

THE ISOLATION OF ISOQUERCITRIN FROM GIANT RAGWEED POLLEN; THE ELECTROPHORETIC PATTERN AND BIOLOGIC ACTIVITY OF THE PIGMENT*

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IN THE course of studies of fractions of giant ragweed extracts, an amorphous precipitate was observed to accumulate if the solutions stood in the refrigerator for a length of time. A crystalline material obtained from this precipitate was analyzed and identified. Then the electrophoretic pattern of this isolated substance was compared with that of normal ragweed extracts. The study was completed by observing the skin reactions with the crude precipitate and the refined crystals in ragweed-sensitive persons.

When the amorphous precipitate recovered from the crude extracts was refined, the yellow needles obtained were found to be isoquercitrin. This glycoside is the 3-glucoside of quercetin, a pentahydroxy-flavone which is one of the most widely distributed natural pigments.¹ It occurs as quercetin in many plants, such as dyers oak, sumac, hops, and red roses, and in glycosidic forms, such as isoquercitrin, which are found less frequently. In the past flavones have been detected in pollens, but the identity of the pigmented derivative has not been clearly established. Quercetin, the hydroxy flavone, may be combined with rhamnose, galactose, or glucose. In isoquercitrin it is combined with glucose. The identity of the glucoside isolated was determined from an analysis for C, H, and O, from its molecular weight, by the isolation from hydrolyzates of quercetin and glucose, and by characteristic melting points.¹

EXPERIMENTAL

Separation of the Precipitate.—The extracts yielding the most voluminous precipitates were made with ether-washed pollen and cold distilled water. Customarily 100 Gm. of giant ragweed pollen were divided into 4 equal portions which were washed successively with 4 lots of the smallest possible volume of distilled water. When these 4 lots were combined and filtered the total volume was about 300 c.c. Such extracts contained from 700 to 800 mg. of nitrogen and from 18 to 19 Gm. of solids determined by drying in vacuo. The pH of the solutions from most samples of ragweed was 5.6 to 5.7. When one of these ragweed extracts was refrigerated a cloud of amorphous precipitate appeared after several days and continued to aggregate and settle for 3 or 4 months. Six to 7 per cent of the 18 or 19 Gm. of solids in a satisfactory solution was carried down as precipitate. The precipitate washed once in a large volume of water and dried in vacuo contained from 0.5 to 0.7 per cent of nitrogen. The unmodified pH of the pollen extracted in water was most satisfactory for the separation as only

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meager precipitation occurred in more alkaline (pH 6.5 to 7.5) or less concentrated solutions, and more acid solutions (pH 3.0 to 5.0) yielded precipitates with higher nitrogen contents.

Analysis of the Precipitate.—The crude precipitate was dissolved in hot ethanol and filtered. Then the fine yellow needles crystallizing from this filtrate were reprecipitated 4 times from boiling water. These needles were insoluble in cold water, soluble in alkalis and alcohol, reduced copper solutions after hydrolysis, and turned green in weak solution on the addition of ferric chloride. The color of a weak solution varied with the pH; it was colorless at 2.0, yellow 4.0 to 6.0, orange 6.5 to 7.5, and green above 8.5. The crystals contained no nitrogen, as determined by micro-Kjeldahl both by titration and colorimetrically, and gave no ash. Duplicate analyses of samples dried at 110° C. were as follows: C, 54.2 to 54.34; H, 4.19 to 4.24; calculated O, 41.5. Samples dried at 75° melted at 214° to 216° with decomposition. From these observations and analyses together with subsequent data the isolated material was believed to be isoquercitrin, a tetrahydroxyflavone³ glucoside (C₂₁, H₂₀, O₁₂). In the following data the theoretical values have been given in parentheses. Fifty milligrams of the dried crystals on hydrolysis in 5 ml. of 10 per cent HCl yielded 33 mg. of solid (theoretical 32.6 mg. calculated as quercetin). A second hydrolysis of 73 mg. in 7 ml. of 10 per cent HCl yielded 46 mg. of solid (theoretical yield of quercetin 47.5 mg.). The dried solid from this hydrolysis melted with decomposition at 312 to 314°. The precipitate from this hydrolysis, believed to be quercetin, was acetylated. Thirty-one milligrams of the precipitate, 30 mg. of anhydrous sodium acetate, and 3 ml. of acetic anhydride were heated on the steam bath for 1.5 hours. After cooling and pouring on ice a white precipitate formed: recrystallization from alcohol produced needles (37 mg.) with a melting point of 192 to 194°. The optical rotation of the supernatant solution from the hydrolysis of the 73 mg. sample was 51.5 (calculated $[\alpha]_D$ 52.8 for 1 mole of glucose per mole of glucoside).

Cutaneous Tests.—Patients strongly reactive to ragweed were tested with solutions of the crystallized isoquercitrin in physiologic salt solution titrated to pH 8.0 with bicarbonate. From data obtained in separating the crude precipitate it was determined that the approximate ratio of nitrogen to isoquercitrin in the ragweed extract was 7 to 11 mg. The solutions of the extract used to test the sensitivity of the patients to ragweed contained .001 and .0001 mg. of nitrogen per c.c. The reactions to these dilutions were compared with those obtained with solutions of the isoquercitrin made to correspond with these concentrations of nitrogen, that is, containing respectively .0016 and .00016 mg. of isoquercitrin. Although the patients tested were extremely sensitive to ragweed no immediate reactions were obtained with the glucoside, but delayed swelling and inflammation were observed the following day if solutions of the glucoside more concentrated than those indicated above were injected. No reactions were obtained with control solutions.

The isoquercitrin used in the preceding tests had been treated drastically with hot alcohol and boiling water. Less drastic methods would not free the

precipitate from nitrogen although the content could be reduced by acid precipitation and resolution of the precipitate by the gradual addition of bicarbonate. For example, a sample containing .67 per cent N was reduced to .36 per cent N by 4 precipitations and subsequently to .15 per cent but at a tremendous loss of material. This sample, which had never been heated and only subjected to a varying pH (4.0 to 8.2), was used for testing. Solutions (0.1 per cent) of the glucoside containing .00036 and .00015 nitrogen were compared with solutions of the original ragweed extract containing .001 and .0001 per cent respectively. Immediate reactions were obtained with the first of the 2 solutions of the glucoside, but with the second, that containing .00015 per cent of nitrogen, only questionable immediate reactions occurred. The reactions with the .00036 per cent solution were peculiar in that only 4 of the 12 strongly sensitive patients showed positive tests. Possibly the glucoside was associated with a particular nitrogenous substance to which all patients were not sensitive. Control tests were negative.

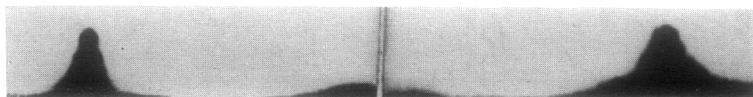


Fig. 1.—Electrophoretic pattern of 0.1 per cent solution of isoquercitrin in borate - NaOH-HCl buffer at pH 8.4 at the end of a 60 minute run. Mobility in ascending limb (left pattern), 11.9×10^{-5} .

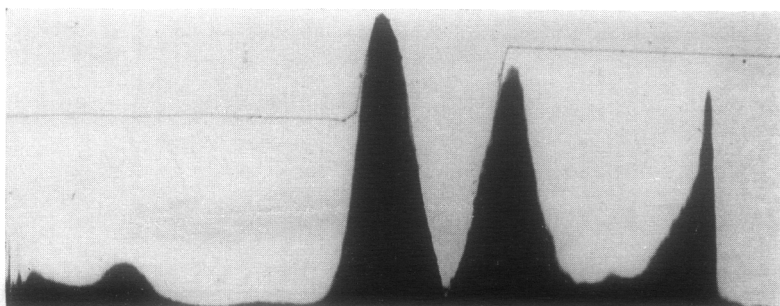


Fig. 2.—Undialyzed giant ragweed extract with added buffer (borate - NaOH-HCl), at pH 8.4 after 60 minutes. Mobility of fast pigment in ascending limb, 11.6×10^{-5} .

Electrophoretic Patterns.—Difficulties were encountered in obtaining the electrophoretic pattern of whole ragweed extracts because in dialysing to bring the extracts to the desired pH and into equilibrium with the buffer solutions used electrophoretically, all the components in the pattern were found dialysable, but by the gradual addition of a concentrated alkaline buffer solution the extracts could be brought to the desired pH, thus avoiding dialysis. In comparing whole ragweed extracts with the solutions of isoquercitrin, a pH more alkaline than that usually employed for electrophoresis was required because of the insolubility of the isolated isoquercitrin. A pH of 8.2 to 8.4 was most satisfactory. Concentrated buffer solutions (borate - NaOH-HCl [pH 8.4]) and concentrated solutions of ragweed were prepared and buffer

was added to the ragweed solutions until the desired pH was attained. The ragweed solutions were then diluted with distilled water to contain 50 mg. of nitrogen per 100 c.c. The buffering solutions employed electrophoretically were made by diluting the solutions of concentrated buffer in the same proportion with distilled water as they were diluted in the ragweed extracts. Isoquercitrin was dissolved in this diluted buffer to make a 0.1 per cent solution. These solutions were satisfactory except for some salt interference in the patterns.

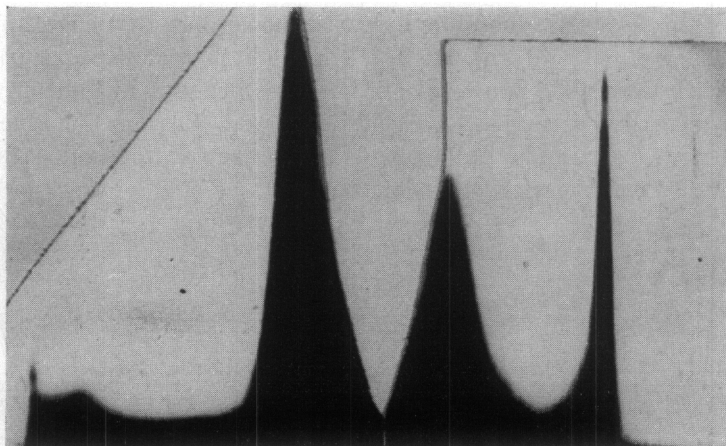


Fig. 3.—Pattern of a concentrated dialyzate containing 50 mg. N per 100 cc., buffered at pH 8.4 with borate - NaOH-HCl, after 61 minutes. Mobility of fast pigment in ascending limb, $11.9 \text{ cm} \times 10^{-5}$.

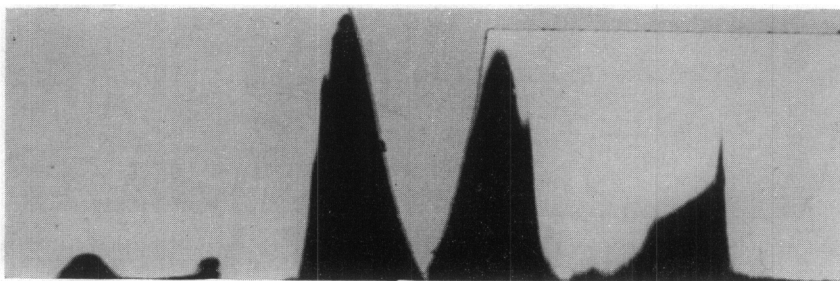


Fig. 4.—Giant ragweed extract with two-thirds of isoquercitrin precipitated. Borate - NaOH-HCl buffer at pH 8.4, 60 minute run. Mobility of fast pigment in ascending limb, $11.9 \text{ cm} \times 10^{-5}$; 50 mg. N per 100 c.c.

The mobility of the isoquercitrin was found to be $11.9 \text{ cm} \times 10^{-5}$ (pH 8.4) in both the ascending and descending limbs of the U tube. This mobility was compared with that of the rapidly moving pigment in solutions of ragweed extracts prepared in several ways. All the ragweed extracts studied contained 50 mg. of nitrogen per 100 c.c. In the first experiment whole ragweed extract was diluted with borate buffer but not dialyzed, while in the second the extract was dialyzed against the borate buffer solution for 24 hours. In the third experiment, extract was used which had been refrigerated for 4 weeks with partial precipitation of the isoquercitrin. In the final experiment

an extract was concentrated by dialyzing under pressure, washed with several volumes of distilled water, and then diluted with borate solution to bring it to the desired pH and concentration of nitrogen. In all 4 experiments the main component in the patterns was clearly delineated but showed fragmentation. At pH 8.4 the mobility of this slowest component was 0.6. In the ascending limb in the first 3 experiments the mobilities of the fast moving pigment were 11.6, 11.9, and 11.9, respectively.² In the fourth experiment this pigment component was absent, apparently having been washed out completely by the dialysis. Because of the identical mobilities of isoquercitrin (11.9 at pH 8.4) and of the fast pigment, this flavone glucoside is believed to be one of the constituents of this pigmented component.

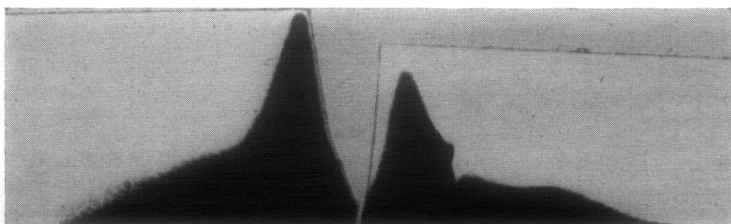


Fig. 5.—Giant ragweed extract dialyzed and concentrated under pressure; rapidly moving pigment dialyzed out, 120 minutes; 45 mg. N per 100 c.c.

SUMMARY

In the preceding experiments isoquercitrin, a tetrahydroxy-flavone glucoside, has been isolated from giant ragweed pollen and found to be a constituent of the most rapidly moving pigment in the electrophoretic pattern of the pollen. The crystalline pigment isolated from the ragweed extracts was inactive biologically on cutaneous testing, a result anticipated because this pigment is so widely distributed in various plants that a specific role in the activity of the pollen could not be expected. But the freshly isolated pigment in amorphous form, before drastic procedures were undertaken to cause its crystallization, contained nitrogenous material with specific biologic action in the skin of ragweed-sensitive patients. When the nitrogen content of the amorphous pigment was reduced fourfold positive cutaneous tests were no longer obtained, presumably indicating that the activity was inherent in the nitrogenous fraction or fractions associated with the pigment. The nature of this association, whether a chemical combination or merely a chemical contamination, is not clear, but numerous unpublished experiments suggest that the active nitrogenous fraction is combined with the pigment. Since the mobilities of the isolated isoquercitrin and the pigment in the whole ragweed solution are identical such a combination would necessarily be through linkage with the glucose of the glucoside.

REFERENCES

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