Spectrophotometric Investigation of Uranil(II)−Rutin Complex in 70% Ethanol

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The composition and stability constant of a $UO_2(II)$-rutin complex in 70% ethanol were determined by suitable spectrophotometric methods and pH measurement. $UO_2(II)$ ion and rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) form a 1:1 complex in which the $UO_2(II)$ ion is linked to rutin through the carbonyl and 5-hydroxyl group. The concentration stability constant of the complex, log β_1, ranged from 6.57 at pH 4.00 to 4.72 at pH 7.00. Conditions for spectrophotometric determination of rutin, by complex formation with $UO_2(II)$ ion, were investigated. Beer's law was obeyed in the concentration range from 1.0×10^{-5} to 2.0×10^{-4} M for rutin. Determination of rutin in Rutinion forte tablets was demonstrated.

INTRODUCTION

Rutin belongs to the group of flavonoids, which are very important plant phenolic compounds, occurring in various kinds of foods (fruits and vegetables) and beverages (coffee, beer, tea, and fruit juices). These compounds display a remarkable array of biological and pharmacological activities: antioxidant, antiinflammatory, antimicrobial, anticancer, cardiovascular protection, etc.; owing to such properties, flavonoids are also used in pharmaceutical preparations.

Rutin ($C_{27}H_{30}O_{16}$) is a glycosidic flavonoid containing four hydroxyl groups in positions 5, 7, 3′, and 4′ and a carbonyl group in position 4 (Figure 1a). This structure enables formation of complex compounds with metals, many of which have been investigated since the 1970s (Katyal and Prakash, 1997). We have also investigated several rutin complexes (Malešev and Radović, 1984−1991). Investigations of these metal−rutin complexes, besides their fundamental significance, may be of considerable importance in the determination of rutin in food, beverages, and drugs. Moreover, interactions of flavonoids with metals occurring naturally in the metabolism, as well as with metals introduced as drugs, influence their biological activities.

Considering uranil's ability to form stable complex compounds with some flavonoids (Malešev et al., 1990), we have investigated properties of the $UO_2(II)$−rutin complex. Fundamental investigations of the $UO_2(II)$−rutin complex, compared to other investigated metal−rutin complexes, revealed that this complex is suitable for quantitative determination of rutin. Therefore, the aim of the present work, besides investigating the complex, is to optimize the complexing reaction and develop a simple spectrophotometric method for rutin determination. Application of this method for rutin determination in tablets is presented.
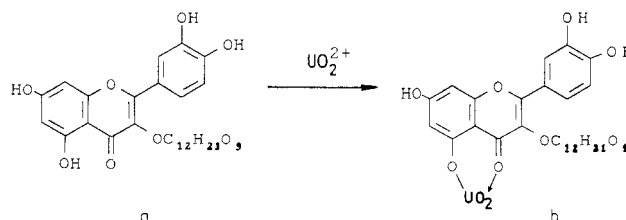

Figure 1. Structure formulas of (a) rutin and (b) $UO_2(II)$−rutin complex.

EXPERIMENTAL PROCEDURES

Spectrophotometric measurements were performed on a Beckman DU-650 spectrophotometer. A quartz cuvette with a 1 cm optical path length was used. For pH measurements a Radiometer pHM 28 pH-meter with a saturated calomel−glass electrode was used. IR spectra of the sample in KBr pellets were recorded on a 983 G Perkin-Elmer spectrophotometer.

The reagents used were $UO_2(NO_3)_2$, ethanol, HNO_3, NaOH (all Merck); $NaNO_3$ (Mallinckrodt Chemica); and rutin (Fluka AG), recrystallized three times from ethanol. Rutinion forte tablets from biomo Natur-Medizin, GmbH, were used.

Because rutin is not soluble in pure water and $UO_2(NO_3)_2$ is not soluble in pure ethanol, 70% ethanol was selected as the most suitable solvent (Malešev and Radović, 1984−1991).

The pH was adjusted by addition of 2.0 M HNO_3 and/or 2.0 M NaOH, and the ionic strength of the final solutions was kept constant at $I = 0.01$ M by addition of 1.0 M $NaNO_3$.

The calibration curve for rutin was obtained from a series of standard solutions of the $UO_2(II)$−rutin complex. In the standard solutions, the amount of rutin was varied from 1.0×10^{-5} to 1.0×10^{-3} M, whereas the concentration of UO_2-$(NO_3)_2$ was kept constant at 2.0×10^{-3} M. Sample solutions were prepared from Rutinion forte tablets in the following way. Ten tablets were weighed and pulverized. The average weight of one tablet is weighed out and dissolved in ethanol, shaken for 15 min in an ultrasonic bath, and filtered through Whatman No. 1 filter paper. Rutin from the sample solution is
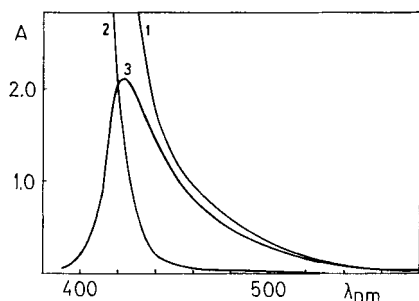
Figure 2. Absorption spectra: (curve 1) mixture of 1.75×10^{-4} M $UO_2(NO_3)_2$ and 3.50×10^{-3} M rutin, blank is 70% ethanol; (curve 2) 3.50×10^{-3} M rutin, blank is ethanol; (curve 3) $UO_2(II)$–rutin complex.

afterward complexed with the same concentration of UO_2-$(NO_3)_2$ as in the standard solutions.

RESULTS AND DISCUSSION

Absorption Spectra. The $UO_2(II)$ ion and rutin react in 70% ethanol, forming a characteristic yellow-orange complex. In Figure 2, we present the absorption spectra of a mixture of rutin and $UO_2(NO_3)_2$ (curve 1), rutin (curve 2), and the $UO_2(II)$–rutin complex (curve 3). Spectra were recorded from 400 to 500 nm, where $UO_2(NO_3)_2$ has no significant absorbance in the investigated concentration range. Because the absorption of rutin is negligible beyond 470 nm, where the complex still absorbs significantly, all following measurements are performed at 475 nm against 70% ethanol as blank.

Effect of pH. Dependence of the absorption spectra of the $UO_2(II)$–rutin complex on pH was investigated in the pH range from 2.80 to 8.00 (Figure 3). It can be seen that the shape of the absorption bands and the position of maxima are unchanged in the pH range from 2.80 to 5.43 (curves 1–6). However, at higher pH values the absorption maxima show a bathochromic shift (curves 7–10). Because it is possible that the bathochromic shift may be due to formation of complexes with different stoichiometric compositions at higher pH values, the composition of the $UO_2(II)$–rutin complex was determined at three pH values.

Composition of the Complex. Composition of the complex at pH 3.60, 7.00, and 8.00 was determined according to two distinct methods: the method of continual variations of equimolar solutions (Irving and Pierce, 1959) and the molar ratios method (Yoe and Jones, 1944).

In the first method, mixed solutions of $UO_2(NO_3)_2$ and rutin with constant total concentrations $c_0 = 2.0 \times 10^{-3}$ M at pH 3.60 and $c_0 = 2.0 \times 10^{-4}$ M at pH 7.00 and 8.00 were used. As can be seen from Figure 4, all obtained Job's curves had a maximum at $x_{UO_2}(II) = 0.50$, indicating the formation of a 1:1 $UO_2(II)$–rutin complex at all investigated pH values.

In the second method, solutions containing a constant $UO_2(II)$ concentration and varied rutin concentrations were used. At pH 3.60 the concentration of $UO_2(II)$ was 6.0×10^{-4} M and the concentration of rutin was varied from 4.0×10^{-4} to 1.6×10^{-3} M. At pH 7.00 and 8.00 the concentration of $UO_2(II)$ was 1.2×10^{-5} M and the concentration of rutin was varied from 8.0×10^{-6} to 3.2×10^{-5} M. As can be seen from Figure 5, straight lines showing $A = f(c_{Rut}/c_{UO_2})$ intercept at $c_{Rut}/c_{UO_2} = 1$ at all investigated pH values. This indicates that the stoichiometric ratio of $UO_2(II)$–rutin = 1:1 in the complex at all investigated pH values.
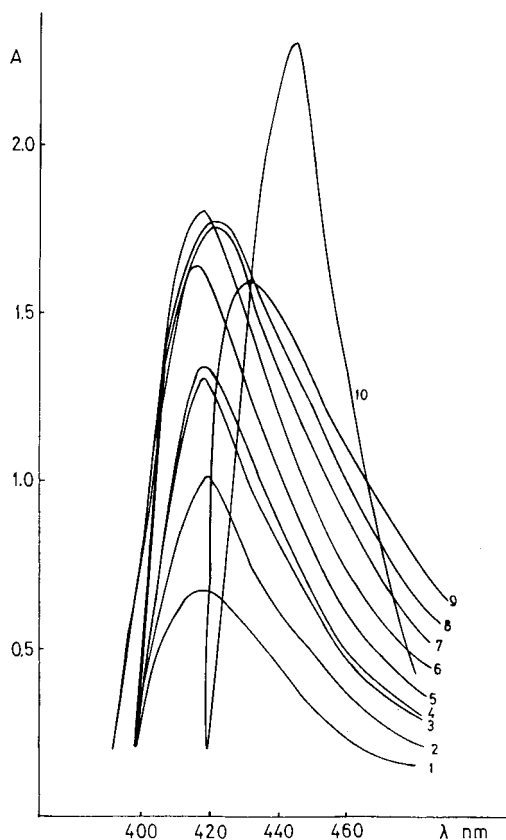

Figure 3. Absorption spectra of the $UO_2(II)$–rutin complex at different pH values, $c_{Rut} = 2.0 \times 10^{-3}$ M, $c_{UO_2} = 1.0 \times 10^{-4}$ M: (curve 1) pH 2.81; (curve 2) pH 3.29; (curve 3) pH 3.85; (curve 4) pH 4.27; (curve 5) pH 4.75; (curve 6) pH 5.43; (curve 7) pH 6.48; (curve 8) pH 6.83; (curve 9) pH 7.41; (curve 10) pH 8.01.
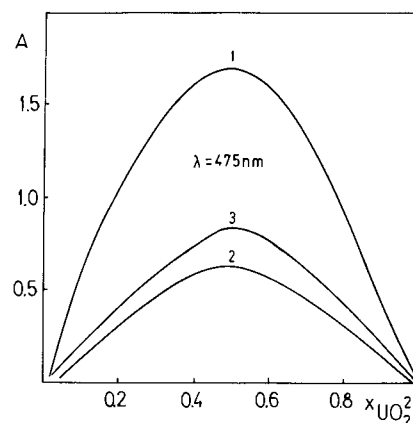

Figure 4. Method of continual variations of equimolar solutions: (curve 1) pH 3.60, $c_{UO_2} = c_{Rut} = 2.0 \times 10^{-3}$ M; (curve 2) pH 7.00, $c_{UO_2} = c_{Rut} = 2.0 \times 10^{-4}$ M; (curve 3) pH 8.00, $c_{UO_2} = c_{Rut} = 2.0 \times 10^{-4}$ M.

Because both methods confirm the formation of a 1:1 $UO_2(II)$–rutin complex at all investigated pH values, the bathochromic shift is not due to formation of different complexes. Rather, the bathochromic shift is due to the dissociation of the complex.

Reaction of Complex Formation. In the investigated solutions rutin reacts in the dissociated form with uranil ions, forming a complex compound. pH measurements in the following solutions revealed that in UO_2-$(NO_3)_2$ solution, $c = 2.50 \times 10^{-3}$ M, pH 2.65; in rutin solution, $c = 2.50 \times 10^{-3}$ M, pH 7.18; whereas in a mixed solution containing $UO_2(NO_3)_2$ and rutin in the same

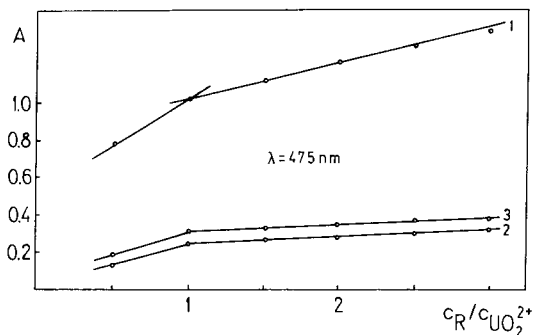Spectrophotometric Investigation of Uranil(II)–Rutin Complex

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Figure 5. Method of molar ratios: (curve 1) pH 3.60, mixture of 6.0×10^{-4} M $UO_2(NO_3)_2$ and rutin (from 4.0×10^{-4} to 1.6×10^{-3} M); (curve 2) pH 7.00, mixture of 1.2×10^{-5} M $UO_2(NO_3)_2$ and rutin (from 8.0×10^{-6} to 3.2×10^{-5} M); (curve 3) pH 8.00, 1.2×10^{-5} M $UO_2(NO_3)_2$ and rutin (from 8.0×10^{-6} to 3.2×10^{-5} M).

concentrations as in the above-mentioned individual solutions, pH 2.70. Therefore, the reaction of complex formation might be presented as

$$UO_2^{2+} + C_{27}H_{29}O_{16}H \leftrightarrow [UO_2(C_{27}H_{29}O_{16})]^+ + H^+$$
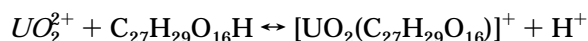
The obtained complex is positively charged, which was ascertained by electrophoresis: the complex is moving toward the negative electrode.

Infrared Spectra of Rutin and the $UO_2(II)$–Rutin Complex. According to the literature (Kohara and Ishibashi, 1967; Paleckite and Finkelsteinaite, 1969), there are two possible places where uranil ions can be bound to rutin: either between the 5-hydroxyl and the carbonyl group or between the 3′- and 4′-hydroxyl groups. To find the position where uranil ion is actually linked to rutin, IR spectra of rutin and of the isolated complex were recorded. In the IR spectrum of rutin a strong band at 1660 cm^{-1} and weak one at 1600 cm^{-1} are observed. The band at 1660 cm^{-1} is assigned to the carbonyl group hydrogen bonded to the hydroxyl group in position 5, because free carbonyl groups absorb at ~50 cm^{-1} toward higher wavenumbers. However, in the spectrum of the $UO_2(II)$–rutin complex these bands have disappeared and one strong band at 1620 cm^{-1} is observed, indicating that the uranil ion forms the complex with rutin through the carbonyl group and the 5-hydroxyl group (Figure 1b).

Stability Constant of the Complex. For calculation of the concentration stability constant, β_1, of the complex, besides the forthcoming equations, Bjerrum's method (Inczedy, 1976) was used. At different pH values, the absorbances of a mixture containing 1.00×10^{-4} M $UO_2(NO_3)_2$ and 2.00×10^{-3} M rutin and of a solution containing 2.00×10^{-3} M rutin alone were measured. Two curves were obtained, and from them the curve of the complex absorption $\Delta A = f(pH)$ was calculated (Figure 6). The highest concentration of the complex was found at pH 7.4. It may be assumed that at this pH the concentration of the complex is approximately equal to the total $UO_2(NO_3)_2$ concentration, $[complex] \approx [UO_2^{2+}]_0$, in view of the fact that the concentration of rutin in the solution is 20 times higher than the $UO_2(NO_3)_2$ concentration. Therefore, the molar absorption coefficient of the complex was calculated from the expression
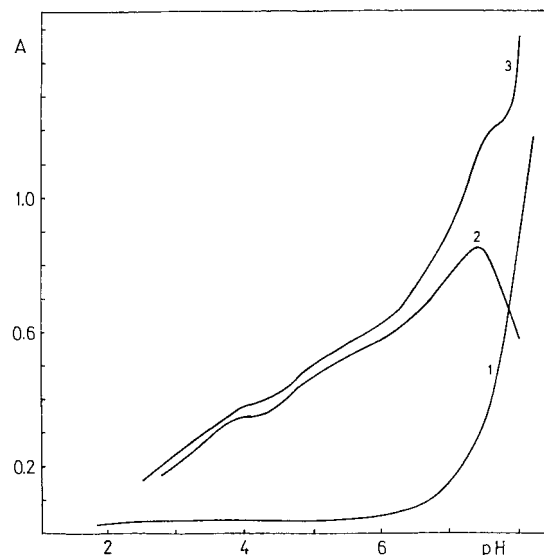
$$a = A_{max}/[UO_2^{2+}]_0 \tag{1}$$

Figure 6. Dependence of absorbance on pH at $\lambda = 475$ nm: (curve 1) 2.0×10^{-3} M rutin, blank is ethanol; (curve 3) mixture of 1.0×10^{-4} M $UO_2(NO_3)_2$ and 2.0×10^{-3} M rutin, blank is 70% ethanol; (curve 2) $\Delta A = f(pH)$.

Table 1. Concentration Stability Constants of the $UO_2(II)$–Rutin Complex

pH	$[UO_2^{2+}]$, M	$[R^-]$, M	[complex], M	a, cm^{-1} M^{-1} L	$\log \beta_1$
4.00	5.9×10^{-5}	1.9×10^{-7}	4.1×10^{-5}	3200	6.57
5.00	4.6×10^{-5}	1.9×10^{-6}	5.4×10^{-5}	4600	5.79
6.00	3.3×10^{-5}	1.9×10^{-5}	6.7×10^{-5}	5700	5.04
7.00	1.0×10^{-5}	1.7×10^{-4}	9.0×10^{-5}	7500	4.72

The concentrations of the complex UO_2^{2+} and $C_{27}H_{29}O_{16}^-$ (denoted $[R^-]$) were calculated from equations

$$[complex] = A/a \tag{2}$$

$$[UO_2^{2+}]_0 = [UO_2^{2+}] + [complex] \tag{3}$$

$$[HR]_0 = [HR] + [R^-] + [complex] \tag{4}$$

$$k_{d_1} = [H^+][R^-]/[HR] \tag{5}$$

where HR denotes $C_{27}H_{29}O_{16}H$ and $k_{d_1} = 9.77 \times 10^{-9}$ is the first dissociation constant of rutin in 70% ethanol (Malešev et al., 1991). The concentration stability constant, β_1

$$\beta_1 = [complex]/[UO_2^{2+}][R^-] \tag{6}$$

is calculated for four different pH values (Table 1).

Quantitative Determination of Rutin. Formation of the complex between $UO_2(II)$ ion and rutin enables the quantitative determination of microamounts of rutin. Standard solutions were prepared as described under Experimental Procedures. At pH 3.80, a linear dependence of the absorbance versus rutin concentration was obtained in the concentration range from 1.0×10^{-5} to 2.0×10^{-4} M for rutin. By the least-squares method, the regression equation $A = 2602.4c_{rutin} + 0.02$ was calculated with a high correlation coefficient of $r = 0.99989$. The accuracy of the method was tested for three different rutin concentrations (Table 2).

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Table 2. Spectrophotometric Determination of Rutin[a]

taken, M	found, M	SD	CV, %
4.0×10^{-5}	4.0×10^{-5}	1.4×10^{-6}	3.5
1.0×10^{-4}	9.8×10^{-5}	2.6×10^{-6}	2.7
1.7×10^{-4}	1.7×10^{-4}	6.3×10^{-6}	3.7

[a] $n = 7$.

Table 3. Spectrophotometric Determination of Rutin in Rutinion Forte Tablets (100 mg)[a]

expected, mg	found x, mg	recovery, %	RSD, %
100	97.9 ± 0.7	97.9	0.75

[a] $n = 7$.

Determination of Rutin in Rutinion Forte Tablets. Stability of the $UO_2(II)$−rutin complex makes possible its application for the determination of rutin in Rutinion forte tablets. Tablet diluents and excipients do not interfere with the complexing reaction. Obtained results are summarized in Table 3.

Conclusion. $UO_2(II)$ ions and rutin form a 1:1 complex in 70% ethanol in a range of pH from 2.80 to 8.00, which is stable enough to be utilized in quantitative determination of rutin. Properties of the complex are investigated by pH measurements, UV−vis spectrophotometry, IR spectroscopy, and electrophoresis. On the basis of the obtained results, a spectrophotometric method for quantitative determination of rutin is proposed. Optimal conditions for rutin determination by this method are at wavelength $\lambda = 475$ nm and pH range from 3.00 to 6.00.

The suggested method, applied for rutin determination in tablets, gave reproducible results. Moreover, as the absorption measurements are shifted to longer wavelengths (visible region), interference with other substances absorbing in the UV region may be avoided.

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