Miklós Gábor

Albert Szent-Györgyi's Studies on Flavones. Impact of the Discovery

In 1936 Albert Szent-Györgyi and his colleagues published several papers on the influence of substances of the flavone group on the permeability of capillaries. The dates are also interesting. The first, 40-line article was published in English, signed by Rusznyák and Szent-Györgyi (27 May, 1936), the second one was published in German (14 August, 1936), and the third one in Hungarian (3 October, 1936). The latter 2 articles listed 5 authors: Armentano, Bentsáth, Béres, Rusznyák and Szent-Györgyi (Fig. 1–5).

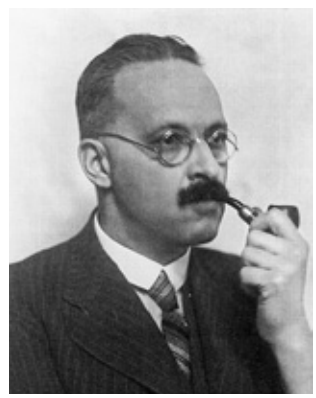

Fig. 1. István Rusznyák

Fig. 2. Albert Szent-Györgyi

Vitamin P: Flavonols as Vitamins

Various chemical and clinical observations have led to the assumption that ascorbic acid is accompanied in the cell by a substance of similar importance and related activity. In absence of both substances, the symptoms of lack of ascorbic acid (scurvy) prevail and conceal symptoms of the deficiency of the second substance. In the lack of suitable experimental animals or conditions, progress was dependent on spontaneous pathological conditions, caused or influenced by this second factor.

In collaboration with L. Armentano and A. Bentsáth, we have found that in certain pathological conditions, characterised by an increased permeability or fragility of the capillary wall, ascorbic acid is ineffective, while the condition can readily be cured by the administration of extracts of Hungarian red pepper ('vitapric') or lemon juice. The extracts were effective in cases of decreased resistance of the capillary wall toward whole blood (vascular type of hæmorrhagic purpura) as well as in cases in which the capillary wall showed an increased permeability towards plasma protein only (various septic conditions). The extracts were fractionated. The active substance was found in the end in a fraction consisting of practically pure flavon or flavonol glycoside. 40 mgm. of this fraction given daily intravenously to man restored in a fortnight regularly the normal capillary resistance. Spontaneous bleeding ceased, the capillary walls lost their fragility towards pressure differences and no more plasma protein left the vascular system on increased venous pressure.

These results suggest that this great group of vegetable dyes, the flavons or flavonols, also play an important role in animal life, and that the dyes are of vitamin nature. The group is not to be confused with the yellow dye, discovered by one of us and termed 'flaves' (like cytoflave), which dye forms the prosthetic group of Warburg's yellow enzyme and has later been renamed by R. Kuhn 'flavins'. We propose to give the name 'vitamin P' to the substance responsible for the action on vascular permeability.

This research is sponsored by the Josiah Macy Jr. Foundation, New York.

St. Rusznyák.
A. Szent-Györgyi.

1. Medical Clinic and
Biochemical Department,
University, Szeged.
May 27.

Fig. 3. Vitamin P: flavonols as vitamins (Rusznyák and Szent-Györgyi: Nature. 1938, 27 (1936).

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MEDIZINISCHE WOCHENSCHRIFT

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NUMMER 33 FREITAG, DEN 14. AUGUST 1936 62. JAHRGANG

Aus der Medizinischen Klinik (Direktor: Prof. St. Rusznyák) und aus dem Institut für Medizinische und Organische Chemie (Direktor: Prof. A. Szent-Györgyi) der Kgl. Ung. Franz-Joseph-Universität in Szeged

Über den Einfluß von Substanzen der Flavongruppe auf die Permeabilität der Kapillaren. Vitamin P[1]

Von L. ARMENTANO, A. BENTSÁTH, T. BÉRES, ST. RUSZNYÁK und A. SZENT-GYÖRGYI

Es war die Analyse pflanzlicher Oxydationssysteme, namentlich des Peroxydasesystems, die zur Entdeckung der Ascorbinsäure führte. Bereits in den frühesten Tagen der Isolierung dieser Säure suchte einer von uns nach der Substanz, die die Ascorbinsäure mit der Peroxydase zu einem gekuppelten Oxydationssystem verbindet.

Der Zitronensaft wurde durch Zugabe von 15% Bariumazetat dezitriert. Die abgetrennte Flüssigkeit wurde mit 2% Bleiazetat versetzt, der inaktive Niederschlag entfernt. Nun wurde mit Ammoniak alkalisiert, bis Bromthymolblau eine bläulich-lila Farbe gab. Das Präzipitat wurde in Wasser durch Salzsäure vom Blei befreit. Die wäßrige Lösung wurde eingeengt und durch Zugabe von Alkohol und Azeton von inaktivem

Fig. 4. Deutsche Medizinische Wochenschrift (1936) *(Armentano et al.)*

80. évfolyam. 40. szám. Budapest, 1936. október 3.

ORVOSI HETILAP

Alapította MARKUSOVSZKY LAJOS 1857-ben.

Folytatták:

ANTAL GÉZA, HÖGYES ENDRE, LENHOSSÉK MIHÁLY, SZÉKELY ÁGOSTON

Szerkesztőbizottság:

HERZOG FERENC, VÁMOSSY ZOLTÁN, ISSEKUTZ BÉLA, VIDAKOVITS KAMILLÓ, GORKA SANDOR, REUTER KAMILLÓ, HÜTTL TIVADAR, JENEY ENDRE

FELELŐS SZERKESZTŐ: VÁMOSSY ZOLTÁN EGYETEMI TANÁR SEGÉDSZERKESZTŐ: FRITZ ERNŐ

EREDETI KÖZLEMÉNYEK

A Ferenc József-Tudományegyetem Belgyógyászati Klinikájának, (Igazgató: Rusznyák István ny. r. tanár) és Orvosi Vegytani Intézetének (Igazgató: Szent-Györgyi Albert ny. r. tanár.) közleménye.

A flavoncsoportba tartozó anyagok befolyása a capillarisok permeabilitására. Vitamin P.*)

Irták: *Armentano Lajos dr., Bentsáth Aladár dr., Béres Tibor dr., Rusznyák István dr. és Szent-Györgyi Albert dr.*

mölcsben az ascorbinsavval együtt van jelen. Ennek az anyagnak további analyzise a következőképen történt:

A ciiromlét 15%-os bariumacetáttal decitráltuk. A különválasztott folyadékhoz 2%-os ólomacetátot adtunk, az inactiv üledéket eltávolítottuk. Ezután ammoniákkal alkalizáltuk, amíg bromthymolkékkel kékes-lila elszíneződést nem adott. A csapadékból az ólmot vízben sósavval távolítottuk el. A vizes oldatot bepároltuk, alkohol és aceton hozzáadásával a többi inactiv anyagoktól megtisztítottuk. Az alkohol és acetonban oldódó fractiót vízben oldottuk fel és alkalmaztuk a klinikai kísérletekre. (1. sz. készítmény).

Fig. 5. Orvosi Hetilap, 1936 *(Armentano et al.).*

Armentano (1936) achieved good therapeutic results in haemorrhagic diathesis with the vitamin C-rich paprika preparation vitapric. However, his group could not reach the same results when using pure vitamin C. Therefore, they suggested that the aforementioned beneficial effect should not be attributed to vitamin C but rather to another substance, which is present in the fruit together with ascorbic acid (Armentano *et al*, 1936).

Interestingly they used lemon instead of paprika to clarify the situation. It is worth mentioning that 200 kilos of lemon was processed and the resulting 70 litres of lemon juice was enough to produce only 2 g of a pure crystalline substance they called 'citrin' (citrus flavone).

Investigati on of the therapeutic effect

The therapeutic effect of citrin was examined in 17 patients, merely 3 of whom were suffering from vascular purpura and 4 from thrombocytopenic purpura. Since bleeding in such patients depends on unpredictable factors and acute thrombocytopenia can resolve spontaneously, chronic cases were examined to prevent flaws, and the course of the disease was followed with exact measurements (number of thrombocytes, capillary resistance). For the latter measurement, the method of Borbély was used, where negative pressure is exerted on the skin of the supraclavicular fossa until the first spot-like haemorrhage (petechia) develops. In addition, the Landis-Michel method was used to examine capillary permeability to plasma and proteins.

It is worth mentioning that the best results were achieved in vascular purpura and the therapeutic effect of citrin was best demonstrated in the above mentioned 3 patients (Armentano *et al*. 1936) (Fig. 6).

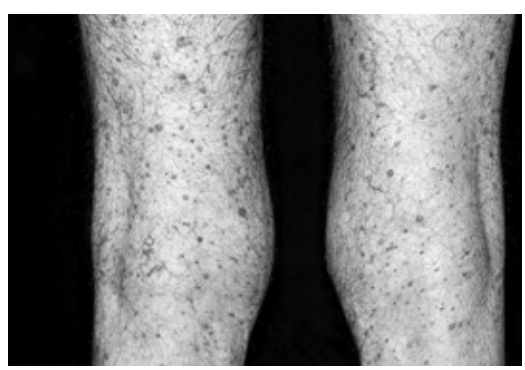


Fig. 6. Vascular purpura

It is also interesting that the findings of a study on only 3 patients drew attention to research on flavonoids worldwide. I should mention that citrin therapy was not found to be beneficial in thrombocytopenic purpura; however, its ineffectiveness was not explained.

The patients received daily intravenous flavone injections (equivalent to 20–40 mg of dry matter) and some days later, usually on the day 8–12, the effect on capillaries was checked. 75 years later, it is know that scorbutogenic guinea pigs were also given flavones, and they outlived the control subjects. It seems likely that these experiments preceded the studies on haemorrhagic patients, which would explain why flavone was administered intravenously to the human patients.

Attentive readers may note Szent-Györgyi's question as to whether the capillary effect described in connection with citrin is a characteristic feature of this substance only, or the other members of the flavone group have similar properties.

Informative experiments with quercitrin and rhamnetin yielded no results. However, Armentano *et al.* (1936) found it surprising that, while all the treated patients tolerated citrin injections well with no unpleasant side-effects, quercitrin and rhamnetin injections led to high fever and collapse in some cases; the treatment therefore had to be stopped early.

Szent-Györgyi and his colleagues concluded that, besides vitamin C, there has to be another capillary-active vitamin in plants (lemon and paprika). They succelded is isolating a substance (citrin) from lemon juice, which turned out to be a flavonoid glycoside. This substance was effective in the treatment of vascular purpura, while it had no beneficial effect in thrombocytopenic purpura. Citrin can also inhibit protein permeability in diseases where the capillaries are more permeable (serous inflammation). This substance was therefore called vitamin P (permeability).

In his foreword to my book on the anti-inflammatory properties of flavonoids, Szent-Györgyi states that he chose a letter on the far unoccupied side of the letters ABC' already being used to designate vitamins in case the bioflavonoids were found not to be true vitamins and correction could be made without confusion (1972).

At the end of 1936, Albert Szent-Györgyi published a paper entitled 'From Vitamin C to Vitamin P', In which he stated that 'If the vitamin character of this substance be firmly established, this will also mean that the great group of vegetable dyes, the flavones, which seem to play such an important role in plant biochemistry, also function in the animal organism.'

The chemical composition of citrin

Szent-Györgyi chose to determine the chemical properties of citrin as his next task. Fortunately, Győző Bruckner, one of the most excellent chemists and scientists of the 20th century, was working in his institute at that time (Fig. 7). According to a legend which survives to the present day, Szent-Györgyi also invited the world-famous professor of chemistry, Géza Zemplén from Budapest to help solve the problem. The discussions and the accompanying feast lasted all day. It was well known that Géza Zemplén needed alcoholic stimulation to set to work. In the evening, Győző Bruckner escorted Géza Zemplén to the railway station, then immediately returned to the laboratory and the same night discovered that citrin is composed of two flavone compounds: hesperidin and eriodictyol glycoside (Fig.s 8 and 9). In the morning Professor Zemplén was sent a telegram to inform him of the result. Bruckner and Szent-Györgyi published the findings of their investigation in Nature (Fig. 10).

The above-mentioned 'legend' is confirmed by a letter I received from Professor Bruckner (1977) (Fig. 11).

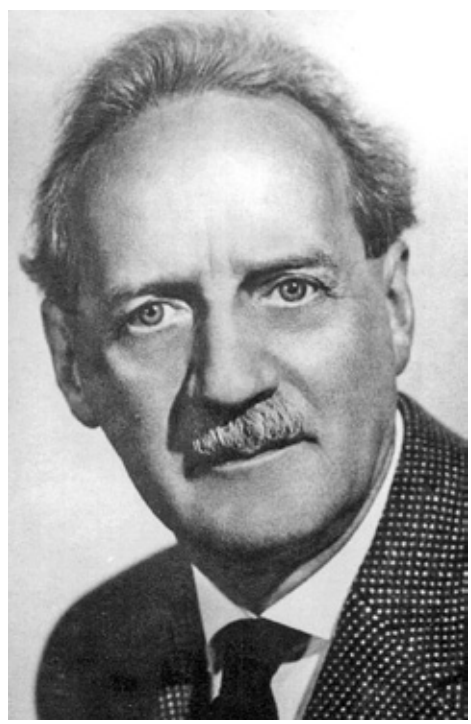


Fig. 7. Prof. Győző Bruckner

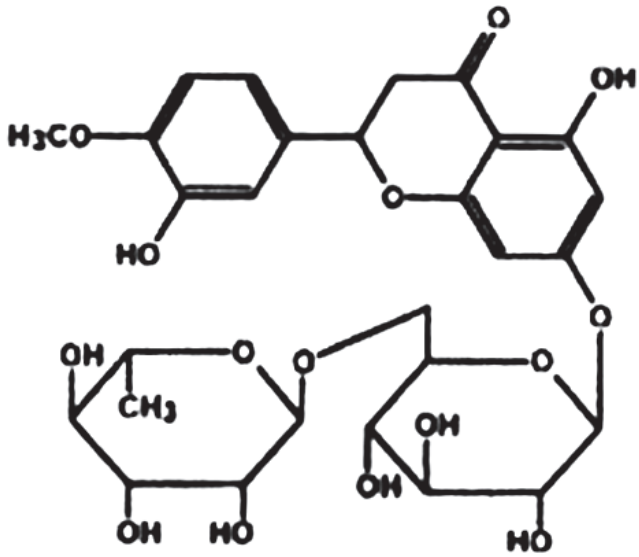


Fig. 8. Hesperidin

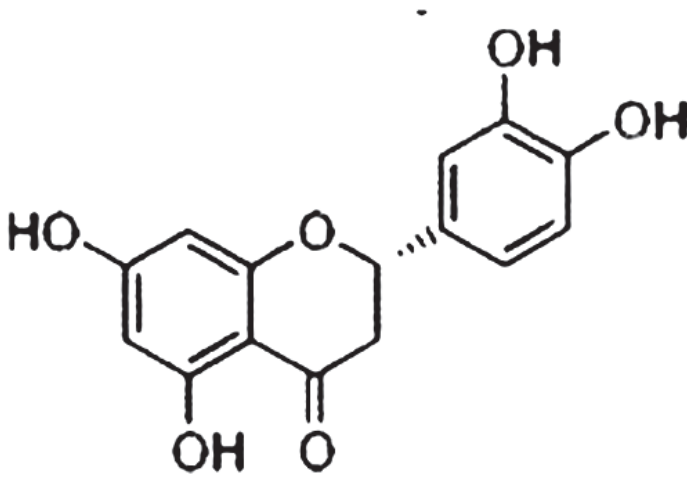


Fig. 9. Eriodictyol

Chemical Nature of Citrin

In a previous note (Rusznyák *et al.*) one of us reported on the isolation and physiological activity (vitamin nature) of the crystalline flavone fraction of lemon juice. The substance, being different from other known flavones, was termed 'citrin'.

Further work has shown citrin to consist of mixed crystals of two different dyes, one being hesperidine (m.p. 261°), the other an eriodictyol glucoside. Hesperidine forms the major part of citrin. The great reactivity and the colour reactions of citrin are due to the eriodictyol glucoside. Citrin contains no free eriodictyol. This substance can be isolated only after complete hydrolysis.

According to its formula, eriodictyol is but a demethylated hesperetine. This makes it probable that both glucosides constituting citrin are but two forms of the same flavanone glucoside. Eriodictyol glucoside was not found in any considerable quantity in unripe oranges, which, however, contain great quantities of hesperidine. This makes it probable that the eriodictyol glucoside is formed from hesperidine by demethylation on ripening of the fruit.

This research is being sponsored by the Josiah Macy Jr. Foundation, New York.

V. Bruckner.
A. Szent-Györgyi.

Inst. Org. and Med. Chem.,
Szeged.
Nov. 21.

Fig. 10. Chemical nature of citrin
(Bruckner and Szent-Györgyi: Nature, 1936).

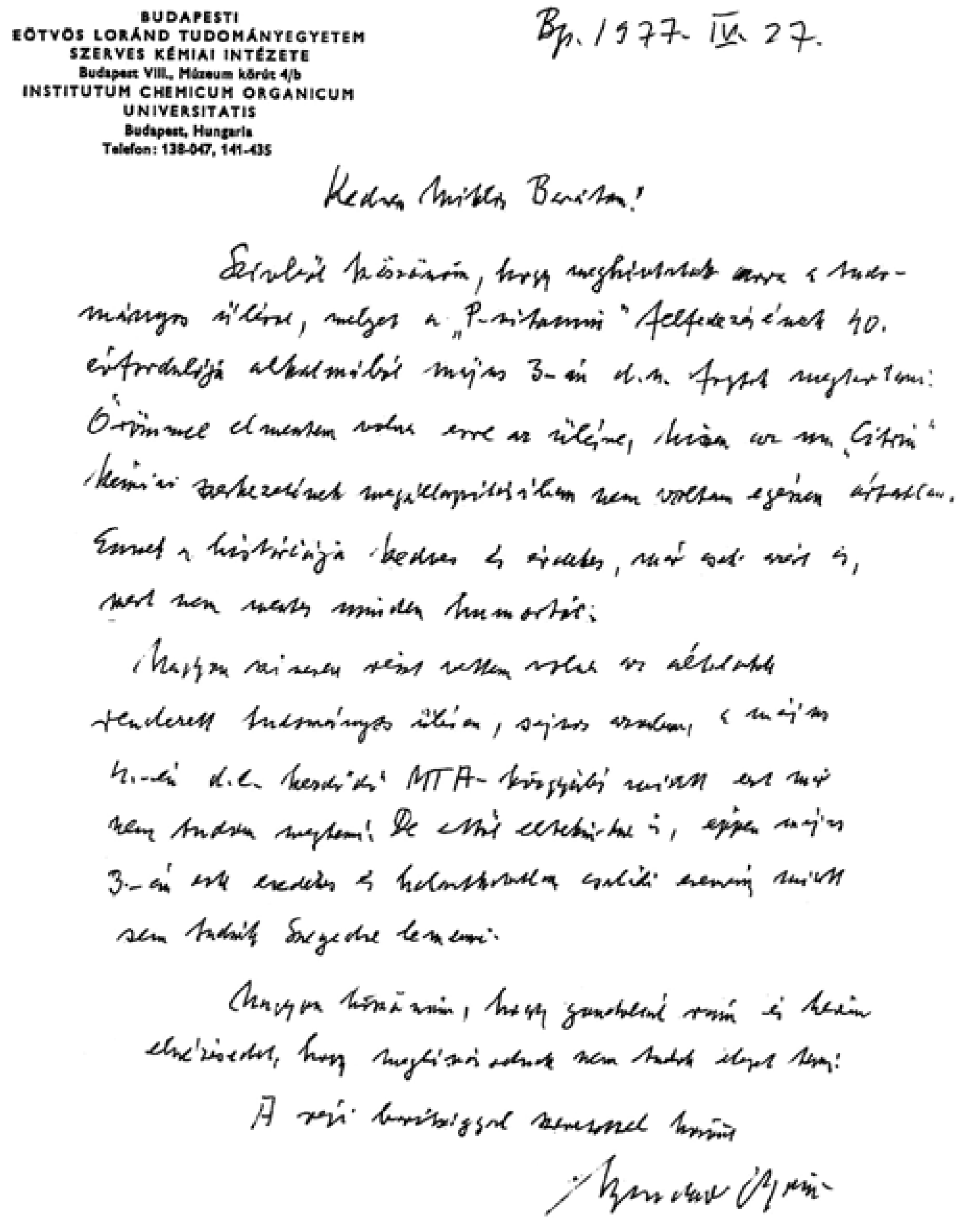


Fig. 11. Letter of Győző Bruckner (1977).

The effect of citrin on experimentally induced scurvy

The vitamin-like properties of flavones were widely researched by Szent-Györgyi and his colleagues (Bentsáth *et al*., 1936), work which was later criticized by many scientists. Guinea pigs were kept on the Sherman, LaMer, Campbell vitamin C-free diet (1922). One group, the untreated controls, died in an average of 28.5 days. The group given one milligram of citrin daily for 6 weeks lived an average of 44 days. All of the animals in both groups showed the typical symptoms of scurvy. Szent-Györgyi and his colleagues concluded 'that these results suggest that experimental scurvy, as commonly known, is a deficiency disease caused by the combined lack of vitamins C and P.'

In another experiment, Bentsáth, Rusznyák and Szent-Györgyi (1937) examined the vitamin P-like effects of hesperidin, demethylated hesperidin and quercitrin in guinea pigs. Animals that also received ascorbic acid showed normal growth patterns. Animals treated with hesperidin and demethylated hesperidin exhibited a similar response to those treated with citrin (Bentsáth *et al*., 1937). Animals that were fed a basic diet or basic diet+quercitrin died of severe scurvy around day 28.

Bentsáth and Das (1937) did not obtain the same results when they repeated their experiments. They concluded that the winter food of the animals was significantly different from their summer food and that studied animals had to be in a good physical condition for the vitamin P test and they could not lack any vital factors. In their opinion, if any vital factor was missing, citrin could not prolong the lifespan of the studied animals.

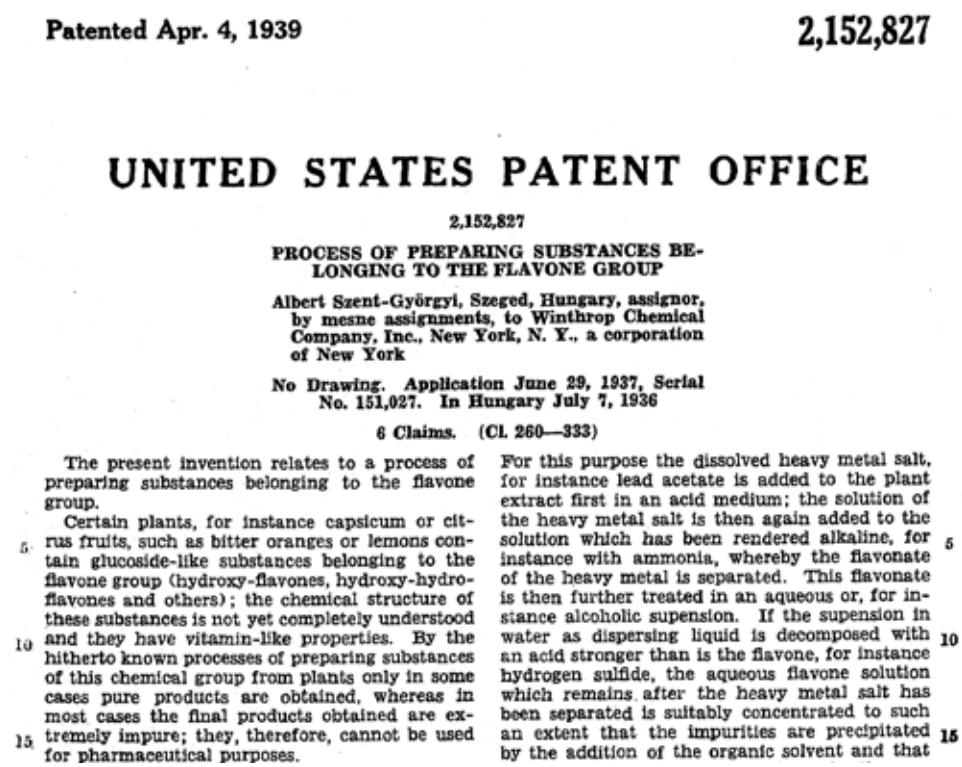
Szent-Györgyi asked other scientists to repeat his studies. The results were partly corroborative and partly negative. In 1937, Szent-Györgyi and Bentsáth stated, 'Vitamin P requires for its activity the presence of ascorbic acid. In the total absence of ascorbic acid, Vitamin P is inactive'.

Flavonoid research in the forefront of Albert Szent-Györgyi's interest

Albert Szent-Györgyi was passionate about flavonoid research, as illustrated by the following features:

1. He wrote an article entitled 'From Vitamin C to Vitamin P'.
2. He devised a method to extract greater amounts of citrin from lemon and published this method in Hoppe-Seylers Z. physiol. Chemie (1938).

3. When receiving the Nobel Prize, he gave an account of his research into flavonoids.
4. He developed a process to produce flavonoids and had this method patented in the United States in 1939 (Fig. 12).
5. At the conference on 'Bioflavonoids and the Capillary,' organized by the New York Academy of Sciences in 1955, he gave an interesting presentation on the perspectives of bioflavonoids.



Patented Apr. 4, 1939 **2,152,827**

UNITED STATES PATENT OFFICE

2,152,827

PROCESS OF PREPARING SUBSTANCES BELONGING TO THE FLAVONE GROUP

Albert Szent-Györgyi, Szeged, Hungary, assignor, by mesne assignments, to Winthrop Chemical Company, Inc., New York, N. Y., a corporation of New York

No Drawing. Application June 29, 1937, Serial No. 151,027. In Hungary July 7, 1936

6 Claims. (Cl. 260—333)

The present invention relates to a process of preparing substances belonging to the flavone group.

Certain plants, for instance capsicum or citrus fruits, such as bitter oranges or lemons contain glucoside-like substances belonging to the flavone group (hydroxy-flavones, hydroxy-hydroflavones and others); the chemical structure of these substances is not yet completely understood and they have vitamin-like properties. By the hitherto known processes of preparing substances of this chemical group from plants only in some cases pure products are obtained, whereas in most cases the final products obtained are extremely impure; they, therefore, cannot be used for pharmaceutical purposes.

For this purpose the dissolved heavy metal salt, for instance lead acetate is added to the plant extract first in an acid medium; the solution of the heavy metal salt is then again added to the solution which has been rendered alkaline, for instance with ammonia, whereby the flavonate of the heavy metal is separated. This flavonate is then further treated in an aqueous or, for instance alcoholic supension. If the supension in water as dispersing liquid is decomposed with an acid stronger than is the flavone, for instance hydrogen sulfide, the aqueous flavone solution which remains after the heavy metal salt has been separated is suitably concentrated to such an extent that the impurities are precipitated by the addition of the organic solvent and that

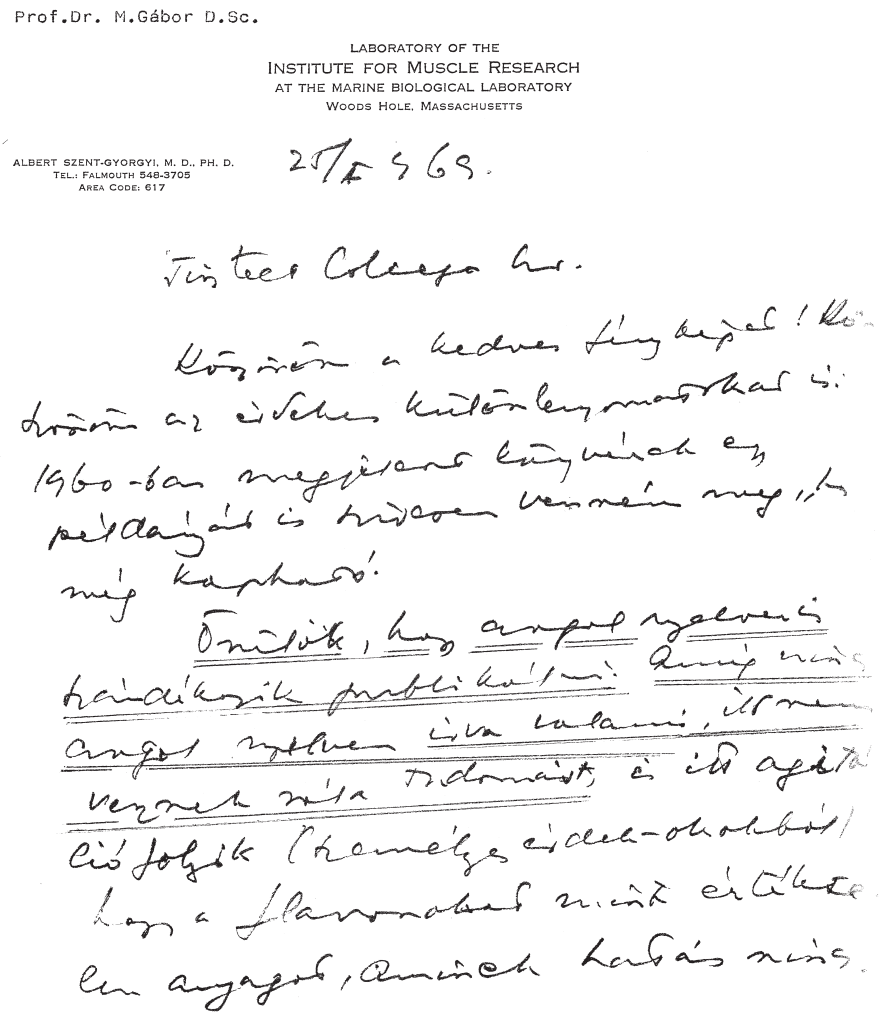
Fig. 12. Patent of Albert Szent-Györgyi (1939).

Correspondence with Prof. Szent-Györgyi

For several decades I had been researching the pharmacological properties of natural substances (plant dyes and flavonoids). By 1969, I considered that I had sufficient enough information to write a monograph on the anti-inflammatory effects of flavonoids. I had the idea to ask Professor Szent-Györgyi to write the foreword to this book. I attached the offprint of my publications,

the review of my monograph published in German by the Hungarian Academy of Sciences (Akadémiai Kiadó, Budapest) in 1960, and a photo showing Szent-Györgyi himself during an interview on the day of the Nobel Prize notification in 1937.

In his response, Szent-Györgyi expressed his delight at my intention to publish my book in English, arguing that everything published in a language other than English was ignored in the United States. He also complained of agitation driven by personal interests to force 'useless and ineffective' flavones out of the market (Fig. 13).


Prof.Dr. M.Gábor D.Sc.

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WOODS HOLE, MASSACHUSETTS

ALBERT SZENT-GYORGYI, M. D., PH. D.
TEL.: FALMOUTH 548-3705
AREA CODE: 617

25/V 69.

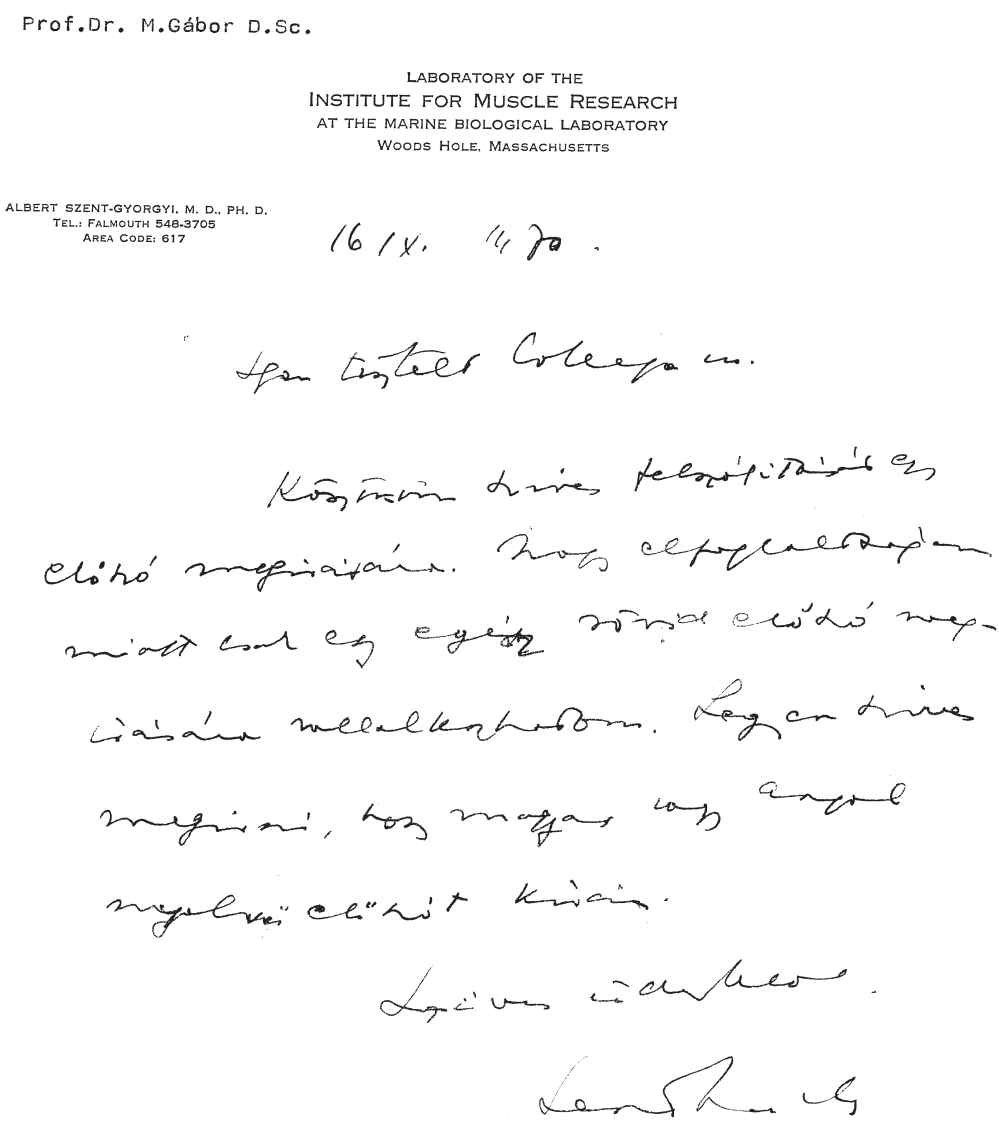
Tisztelt Colléga úr.

Köszönöm a kedves fényképet! Köszönöm az értékes különlenyomatokat és 1960-ban megjelent könyvének egy példányát is [illegible] még kapható.

Örülök, hogy angol nyelven is [illegible] publikálni. Amíg nincs angol nyelven írva valami, itt nem vesznek róla tudomást, és itt agitáció folyik (személyes érdek-okokból) hogy a flavonokat mint értéktelen anyagot, aminek hatása nincs.

Fig. 13. First letter of professor Albert Szent-Györgyi.

In 1970, I asked Szent-Györgyi to write the foreword to my English-language book, 'The Anti-inflammatory Action of Flavonoids.' In his response, Szent-Györgyi thanked me for the request, adding that he only had time for a short one (Fig. 14). The foreword and the accompanying letter arrived in January, 1971 (Figs. 15, 16).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16/X. '70.

Fig. 14. The response by Professor Szent-Györgyi, in wich he promised to write the foreword.

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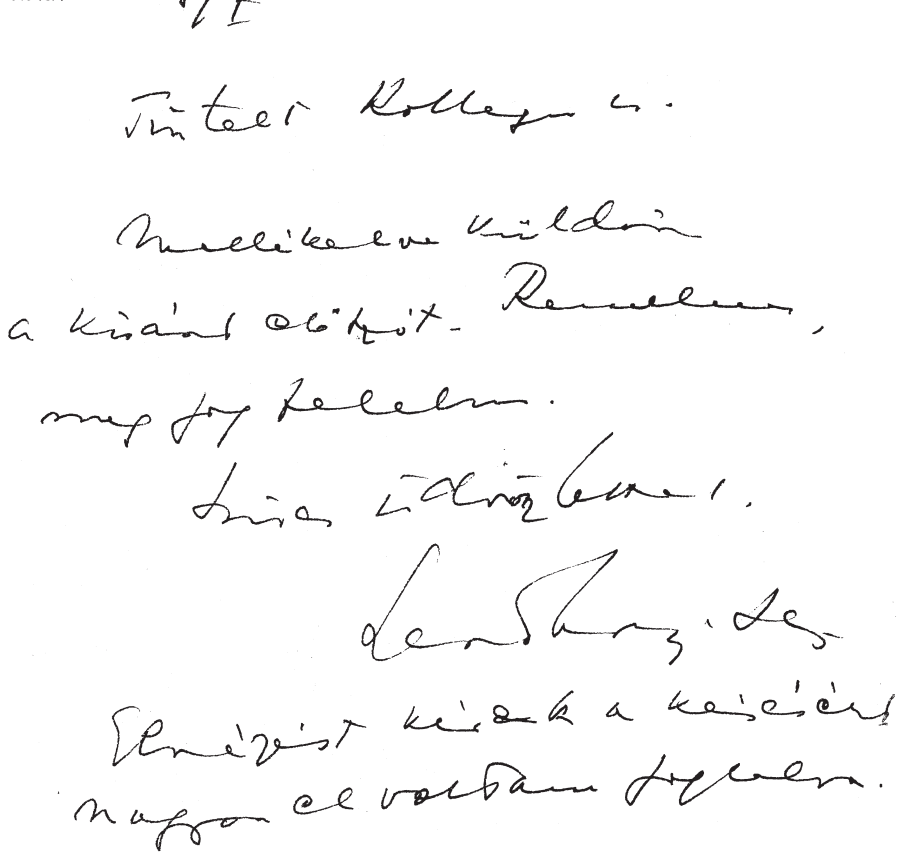


Fig. 15, 16. The letter that accompanied the foreword.

In 2008, our correspondence was published in Orvosi Hetilap, the oldest medical journal in Hungary, as requested by János Fehér, editor-in-chief.

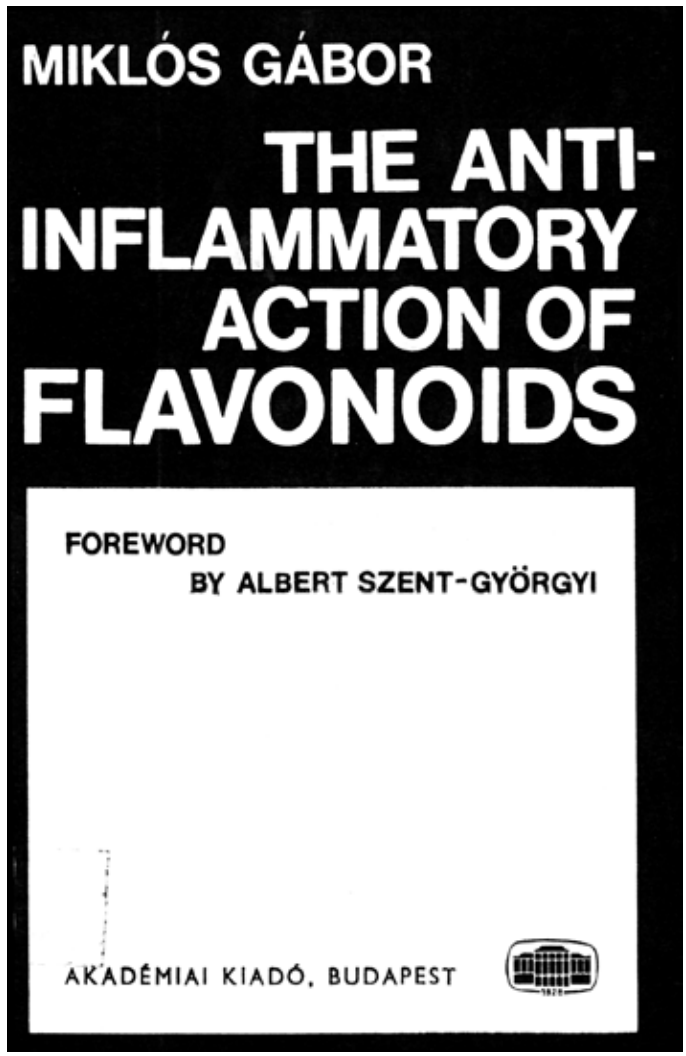
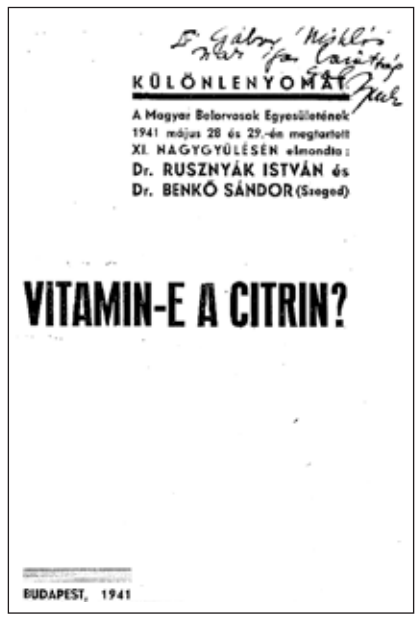


Fig. 16. The anti-inflammatory action of flavonoids (Akadémiai Kiadó, Budapest, 1972).

Can citrin be considered a vitamin?

Rusznyák and Benkő gave a lecture at the 10th Convention of the Hungarian Association of Internists {Magyar Belorvosok Egyesülete} on 28 May, 1941. Interestingly their lecture, 'Is citrin a vitamin?', was published as an offprint (Fig. 17).


KÜLÖNLENYOMAT

A Magyar Bolorvosok Egyesületének
1941 május 28 és 29.-én megtartott
XI. NAGYGYŰLÉSÉN elmondta:
Dr. RUSZNYÁK ISTVÁN és
Dr. BENKŐ SÁNDOR (Szeged)

VITAMIN-E A CITRIN?

BUDAPEST, 1941

Fig. 17. Is citrin a vitamin? (Rusznyák and Benkő, 1941).

Guinea pigs and rats were kept on the scorbutic Sherman – La Mer – Campbell deficiency diet and capillary resistance was measured on the shaved and depilated back of the animals with a glass cup 8 mm in internal diameter (Borbély method). In their guinea pig experiments they determined the minimum vacuum required to produce the first spot-like haemorrhage (petechia) in 15 sec. In rats they applied a vacuum of 250 mm Hg and measured the time until the first petechia appeared. As a result of the deficiency diet, the normal capillary resistance of the guinea pigs dropped significantly, and this decrease continued in spite of the fact that they were given 3 mg of ascorbic acid daily. However, the capillary resistance resumed its baseline level after the animals were injected with 4 mg of subcutaneous citrin (extracted according to the original Szent-Györgyi method). Here I would like to present the results with the original figures of Rusznyák and Benkő's article (1941) (Figs 18 and 19)

On the basis of their experiments, Rusznyák and Benkő (1941) drew the following conclusions:

- the scorbutic diet is low in both ascorbic acid and flavonoids,
- an insufficient intake of flavonoids leads to a decrease in capillary resistance, regardless of the vitamin C deficiency, but the resistance can be normalized by increasing the flavonoid intake,
- experimental scurvy is double avitaminosis.

As a result of a scorbutogenic diet, the capillary resistance of rats decreases significantly, often as rapidly as in 2 weeks.

Next, Rusznyák and Benkő (1941) described the course of an experiment. At the beginning of the experiment, the rat's capillary resistance was low and it took more than 5 min to induce petechiae with a vacuum of 25 cmHg. As a result of the diet this value decreased to 15 sec in 4 weeks. Daily administration of 4 mg of citrin (Richter-Budapest) increased the capillary resistance to the baseline level in 3 weeks (Fig. 19).

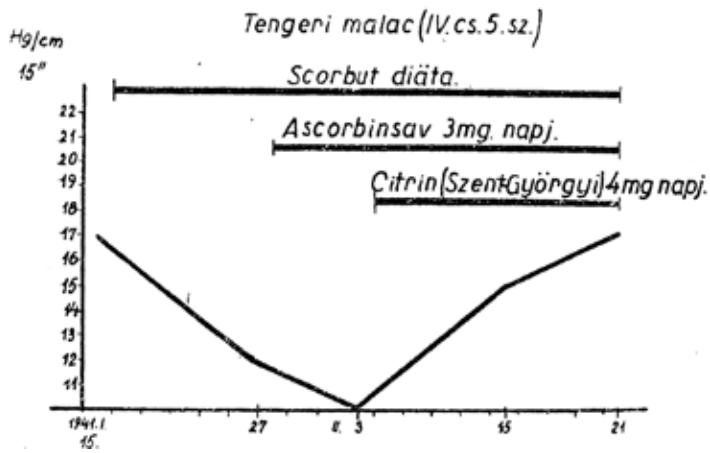


Fig. 18. Effects of citrin in guinea pigs kept on scorbutogenic diet (fig. original).

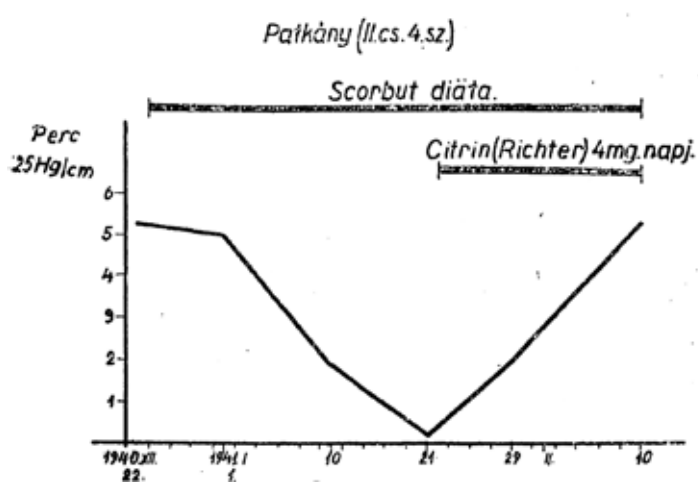
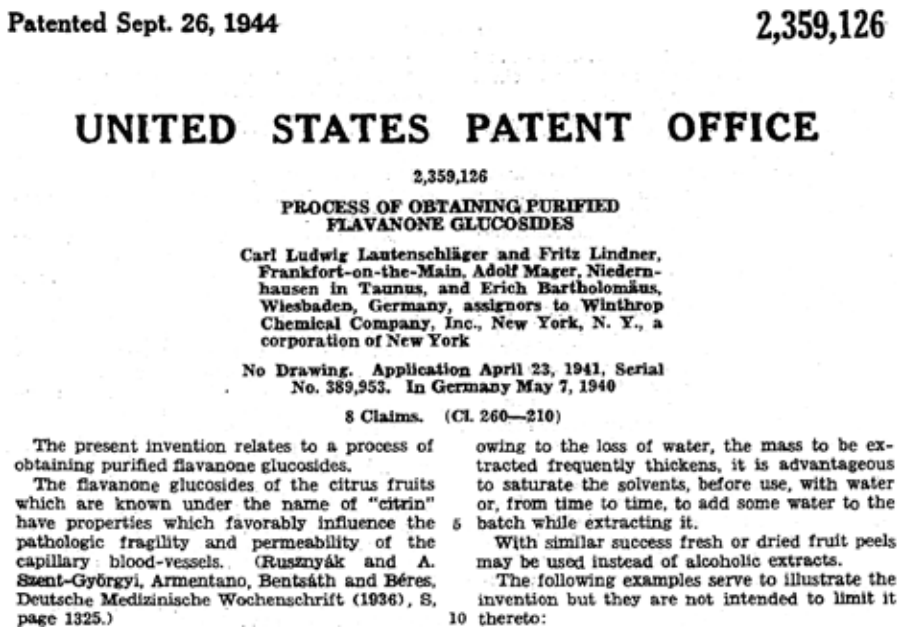


Fig. 19. Effect of citrin on rats kept on scorbutogenic diet (fig. original).

It is worth mentioning that Rusznyák and Benkő (1941) reached similar results when working with various citrin preparations (Richter, Budapest; Bayer, Leverkusen; Hoffmann-La Roche, Basel). These data demonstrate that citrin was produced by both Hungarian and foreign pharmaceutical companies as early as 1940.

The interest of pharmaceutical companies' in citrin is also revealed by the fact that Lautenschläger and Lindner (Winthrop Chemical Company, Inc., New York) submitted a request in 1941 to derive purified flavanone glycosides. The process was patented in 1944. Patent number: 2,359,126 [14] (Fig. 20).



Patented Sept. 26, 1944 2,359,126

UNITED STATES PATENT OFFICE

2,359,126

PROCESS OF OBTAINING PURIFIED FLAVANONE GLUCOSIDES

Carl Ludwig Lautenschläger and Fritz Lindner, Frankfort-on-the-Main, Adolf Mager, Niedernhausen in Taunus, and Erich Bartholomäus, Wiesbaden, Germany, assignors to Winthrop Chemical Company, Inc., New York, N. Y., a corporation of New York

No Drawing. Application April 23, 1941, Serial No. 389,953. In Germany May 7, 1940

8 Claims. (Cl. 260—210)

The present invention relates to a process of obtaining purified flavanone glucosides.

The flavanone glucosides of the citrus fruits which are known under the name of "citrin" have properties which favorably influence the pathologic fragility and permeability of the capillary blood-vessels. (Rusznyák and A. Szent-Györgyi, Armentano, Bentsáth and Béres, Deutsche Medizinische Wochenschrift (1936), S, page 1325.)

owing to the loss of water, the mass to be extracted frequently thickens, it is advantageous to saturate the solvents, before use, with water or, from time to time, to add some water to the batch while extracting it.

With similar success fresh or dried fruit peels may be used instead of alcoholic extracts.

The following examples serve to illustrate the invention but they are not intended to limit it thereto:

20. kép. Patent of Lautenschläger *et al.* (1944).

Recommendation to drop the name 'vitamin P'

The American Society of Biological Chemists and The American Institute of Nutrition Committee on Nomenclature recommended dropping the name 'vitamin P', arguing that there was no evidence at all that flavonoids increased the life expectancy of rats suffering from scurvy or fprocessed vitamin-like properties. The recommendation was signed by Vickery, H.B., Nelson, E.M., Almquist, H.J. and Elvehjem, C., on behalf of the Committee on Nomenclature, 1950.

Retrospective justification of the recommendation

From the beginning of the 1950s, we performed several series of experiments on the pharmacological effects of indenochromene derivatives (haematoxylin, haematein, brazilin and brazilein) (Fig. 21). Similarly to Rusznyák and Benkő (1941) we examined the effect of haematoxylin on capillary resistance in rats the kept on a scorbutogenic diet (Gábor and Dux, 1952). As a result of the diet, capillary resistance stagnated at a low level for several weeks and petechiae appeared following a vacuum of seconds (Fig. 22, curve C).

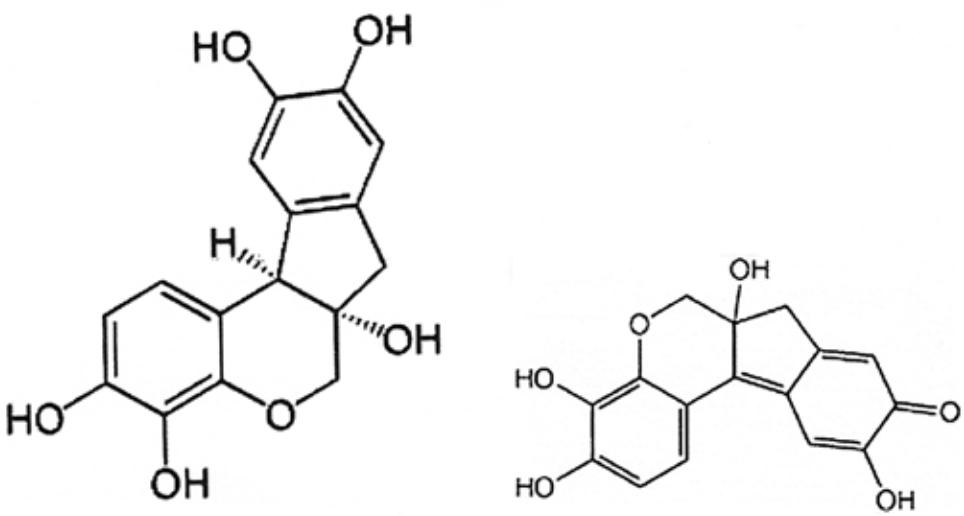


Fig. 21. Haematoxyline and haemateine

Rats weighing 190–210 g received a subcutaneous (occipital region) injection of 15 mg of haematoxylin every third day. Only 2 weeks following the onset of the therapy it took more than 5 minutes to induce petechiae. Measurements performed 12 days after the completion of the therapy showed minimum capillary resistance (Fig. 22, curve A). Haematein therapy yielded similar results (Fig. 22, curve B).

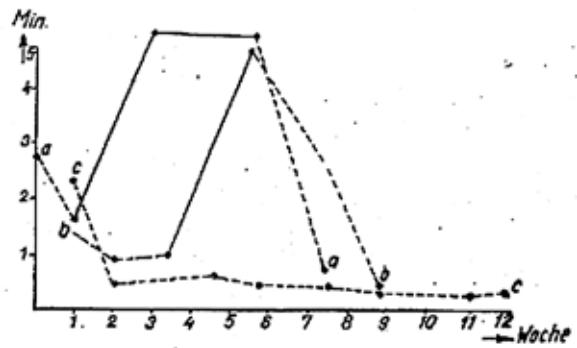


Abb. 1

Abb. 2

DIE EXPERIMENTELLE BEEINFLUSSUNG DER KAPILLARRESISTENZ MIT HÄMATOXYLIN UND VERWANDTEN DERIVATEN

Von

M. GÁBOR und E. DUX

PHARMAKOLOGISCHES INSTITUT DER MEDIZINISCHEN UNIVERSITÄT, SZEGED

(Eingegangen am 25. Januar 1952.)

Fig. 22. Effects of haematoxylin and haematein on rats kept on scorbutogenic diet (fig. original, Gábor and Dux, 1952).

Our experiments confirmed that the resistance-increasing effect of a drug ('pharmacon') does not justify its vitamin-like properties, and it therefore cannot be named a vitamin.

The first conference on bioflavonoids

The conference on 'Bioflavonoids and the capillary' was organized by the New York Academy of Sciences and held on 11 February, 1955. As revealed by the programme of the symposium, the term 'Vitamin P' was no longer used.

We should also note that Albert Szent-Györgyi gave a presentation entitled 'Perspectives for the Bioflavonoids' at the conference.

BIOFLAVONOIDS AND THE CAPILLARY*

Conference Co-Chairmen: Gustav J. Martin and Albert Szent-Györgyi

Consulting Editor: Gustav J. Martin

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* This series of papers is the result of a conference on *Bioflavonoids and the Capillary* held by the Section of Biology of The New York Academy of Sciences, February 11, 1955.

Fig. 23. Bioflavonoids and the capillary (Conference in New York, 1955).

The mechanism of action of flavonoids

Exploration of the mechanism of action of flavonoids is an important field.

In connection with this, it is worth citing Szent-Györgyi's presentation (1955): 'As a chemist, I am deeply impressed by the reaction of flavonoids with metals, while as a biochemist, I am increasingly impressed by the central role metal atoms play in biological function. So it seems possible that the reactions of flavones with metals hold the key to the understanding of their biological function, while the flavonoid metal complexes may hold the key to a better understanding of the working of the machinery of life.' (Szent-Györgyi, 1955). To illustrate his ideas for the sake of better understanding, I would like to introduce the formation of quercetin-copper chelate complexes, published by Clark and Geissman (1949). 3',4'-Dihydroxy and 3-hydroxy-4-keto groups are important constituents of flavonoid compounds (Fig. 24).

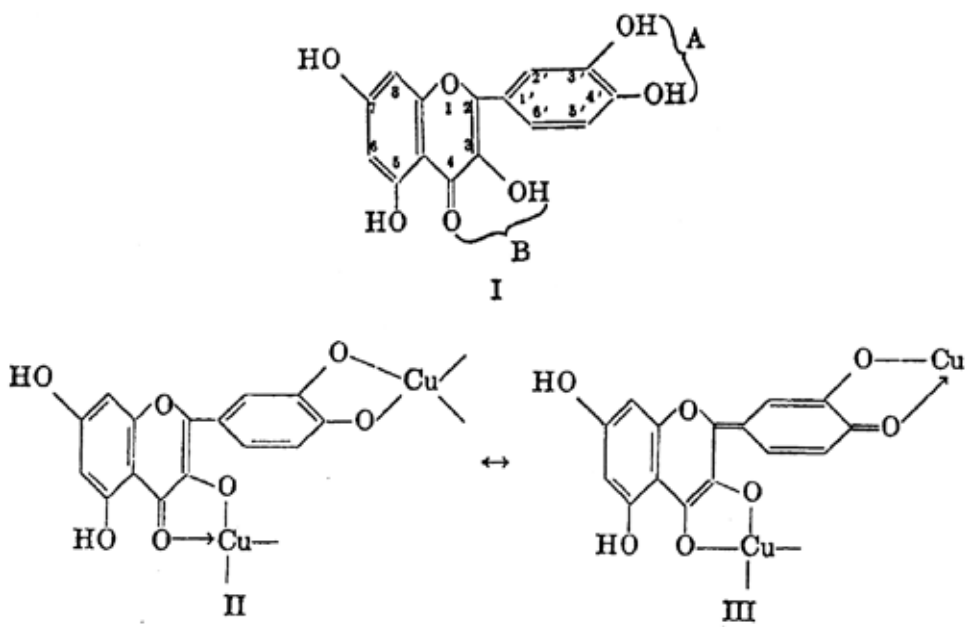

Fig. 24. Structure of quercetine-copper chelate complexes.

Modern ideas concerning the mode of action of flavonoids are illustrated with a figure taken from the paper of Kim *et al.* (2004) (Fig. 25).

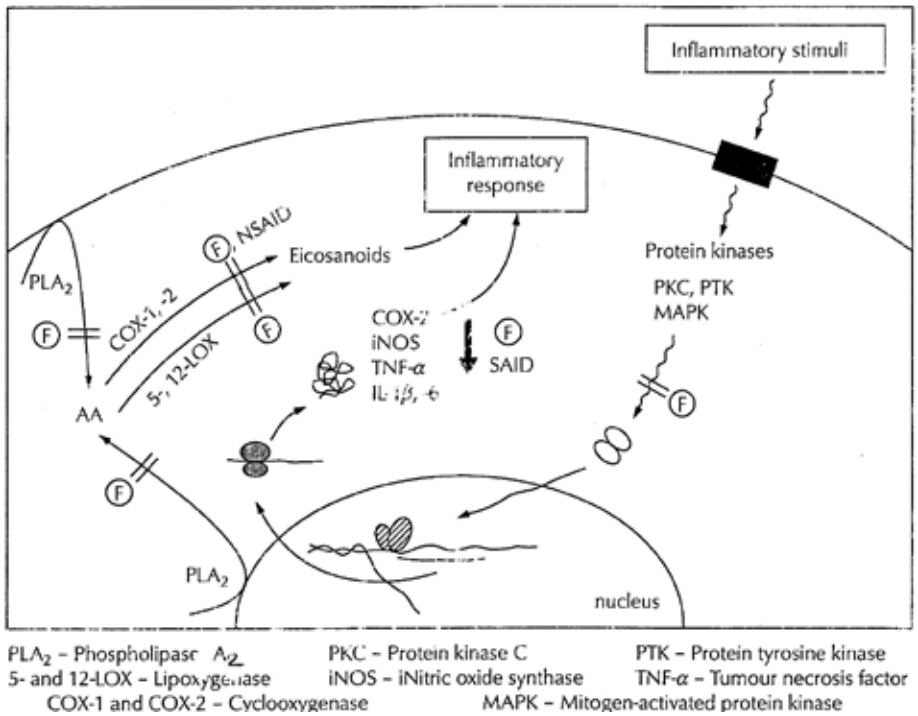

Fig. 25. Mechanism of action of flavonoids (Kim *et al*., 2004).

Phenylbenzopyrone, chromone and chromane derivatives in medications

Threeimportant chemical formulae may be given (Fig. 26).

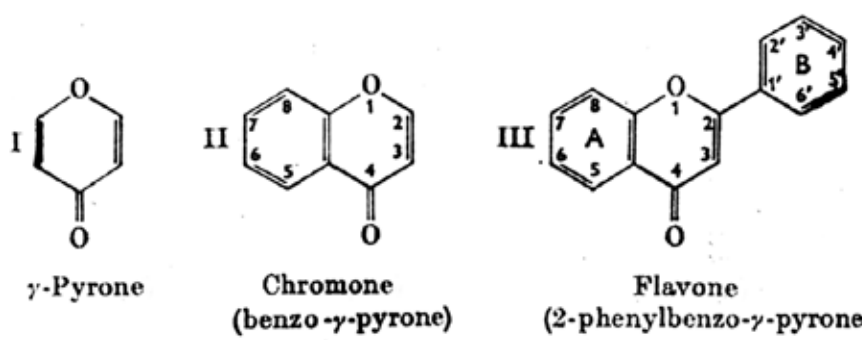

Fig. 26. Structural formulae of pyrone, chromone and 2-phenylbenzo-γ-pyrone (flavone).

Nowadays there is no doubt that it was the result of Szent-Györgyi's research that focused attention on the study of phenylbenzopyrones (flavones) and related derivatives.

The research into phenylbenzopyrone, chromone and chromane derivatives is of outstanding importance; it has already led to the discovery of a number of medications with various pharmacological effects. I have described the pharmacology of these derivatives and other related compounds in my books (Gábor, 1986, 1988) (Fig. 27).

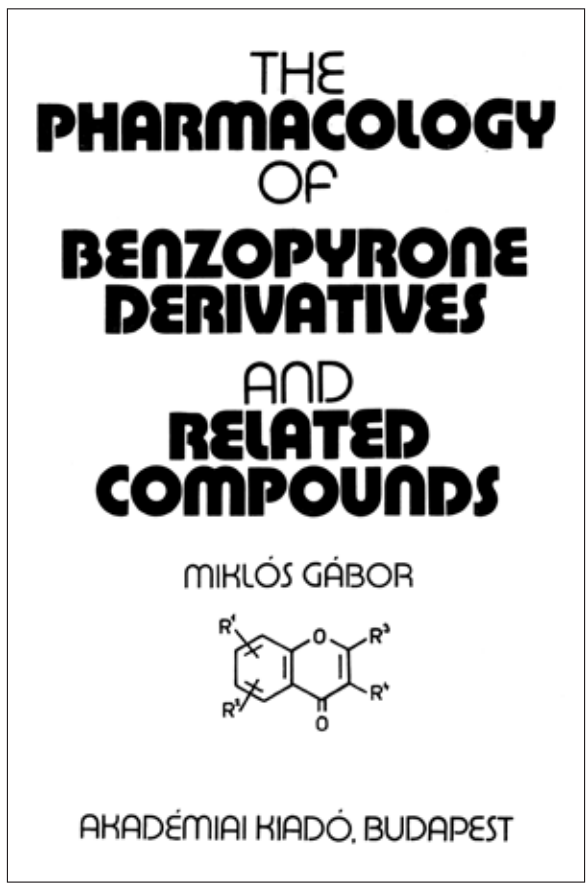

Fig. 27. The Pharmacology of Benzopyrone derivatives and Related Compounds (Gábor, 1986).

It is worth mentioning the monograph by Casley-Smith and Judith Casley-Smith, published in Australia in the same year: High-protein oedemas and the benzo-pyrones' (1986) (Fig. 28).

The pharmacological study of benzopyrones led to the discovery of new medications, many of which are available on the market. I would like to mention some which are used in medical treatment.

Flavone-containing medications marketed in Hungary

Rutascorbin

Rutascorbin is one of the flavonoid drugs with the longest history. It is used in the treatment of a chronic venous insufficiency and haemorrhoids. In ophthalmology, the range of application includes subconjunctival haemorrhage and diabetic retinopathy.

Detralex

The active substance is a purified and micronized flavonoid fraction, which contains diosmin and other flavonoids expressed as hesperidin. It is recommended for the treatment of chronic venous insufficiency of the lower limb and haemorrhoids. In some countries a micronized purified flavonoid fraction containing 450 mg of diosmin and 50 mg of hesperidin is also marketed under the name Daflon 500 mg.

Venoruton

This has been used for decades in the treatment of venous insufficiency, venous circulatory disorders, leg ulceration, haemorrhoids and diabetic retinopathy. Composition: 0-(β-hydroxyethyl) rutosides (oxerutin).

Medications containing benzopyr one or its derivatives on foreign markets

Earlier gave a detailed description of the pharmacology of benzopyr one derivatives and some related compounds (Gábor, 1986). (For the sake of brevity, I reform here to this monograph.) I mention merly ormeloxifene, the selective oestrogen receptor modulator, which is interesting from a pharmacological aspect.

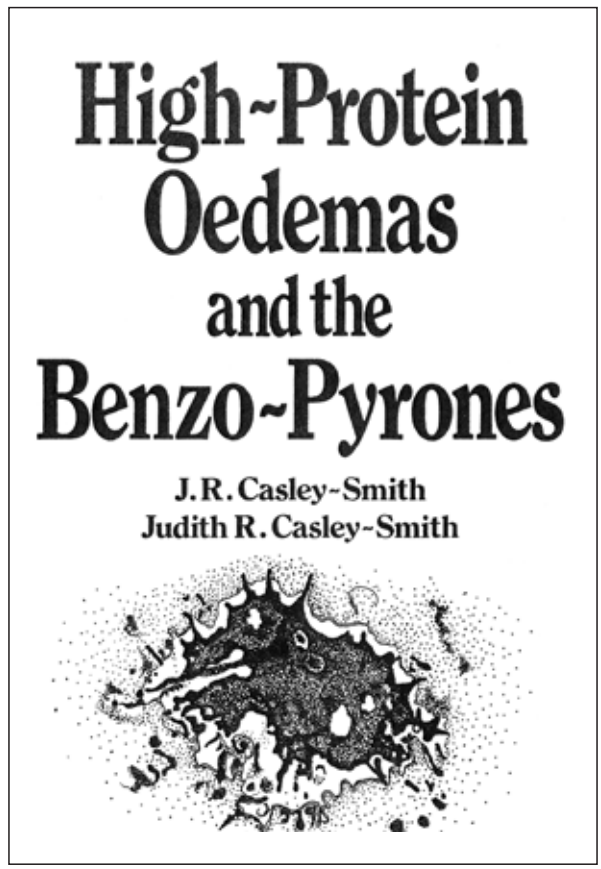

Fig. 28. High protein oedemas and the benzo-pyrones (J. R. Casley-Smith and J Casley-Smith, 1986).

Ormeloxifene (Centchroman)

It is 3,4-trans-2,2-dimethyl-3-phenyl-4-(p-(β-pyrrolidinoethoxy)-phenyl)-7-methoxychroman. Its anti-inflammatory effect was first reported by Dhawan and Srimal in 1973. It is worth mentioning that in India it has been available since the beginning of the 1990s as a contraceptive under various names (Saheli, Novex; Hindustan Latex Ltd). For the treatment of dysfunctional haemorrhages, it is marketed under the name Sevista by Torrent Pharmaceuticals.

As a contraceptive, ormeloxifene is recommended to be taken orally, once a week (Twice a week in the first 12 weeks). Its contraceptive use was legalized in India in 2009.

Flavonoid-containing herbal combinations used as drugs

Scutellaria species are widespread in nature *(S. lateriflora, S. hastifolia, S. altissima and S. baicalensis).* In the last decade, *S. baicalensis* was used in the development of 2 medications.

SK Ato formula

Lim *et al.* (2006) reported a topical anti-inflammatory flavonoid preparation, SK Ato Formula. The preparation contains flavonoid mixtures from *Scutellaria baicalensis,* Georgi roots and *Ginkgo biloba* L. leaves with an extract of *Gentiana scabra.*

Several flavonoid compounds have been isolated from *Scutellaria baicalensis* (baicalin, wogonin, oroxylin A, etc.). The leaves of *Ginkgo biloba* contain myricetin, quercetin, and the biflavone, ginkgetin among other.

Flavocoxid (Limbrel®)

Burnett *et al.* (2007) analysed the cyclooxygenase-1, 2 and 5-lipoxygenase inhibitor effect of the mixed extract of *Scutellaria baicalensis* and *Acacia catechu* (baicalin and catechin). The mixed extract (flavocoxide) is used in the treatment of inflammatory knee diseases (osteoarthritis).

Bioflavonoid conferences, international polyphenol associations

As I mentioned earlier, the first conference on bioflavonoids was organized by the New York Academy of Sciences in 1955. I am glad to report that the Hungarian Academy of Sciences founded the Committee for Flavonoid research in 1964 to coordinate and publish the findings of Hungarian flavone research. It was at one of the first sessions that we decided to replace the term 'vitamin P' with 'bioflavonoids' when referring to biologically active flavone compounds. This terminology has not changed in the past 4 decades.

The following institutions have contributed most to the work of the Committee: Budapest University of Technology and Economics, Department of Organic Chemistry at the University of Debrecen, and the Department of Microbiology, the Institute of Pharmacognosy and the Department of Pharmaceutical Technology at the University of Szeged.

The first president of the Committee for Flavonoid research was Academician Rezső Bognár. Later the Committee was presided by Academicians Loránd Farkas and Sándor Antus.

The first and second symposia (1965 and 1967) were held in Szeged, while the next one was hosted by Debrecen (1970). Since then, the national symposia have been held every year and the international ones every four-to-five years. The lectures presented at the international symposia are published in English by the Hungarian Academy of Sciences.

I may note that the 6th Hungarian Bioflavonoid symposium was held in Munich (1977), the 7th in Szeged (1985) Fig. 30, and the 9th in Vienna (1995).

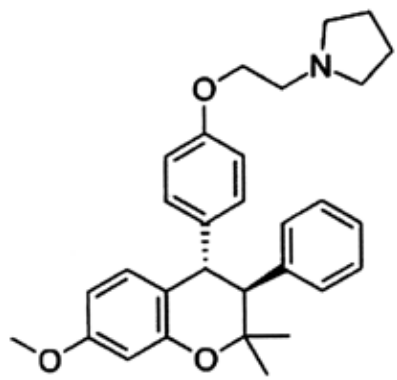

Fig. 29. Ormeloxifene

In 2012 the Committee received new tasks under the new name 'Committee on Alkaloid and Flavonoid Chemistry'. Its sessions are held every year following in the footsteps of Hungarian flavonoid research.

I would like to mention the Venoruton symposia (in the German literature: O-(β-hydroxyethyl) rutoside) where experimental and clinical results were presented. (Nyon, 1972, Mayschoss, 1978, Ludwigsburg, 1982, and Darmstadt, 1990). The presentations were also published by Springer Verlag and Medikon Verlag.

The Groupe Polyphénols (GP) society is an international association founded in France in 1972 with the aim of promoting research on plant polyphenols. Its head office is in Bordeaux and its conferences (International Conference on Polyphenols, ICP) are held every 2 years.

The research on polyphenols is still at the focus of attention, as indicated by the 7th World Congress of the International Society of Antioxidants in Nutrition and Health, entitled Polyphenols Applications, held in Bonn in 2013. (Fig. 31). These congresses have been organized yearly since 2004.

Fig. 30. The 7th Hungarian Bioflavonoid Symposium (Szeged, 1985).

I conclusion, we can say that the intensity of flavone (polyphenol) research is still unbroken, following in the path of Szent-Györgyi and his colleagues. It gives me special joy to see that Hungarian scientists continue to play a significant role in this research. I think it is most appropriate to finish my presentation with a quotation from the Nobel-laureate Albert Szent-Györgyi (1955): 'I hope to leave the reader with the impression that flavonoids comprise one of the most exciting, broad, and hopeful fields of biological inquiry, and I am glad to close on such an optimistic note.' (Szent-Györgyi, 1955).

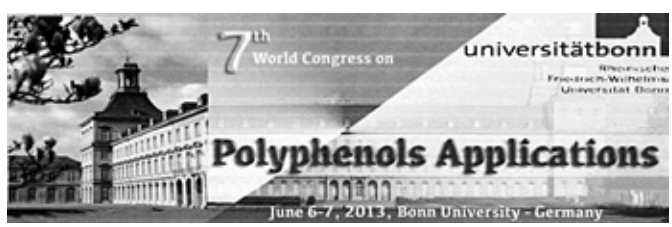

Fig. 31. International Society of Antioxidants in Nutrition and Health, Polyphenols Applications, 7. World Congress (Bonn, 2013).