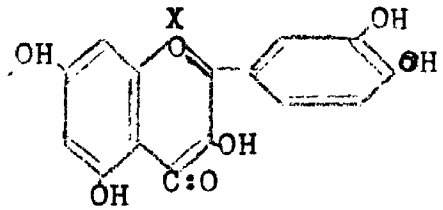
Hesperetol



Friodictyol



Quercetol



Cyanidol

The action of the catechins is interesting. Catechin exists in two epimeric forms of which only the d-epicatechin is active. Its activity was found to be such that 0.01 milligram injected into guinea pigs increases the capillary resistance from 17 to 35 cm of mercury.

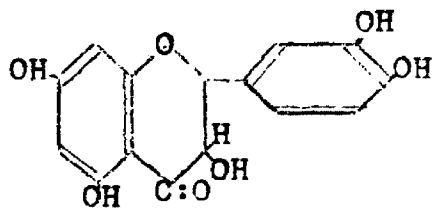
In another experiment Javillier and Lavollay found that the glycoside phlorizin and its aglycone phloretol are highly active. The action of phloretol is interesting because the structure of this product may be regarded as a flavanone, whose heterocycle can be opened by hydrogenation.

Another product, esculetol (6:7-dihydroxycoumarin) and its glycoside esculoside (aesculin) were found to possess strong activity.

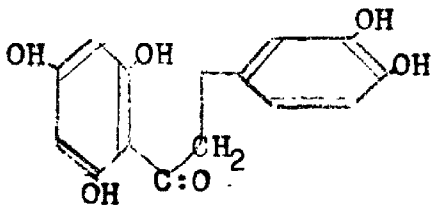
In attempting to correlate structure and activity Javillier and Lavollay raise the following questions:

(1) In the group of active products with a flavonic structure, does the opening of the heterocycle influence the activity?

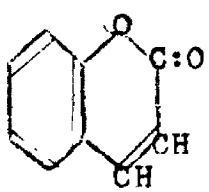
(2) During the epimerization of the inactive d-catechin, is the transformation into an active substance due to the opening of the oxygenated ring, leading to a product of chalcone structure?



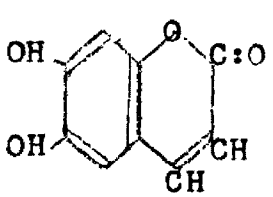
Catechin



Phloretol

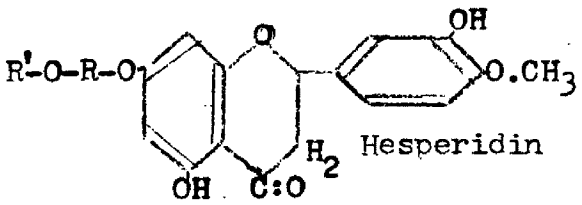


Coumarin

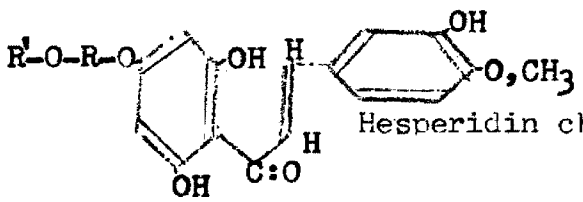


Esculetol

Higby[2] reports that pure hesperidin is inactive and according to him this should be due to its insolubility. The transformation of the glycoside into its chalcone yields a water soluble and active product.



Hesperidin

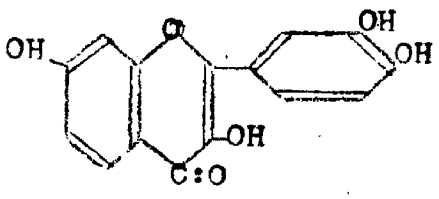


Hesperidin chalcone

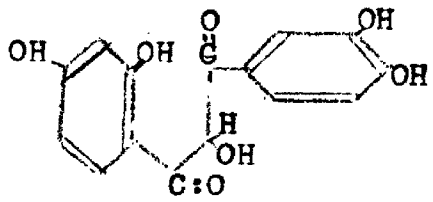
Rutin has been found to have vitamin P properties but according to clinical reports it is often necessary to increase the usual dosage before any therapeutic action can be observed. This may be due to the fact that rutin like hesperidin is insoluble in water. It is a known fact that both of these compounds are soluble in alkaline solution and given orally it may be possible that they are slightly absorbed within the intestinal tract because of the slight alkalinity of its content. This may be also the reason why some workers have found pure hesperidin to be active.

CHEMISTRY

The transformation of hesperidin into its chalcone seems to be the answer to the problem of its insolubility. In this transformation the change of structure consists of the shifting of a hydrogen from position three to position one to form a hydroxyl accompanied by fission of the heterocyclic ring. In rutin, however, due to the unsaturation between carbons 2 and 3 transformation should be different. Wilson[3] in his study of the boric acid color reaction assigns the following structures to fisetin and alkali treated fisetin.

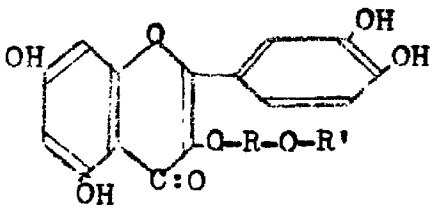


Fisetin

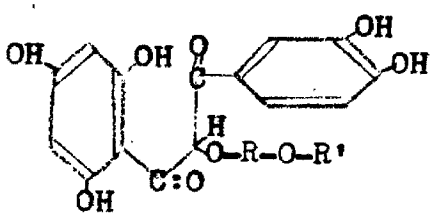


Alkali treated fisetin

Considering the similarity of structure of fisetin and rutin, it seems reasonable to assume that a compound of similar structure should be formed from rutin by alkali treatment.



Rutin



Alkali treated rutin ?

EXPERIMENTAL

10 gm of rutin were dissolved in 250 cc of methanol with the aid of gentle heat. This solution was mixed and allowed to stand ten minutes with a solution of 10 gm of potassium hydroxide in 50 cc of methanol. During this operation the color of the mixture changed to a yellowish red. 200 cc of purified ether were then added, resulting in the formation of an abundant yellow precipitate. On centrifugation the liquid layer was removed and discarded. The precipitate was suspended in ether and centrifugated again, this procedure being repeated three times. After removing the last liquid layer the remaining ether was evaporated and the product dried in vacuum at

low temperature (30°C). The compound was further purified by redissolving in methanol, reprecipitation with ether and drying in vacuum at low temperature as previously.

The final product was a fine yellow light powder which upon examination under a microscope was found to be devoid of crystalloidal structure. The compound was freely soluble in water and decomposed between 214-216° C without yielding a definite melting point.

In order to determine the close relationship of the new compound to that of rutin, the boric acid color test was performed according to the technique of Wilson[3]. The reaction was positive as it is for rutin. Furthermore, the precipitate obtained by slightly acidifying an aqueous solution of the product showed on microscope examination the typical crystalloidal structure of rutin.

PHARMACOLOGY

Experimental studies of vitamin P related products have been handicapped by the lack of an accurate method for measuring the effects produced on capillar fragility. However, three different methods have been reported.

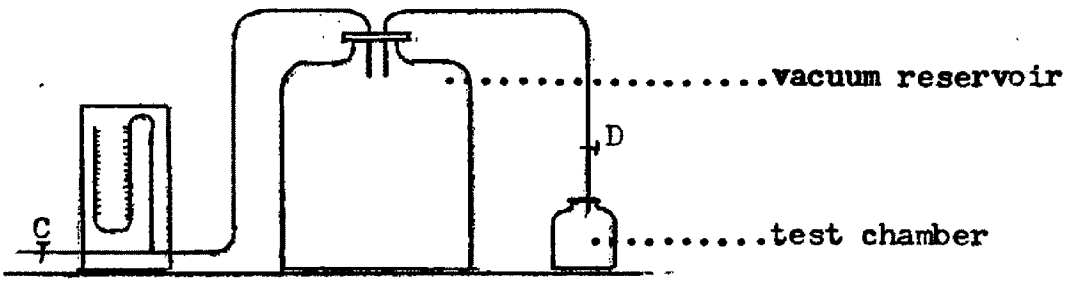
The first one, fully described by A. M. Ambrose and F. De Eds[4] is based on the fact that capillary whose resistance is decreased allow colloidal dyes to pass through. Blue trypan is injected in the circulatory system of rabbits, chloroform is applied on the shaved skin which produces irritation. If there is no capillary resistance a blue color appears in the chloroform treated patch. In case of increased capillary resistance no color appears or appears later.

The second method, described by A. L. Bacharach[5] uses a suction cup applied on the shaved skin of guinea pigs. The strength of the "negative pressure" required to produce the appearance of petechiae gives a measure of the capillary fragility.

The third method is described by G. J. Majovski and his collaborators[6]. In this method, mice are subjected to a sudden reduction of atmospheric pressure. The extent of the lung hemorrhage is observed in control and test mice. Due to its simplicity and the apparent good results obtained with it, this method was used here.

EXPERIMENTAL

The apparatus used is described below.

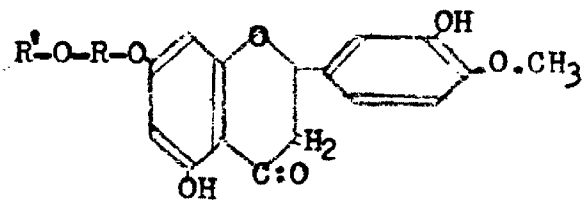


White mice of approximately 20 grams were used. Mice of the same sex and weight, fed at the same time were used as control and test. Several assays showed that the minimum "negative pressure" required to produce lung hemorrhage was 34-35 mm of mercury, this pressure rose to 65 mm when the stopcock C was closed and the stopcock D opened.

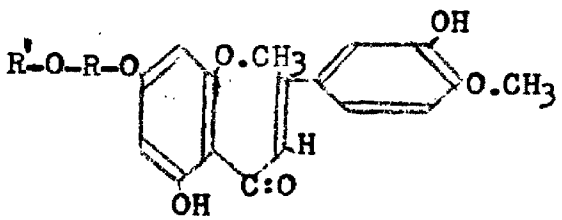
The rutin derivative in an aqueous 2.5% solution was injected parenterally at a dose of one mg per gram of weight. The pH of the solution was 7.2. The control mice received the same volume of water. After a pressure of 35 mm of mercury was reached in the vacuum reservoir the stopcock C was closed. Both mice were placed in the test chamber, the connection was made

by opening the stopcock D for one minute the mice remained in the test chamber one minute longer. They were then removed, the thorax was opened and the extent of the pulmonary hemorrhage was observed and rated from 0 to ÷ ÷ ÷ ÷ .

In every case the urine presented a dark yellow color which showed that absorption took place. However, in several cases a yellow precipitate was present in the peritoneal cavity. This yellow precipitate had the same appearance and the same crystalloidal structure as one obtained by slight acidification of an aqueous solution of the rutin derivative. This reversibility seems to be the same problem that Higby found with hesperidin chalcone, in the Higby work this problem was solved by methylation of the nascent OH during the chalconization.



Hesperidin



Hesperidin methyl chalcone

All the results obtained with the rutin derivative are presented in Table I. In Table II are presented the results obtained with hesperidine methyl chalcone injected at the same dose in an aqueous 2% solution.

The results presented here are very irregular. In some instances the responses were similar to those obtained by Majovski and conformed to the color photography presented in his report, but in other cases hemorrhage was found in both control and test mice and sometimes only a small hemorrhage occurred in the control mice.

In his report the same author presents results apparently favorable

TABLE I

RESULTS OBTAINED BY INTRAPERITONEAL INJECTION OF SOLUBLE RUTIN DERIVATIVE

2.5% aqueous solution - 1 mg per gram of weight

Test No.	Weight	Vol. Inj.	Test	Control
1	21 gm	.84 cc	+	+ + + +
2	25	1.	+ + +	+ + +
3	23	.92	+ +	+ + +
4	23	.92	+ +	+ + +
5	23	.92	+ + +	+ + +
6	23	.92	+ +	+ + +
7	17	.68	+ + +	+ +
8	22	.88	+ +	+ + +
9	18	.72	+	+ + +
10	19	.76	+	+ +
11	15	.60	+	+
12	24	.96	+ +	+ +
13	18	.72	+ +	+ + +
14	17	.68	+	+ + +
15	21	.84	+ +	+ +
16	23	.92	+ +	+
17	22	.88	+ + +	+ + + +
18	19	.76	+	+ + +
19	20	.80	+ +	+ +
20	19	.76	+ +	+ + +

TABLE II

RESULTS OBTAINED BY INTRAPERITONEAL INJECTION OF HESPERIDINE METHYL CHALCONE

2% aqueous solution - 1 mg per gram of weight

Test No.	Weight	Vol. Inj.	Test	Control
1	18 gm	.9 cc	÷ ÷	÷ ÷ ÷ ÷
2	23	1.15	÷ ÷ ÷	÷ ÷ ÷
3	19	.95	÷ ÷	÷ ÷ ÷
4	19	1.1	÷ ÷	÷ ÷
5	22	.95	÷	÷ ÷ ÷
6	19	.95	÷ ÷	÷ ÷
7	19	.95	÷ ÷ ÷	÷ ÷ ÷
8	19	.95	÷ ÷	÷ ÷ ÷
9	20	1.	÷ ÷	÷ ÷ ÷
10	18	.9	÷ ÷ ÷	÷ ÷ ÷
11	18	.9	÷ ÷ ÷	÷ ÷

but in his discussion he mentions that there have been occasional mice which failed to exhibit pulmonary hemorrhage and sometimes axillary or other hemorrhage occurred, in such mice the pulmonary hemorrhage was slight but these animals were discarded from the experiments. It would be interesting to know how many such cases were encountered in those experiments. It may be concluded that this method leaves too great an opportunity for personal interpretation.

As for the rutin derivative itself, this preliminary study does not lead to definite results, it is probable that by intraperitoneal injection only a small part of the product is absorbed. It is possible that by the oral route the absorption may be greater.

CONCLUSIONS

In many reports, water insolubility of rutin has been mentioned to be an important obstacle to its experimental study and clinical use. A water soluble derivative has been prepared here. A first assay has not shown definite results. However, the same assay conducted with hesperidin methyl chalcone did not give better results and another method should be used for further assay.

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