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Some Physical Properties of Rutin*

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Approximate solubility of rutin in common solvents and solvent-water mixtures has been determined at room temperature and near boiling temperatures. Data are presented showing the formation of rutin solvent complexes. Rutin of high purity, free of quercetin and other flavonoids, has been prepared using this principle. Values for the optical rotation of rutin in various solvents and water-solvent mixtures are given.

SINCE the first report by Griffith, Couch, and Lindauer (1) that rutin is effective in the treatment of spontaneous increased capillary fragility in humans, numerous articles have appeared on its pharmacology and clinical applications. These have recently been summarized (2). Although rutin has been known for over a century, very little has been published on its physical properties. Some of its chemical properties have been brought to light in the elucidation of its structure (3, 4).

Rutin has been isolated and prepared in the pure state from over sixty species of plants. However, the techniques used did not lend themselves to large-scale production of a pure substance for medicinal use. During our development of a successful method for the extraction and purification of rutin from domestic plants (9) it became necessary to acquire considerable data concerning its physical properties. There was a special need for information concerning the approximate solubilities of rutin in water, common organic solvents, and water-solvent mixtures in order to develop optimum conditions for extraction and purification. This paper is a report of some of the studies made on the solubility of rutin in various solvents, its purification, the formation of solvates of crystallization with organic solvents, and its optical rotation in various solvents.

EXPERIMENTAL

Approximate Solubility of Rutin in Various Solvents

Procedure.—An excess of finely powdered rutin of N. F. IX (5) quality, dried eighteen hours at 110° [not anhydrous (10)], was added to 100 cc. of solvent (technical grade) and refluxed for ten minutes. The undissolved rutin was removed by filtering the mixture while hot through a sintered glass disc, using applied pressure to prevent concentration of the filtrate by evaporation.

The amount of rutin dissolved was determined by evaporating the solvent from an aliquot, moistening the residue with water to remove traces of solvent, and finally drying at 110°. Although this procedure does not give the solubility at the exact boiling temperature, it does give a value sufficiently close to the boiling point to be of practical use. To determine the solubility at room temperature, the remainder of the filtrate was placed in a stoppered flask and left at 25–27° for three days. All samples from which rutin precipitated on standing were filtered, and the rutin remaining in solution was determined by evaporating an aliquot to dryness as described above. The data obtained for various solutions of methanol, ethanol, isopropanol, and acetone with water are plotted in Figs. 1–4.

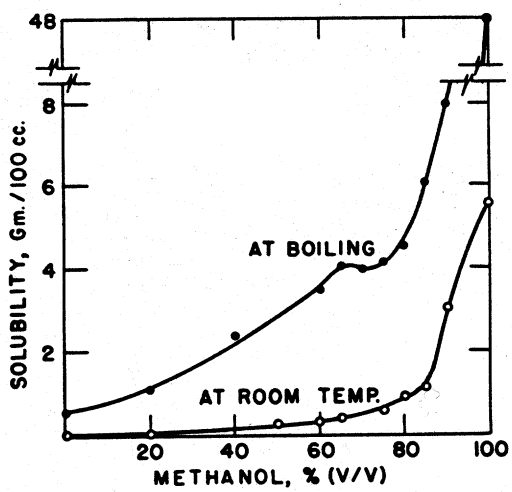

Fig. 1.—Approximate solubility of rutin in aqueous methanol.

Solubility of rutin in water below boiling was determined by maintaining the suspension of rutin in a water bath with stirring for four to six hours. Approximate values for solubility at elevated temperatures were obtained by treating under increased pressures as follows:

The rutin was suspended in water in a sintered glass Büchner funnel which was fitted to an Erlenmeyer flask. This was heated in a steam autoclave for seventy-five minutes. During the heating, steam displaced the air in the flask which, in escaping through the sintered glass, agitated the rutin solution. When the autoclave was turned off, condensation of the steam in the flask created a vacuum and automatically filtered the rutin solution at the elevated temperature. Data obtained

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at various temperatures are plotted on the curve shown in Fig. 5.

Discussion.—The solubility curves (Figs. 1–4) with the organic solvents near the boiling point showed two maxima. One occurred in the anhydrous solvent and the other in water solutions containing 50–70% solvent. The minimum solubility was with solvents of 70 to 98% concentration. Of the four solvents compared, rutin was most soluble in anhydrous methanol, and was soluble in decreasing amounts in strong ethanol, isopropanol, and acetone. Rutin did not precipitate from solutions of ethanol and acetone in the range of the minimum solubility when cooled to room temperature. Thus, values could not be obtained by this procedure for the solubility of rutin at room temperature in these solvents at these concentrations. This is illustrated by the breaks in the curve shown in Figs. 2 and 4. Also, rutin did not precipitate from strong isopropanol (98–99%) solutions, and the break in the curve occurred near the maximum solubility (Fig. 3). Rutin precipitated from solutions of 96–99% ethanol only when seeded with rutin ethanolate (6). However, rutin did not precipitate from a 95% ethanol solution even after seeding with either rutin ethanolate or rutin hydrate. For practical purposes, rutin is insoluble at room temperature in aqueous solutions containing less than 30% solvent.

It is to be noted that the solubility of rutin in water was influenced very greatly by the temperature, especially near the boiling point. Elevating the temperature by raising the pressure to 15 p. s. i. increased the solubility threefold.

Some additional data have been obtained on the solubility of rutin in other solvents. Rutin was

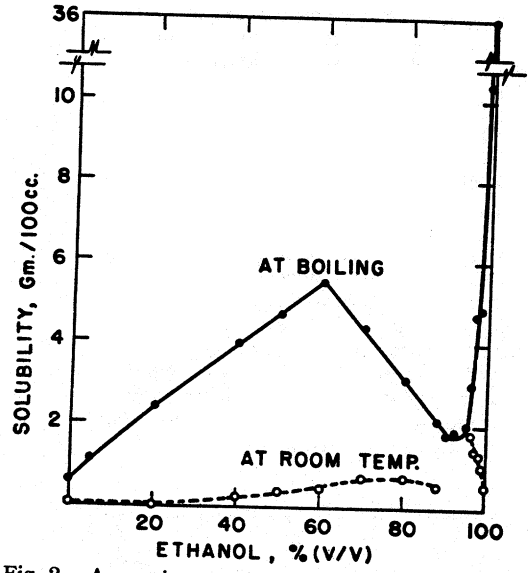

Fig. 2.—Approximate solubility of rutin in aqueous ethanol.

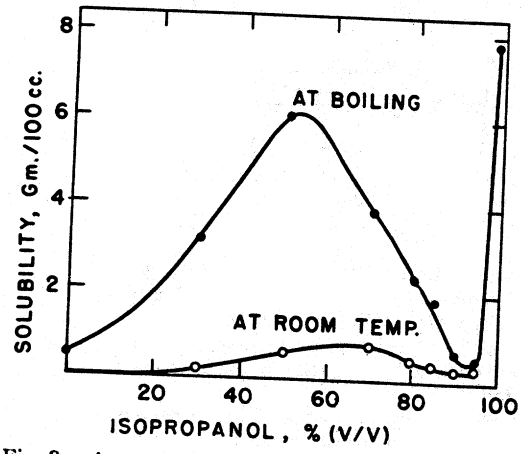

Fig. 3.—Approximate solubility of rutin in aqueous isopropanol.

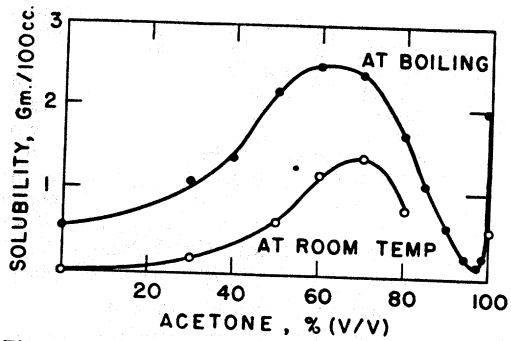

Fig. 4.—Approximate solubility of rutin in acetone.

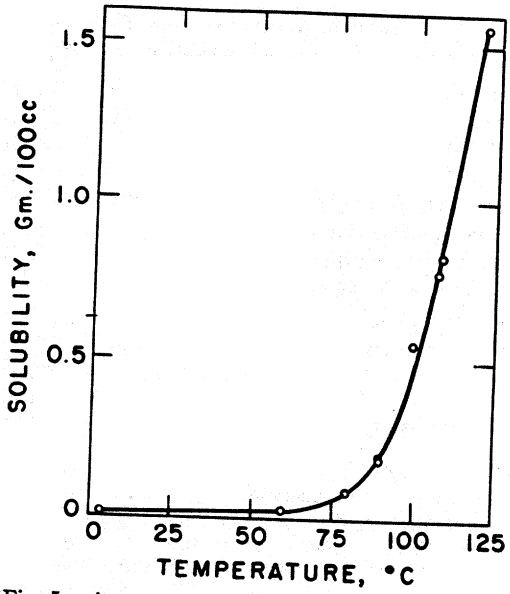

Fig. 5.—Approximate solubility of rutin in water.

TABLE I.—APPROXIMATE SOLUBILITIES OF RUTIN IN WATER AND VARIOUS ANHYDROUS SOLVENTS AT ROOM TEMPERATURE AND NEAR THE BOILING POINT

Solvent	Sol. at Room Temp., Gm./100 cc.	Sol. near Boiling Temp., Gm./100 cc.
Water	0.013	0.50–0.55
Methanol	5.5	48.0
Ethanol	0.55	36.0
Isopropanol	1.45	7.0
n-Propanol	0.60	4.0
n-Butanol	...	70.0
Acetone	0.56	2.0
1,4-Dioxane	...	13.6
Pyridine	37.3	...

exceedingly soluble in hot glycerol (U. S. P., 98% strength) and in ethylene glycol. No attempt was made to dissolve more than 50 Gm./100 cc. of glycerol because of difficulties in obtaining uniform heating. Rutin crystallized readily from diluted glycerol solutions on cooling. It was soluble to the extent of 1.2, 1.4, 1.7, 3.8, and 14.7 Gm./100 cc. in 15, 20, 25, 40, and 72% (v/v) boiling aqueous glycerol solutions, respectively. Boiling *n*-butanol (technical) dissolved 70 Gm. of the rutin used per 100 cc. (The solution gelled on cooling; *anhydrous* rutin may be even more soluble.) The presence of water in the *n*-butanol greatly depressed the solubility. This solvent, to which 5% water was added, dissolved only 0.4 Gm./100 cc., while *n*-butanol saturated with water dissolved 0.7 Gm./100 cc. Boiling absolute *n*-propanol dissolved 4.0 Gm./100 cc., but this alcohol dissolved only 0.6 Gm./100 cc. at room temperature. Boiling 1,4-dioxane (technical) dissolved 13.6 Gm./100 cc. In Table I are listed solubilities of rutin in some of the more important solvents.

Rutin was soluble in such solvents as pyridine, acetic acid, and solutions of inorganic bases. In pyridine it was extremely soluble at room temperature, 37.3 Gm./100 cc. Glacial acetic acid behaved toward rutin in a manner which suggested that it also formed a solvate with rutin.[1]

Rutin has been found insoluble in ether, benzene, toluene, petroleum-ether, carbon tetrachloride, chloroform, chlorobenzene, ethylene dichloride, and 1-nitropropane; it was slightly soluble in butyl acetate at boiling temperatures.

Solvents of Crystallization

No data are available in the literature for rutin on the existence of other solvents of crystallization in addition to water of hydration. This may be because rutin, when not highly purified, does not crystallize from ethanol with ease unless it is seeded with the alcoholate, and then only from solutions containing more than 96% ethanol by volume. During the course of removing alcohol insolubles (7–9) from rutin, an alcoholic solution of rutin suddenly precipitated crystals and quickly set to a solid mass. The product so formed proved to be rutin alcoholate, i. e., rutin with ethanol of crystallization. This product when isolated dissolved easily and abundantly in cold water, a spectacular difference from rutin's normal behavior with water. However, rutin persisted in this solubilized condition only a short time; then the water-insoluble hydrate formed and precipitated out.

Investigation of other solvents showed that rutin crystallized in the same manner from absolute methanol, from acetone, and from *n*-propanol with increasing order of difficulty. Rutin formed gels with *n*-butanol and isoamyl alcohol which did not crystallize: no precipitation of rutin has occurred from strong (98–99%) isopropanol solutions.

Procedure.—Purified rutin of N. F. IX (5) quality (10.0 Gm./100 cc. of solvent) was permitted to crystallize from the desired solvent. The crystals were collected on a Büchner funnel with gentle suction, and the wet cake was distributed into weighing bottles for drying. The quantities of various solvents retained under different drying conditions are presented in Table II.

Discussion.—It was difficult to remove organic solvents from rutin. Although the values obtained do not agree exactly with theory, they do indicate the existence of rutin as trisolvate. Rutin hydrate is practically tasteless, but the alcoholate is extremely bitter. This is in line with expectation for the taste of a glycosidic compound. The contrast is undoubtedly due to the extreme difference in solubility in cold water between the hydrate and alcoholate.

The effect of solvent strength on the preparation of rutin ethanolate was investigated in some detail. Aqueous solutions containing 96% (v/v) or more of ethanol yielded rutin ethanolate while those below 90% ethanol yielded the hydrate. Solutions of 90 to 95% (v/v) ethanol saturated with rutin at boiling temperatures did not precipitate rutin in any form even when seeded with ethanolate or hydrate at 5°.

Purification of Rutin

The fact just reported that rutin forms solvates with various organic solvents led to a new method for the preparation of highly purified rutin (6). Buckwheat rutin (N. F. quality) prepared by the present accepted procedure (5) contains small amounts of quercetin and other related flavonoids. These minor flavonoids interfered in the spectrophotometric analysis, and values greater than 100% were obtained for the sum of rutin and quercetin. Repeated recrystallizations from water or aqueous solvents as the hydrate did not significantly reduce the quantities of these impurities. However, when rutin crystallized as the solvate, the major portion of the impurities remained in the organic solvent solution. The separation and identification of these minor flavonoids will be the subject of a later paper.

The effect on the purity of rutin when samples were crystallized from technical absolute methanol, ethanol, *n*-propanol, and acetone is shown in Table III.

[1] When glacial acetic acid was added to 2.000 Gm. of rutin (moisture, 7.7%) burette-wise with heating under reflux, 15 cc. of the solvent completely dissolved the rutin at boiling temperature. Immediately after the rutin dissolved, a precipitate formed. The quantity of this precipitate increased with the addition of more solvent. Finally 44.1 cc. of acid was required to completely dissolve this precipitate; solubility of rutin on this basis was 4.2 Gm./100 cc. However, in a repeat trial where 20 cc. of glacial acetic acid was run in all at once and the solution was boiled, the rutin dissolved completely. Addition of more acetic acid did not cause any precipitation nor did a precipitate form on cooling; approximate solubility on this basis, 9.2 Gm./100 cc.

TABLE II.—REMOVAL OF ORGANIC SOLVENTS FROM RUTIN BY DRYING

	Drying Conditions	Solvent Retained by Solvated Rutin %	Solvent Retained by Solvated Rutin Moles/Mole
Ethanol [a]	Desiccator, $CaCl_2$, room temp., 2 mo.	17.1	2.7
	Desiccator, H_2SO_4, room temp., 2 mo.	6.9	1.0
	Vacuum oven, 65°, 48 hr.	6.3	0.9
	Mech. conv. oven, 110°, 48 hr.	3.6	0.5
Methanol [b]	Vacuum desiccator, $CaCl_2$, room temp., 10 days	14.0	3.1
	Vacuum desiccator, H_2SO_4, room temp., 10 days	1.6	0.3
	Vacuum oven, 65°, 48 hr.	0.4	0.1
Acetone [c]	Acetone atmosphere, 3 days	31.0	3.6
	Vacuum, room temp., 3 days	6.2	0.7
	Vacuum, room temp., 8 days	4.6	0.5
	Vacuum, room temp., 15 days	4.2	0.5
	Vacuum, room temp., 22 days	3.9	0.5

[a] Ethanol content determined, using the technique of Zeisel (15).
[b] Methanol by difference after determination of rutin, using spectrophotometric method (11).
[c] Acetone determined by method of W. M. Marriott (16).

Procedure.—The procedures used for these purifications are described below:

Rutin Methanolate.—Ten grams of rutin dried at 110° was dissolved by heating in 50 cc. of absolute methanol and filtered through a heavy filter pad.[2] The pad was washed with 10 cc. of solvent. Crystallization took place immediately on cooling. Crystals were filtered off after two hours of standing at room temperature; they were slurried with solvent and filtered with suction, and the filter cake was added to 400 cc. of boiling water. The product dissolved immediately, then quickly precipitated as the rutin hydrate. The latter was recovered by filtration after standing sixteen hours and dried at 110°; yield was 5.2 Gm. (52%). Upon evaporation, the mother liquor and washings yielded 4.1 Gm. of additional rutin.

Rutin Ethanolate.—Technique was repeated as described above for methanol except that 5.0 Gm. of dried rutin and absolute ethanol was used. Crystallization occurred immediately when the cooled solution was seeded with rutin ethanolate. Also, the rutin ethanolate cake was placed in 100 cc. of boiling water for hydrate formation. The yield of purified rutin was 3.7 Gm. (74%).

This procedure was applied successfully to the purification of more than 20 kilogram-batches. Yields ranged from 745 to 924 Gm. Concentration of the mother liquors yielded from 50 to 200 Gm. of additional rutin of inferior quality to the main crop.

Rutin n-Propanolate.—The technique was the same as that described for methanol except that only 2 Gm. of rutin was used and crystallization occurred after 7 days of standing at room temperature. In the hydrate preparation step, 150 cc. of boiling water was used; yield was 1.6 Gm. (80%). However, this was still impure as shown by data in Table III.

Rutin Acetonate.—Five grams of dried rutin was dissolved in 300 cc. of acetone (99.5%) and filtered. The product crystallized on cooling in the form of extremely long thin needles. The hydrate was prepared by dissolving the acetonate thus formed in 150 cc. of boiling water in which the acetonate was immediately soluble; yield of rutin was 3.5 Gm. (70%).

Discussion.—Of the four solvents used in purification of rutin, ethanol and acetone gave the purest products. The yields were good since the second crop of rutin obtained from mother liquors may be reprocessed. Ethanol is the most practical of these two to use because the solubility of rutin is greater in ethanol than in acetone and the amount of moisture present in the solvent is not as critical. The solubility of rutin in absolute ethanol is greater than 10%, but more concentrated solutions were difficult to handle especially after the laboratory became seeded with alcoholate. When the laboratory did become seeded, the alcoholate precipitated from absolute alcohol containing more than 10% rutin, even while the solution was boiling under reflux.

Purification of rutin by alcoholate procedure is now in operation on a commercial scale for the production of highly purified rutin required to make soluble preparations of rutin for parenteral use. Rutin prepared through the alcoholate procedure was the only rutin we have obtained which did not discolor when exposed to light; rutin recrystallized 15 times from aqueous solvents became discolored.

Optical Rotation of Rutin

Buckwheat rutin of N. F. IX (5) quality was specially purified for this work by three recrystallizations through the alcoholate procedure as described in the previous section. A determination of its purity was made by spectrophotometric analysis (11) and found to be 100.1% rutin and 0.0% quercetin. Since anhydrous rutin cannot be weighed accurately the sample was equilibrated with air-moisture (a moisture determination showed 7.7%). The solvents used for dissolving the rutin were C. P. reagents. Pyridine was further purified by twice distilling, using only center fractions; the

TABLE III.—EFFECT OF RECRYSTALLIZATION FROM ORGANIC SOLVENTS ON PURITY OF RUTIN[a]

Recrystallized From	Spectrophotometric Analysis Rutin, %	Spectrophotometric Analysis Quercetin, %
Methanol	98.3	1.4
Ethanol	100.0	0.0
n-Propanol	100.6	1.3
Acetone	100.5	0.0

[a] Original rutin analyzed 97.3% rutin and 4.3% quercetin

[2] Either the S-1 or S-3, Republic-Seitz Filter Corp., Newark, N. J., is suitable. (Mention of this company does not constitute an endorsement of its products by the Department of Agriculture to the detriment of other companies not mentioned.)

TABLE IV.—OPTICAL ROTATION OF RUTIN (MOISTURE CONTENT 7.7%) IN VARIOUS SOLVENTS AND SOLVENT-WATER MIXTURES

Solvent	Solvent Strength, % by Wt.	Optical Rotation $[\alpha]_D^{75° \pm 1° F.}$ at Rutin Concentration of 0.5000 Gm./100 cc.	1.000 Gm./100 cc.	2.000 Gm./100 cc.
Ethanol	99.7	+11.46	+12.19	+13.26
Ethanol	95.0	+ 6.77	+ 7.58	...[a]
Ethanol	49.9	− 9.10	...[a]	...[a]
Methanol	99.8	+ 4.63	+ 5.35	+ 6.68
Methanol	49.9	− 1.52	...[a]	...[a]
Isopropanol	98.2	+ 3.52	...[a]	...[a]
Isopropanol	49.1	− 4.60	...[a]	...[a]
n-Butanol	99.9	+20.77	+18.46	+18.25
Acetone[b]	49.6	−16.03	−11.02	...[a]
1,4-Dioxane	100.0	+21.37	...[a]	...[a]
1,4-Dioxane	50.0	− 9.21	−14.73 (−11.98)[c]	−12.52[c]
Pyridine[d]	100.0	−38.35	−38.83	−40.01
Pyridine	80.0	−41.98	−47.10	−48.12
Pyridine	25.0	−33.47	−34.56	−34.06
Acetic acid	99.8	+42.98	+42.25	+37.97
Acetic acid	49.9	− 3.63	...[a]	...[a]

[a] Indicates rutin not soluble to this extent.
[b] Acetone, 99.1%, would not dissolve this rutin in sufficient quantity to make a 0.5% solution.
[c] Values with technical grade 1,4-dioxane.
[d] Since pyridine-rutin solutions react with metals, contact with metal parts of the polarimeter tube must be avoided.

1,4-dioxane was purified according to the method of Fieser (12). The percentage water by weight in each was determined by use of the Karl Fischer reagent.

Procedure.—When possible, a 2% solution of rutin was prepared by dissolving 1.0000 Gm. of rutin in 40 to 45 cc. of solvent (warming when necessary) and, after cooling, diluting to 50 cc. in a volumetric flask. One per cent and 0.5% solutions were prepared by dilution (pipetting 25 cc. and diluting to 50 cc. in a volumetric flask). Where rutin was not sufficiently soluble in a solvent to make a 2% solution, the correct quantity of rutin was weighed directly to prepare the 1% or 0.5% solution. All measurements were made with a sodium light, using a 4-dm. tube in a Bellingham-Stanley polarimeter. The results obtained with a variety of solvents and water solutions of the solvents are presented in Table IV.

Discussion.—The few published values for the optical rotation of rutin are at variance. This disagreement is no doubt attributable to the impure condition of the rutin samples used. Sosa and Plouvier (13) made a study of the optical rotation of *Forsythia viridissima L.* rutin in various solvents but purified the rutin by only three recrystallizations from aqueous solvents. Our experience with *Forsythia* rutin (14) would indicate that their rutin was not free of quercetin and other contaminants (unidentified sterol-like material). In general, their results with "anhydrous rutin" in the solvents pyridine, ethanol, methanol, and dioxane are slightly lower than ours.

From our data the following conclusions with respect to optical rotation may be suggested: of the solvents tried, rutin gives the largest angle of rotation in pyridine (80 or 100%) and glacial acetic acid, and these solvents appear to be the best for measuring optical rotation. With these, 0.5000 to 2.000 Gm./100 cc. concentration of rutin is satisfactory, especially when a 4-decimeter tube is used. The third choice solvent is *n*-butanol in the same concentration range. Rutin was found to be dextrorotatory in neutral (and anhydrous acid) solvent and levorotatory in dilute neutral, acid, and basic[3] solvents.

SUMMARY

The approximate solubility of rutin in various common solvents such as water, methanol, ethanol, isopropanol, and acetone is reported. Curves for the solubility of rutin in water-solvent mixtures are given for solutions at room temperature and near the boiling point. One maximum solubility occurred in the anhydrous solvent, and another at solvent concentration of 50–80%. The minimum solubility occurred at a solvent concentration of 90–98%. Some data are given on the solubility of rutin in glycerol, *n*-propanol, *n*-butanol, 1,4-dioxane, pyridine, and glacial acetic acid.

Data are presented to illustrate the formation of solvates of rutin with methanol, ethanol, *n*-propanol, and acetone. Use has been made of this phenomenon in the development of a method for the purification of rutin. The method is specially effective in the removal of quercetin and minor flavonoid constituents, and is useful in the preparation of rutin suitable for paranteral injection.

The optical rotation of rutin in methanol, ethanol, isopropanol, *n*-butanol, acetone, 1,4-dioxane, pyridine, and glacial acetic acid has been determined. Readings were made with rutin dissolved in pure solvents and water solutions. Rutin was dextrorotatory in neutral and acidic solvents. Dilution with water de-

[3] Earlier in our work on rutin, levorotatory values were obtained for morpholine; these are not reported because the rutin used was not of high purity.

creased the rotation; in 50% dilution, rotation is levorotatory. Rutin was levorotatory in the alkaline solvents used.

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