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# Identification and Structural Assessment of Alkaline-Earth Metal Complexes with Flavonols by FAB Mass Spectrometry

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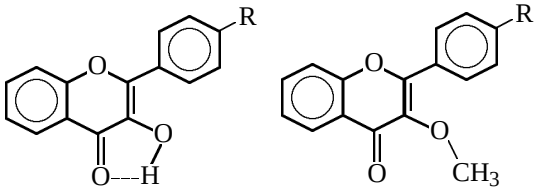
**Abstract** — Complexation of a series of flavonols with $Mg^{2+}$, $Ca^{2+}$, and $Ba^{2+}$ ions was studied by FAB mass spectrometry. The mass spectra of flavonol solutions containing magnesium ions revealed formation of 1 : 2 complexes $ML_2$, undetectable by spectrophotometric and spectrofluorimetric methods. Experiments with a crown-substituted flavonol revealed formation of not unly previously known chelate complexes MgL and $Mg_2L$, but also of the 1 : 2 complex $ML_2$ and crown complex MgHL. The barium ions form with the crown-substituted flavonol two types of "sandwich" complexes: $Ba(HL)_2$ and $Ba_2(HL)_2$. With the $Ca^{2+}$ ions, such complexes are not formed, and only the fragmentation product of the complex $Ca(HL)_2$ was detected.

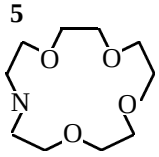
Flavonols are widely occurring vegetable dyes responsible for the yellow color of fruits and flowers [1]. Complexation of flavonols with metal ions enhances the color intensity and the fluorescence properties [2].

The differences between the spectral characteristics of flavonols and their complexes are used in analytical chemistry for determining the concentrations of $Al^{3+}$, $Sc^{3+}$, and $Th^{4+}$ ions [3–5], and also for identifying flavonols in vegetable extracts in the course of chromatographic separation [1, 6]. Recent studies showed that flavonols can be used as probes for cell membranes [7] and micelles [8], and also as agents for estimating the content of alkaline-earth metal ions in biological systems.

As investigation objects we chose the flavonols shown below. To select the best probes for determining the concentrations of alkaline-earth metal ions, we performed spectrophoto- and spectrofluorimetric studies of the structures of $Mg^{2+}$ and $Ba^{2+}$ complexes with these flavonols. These studies revealed formation of two types of complexes depending on the ion size [9–11]. The $Mg^{2+}$ ions form the chelate complexes ML by substitution of the hydroxyl hydrogen atom in the 3 position (structure **a**, see below). The larger $Ba^{2+}$ ions form "external" complexes MHL. In these species, the metal ion coordinates with the ligand via an "external" (not involved in hydrogen bonding with the 3-OH group) electron pair of the carbonyl oxygen atom (structure **b**). Experiments with 4'-monoaza-15-crown-5-flavonol **4** (hereinafter, crown-substituted flavonol) showed that complexation in this case occurs in two steps: complexes in which the metal coordinates with the crown ring are formed along with the "external" and chelate complexes [9].




**1–4** **5**

R = H (**1, 5**), $OCH_3$ (**2**), $N(CH_3)_2$ (**3**),  (**4**).

Unfortunately, the spectroscopic studies do not give a complete pattern of complexation of flavonols with alkaline-earth metal ions. For example, the 1 : 2 chelates $ML_2$ (structure **c**) were not revealed in solutions, although data on similar complexes with structurally related ligands can be found in numerous papers [2, 12–14]. Problems also arise when studying the $Ca^{2+}$ complexes. For example, in some cases, spectrophotometric methods do not allow assignment

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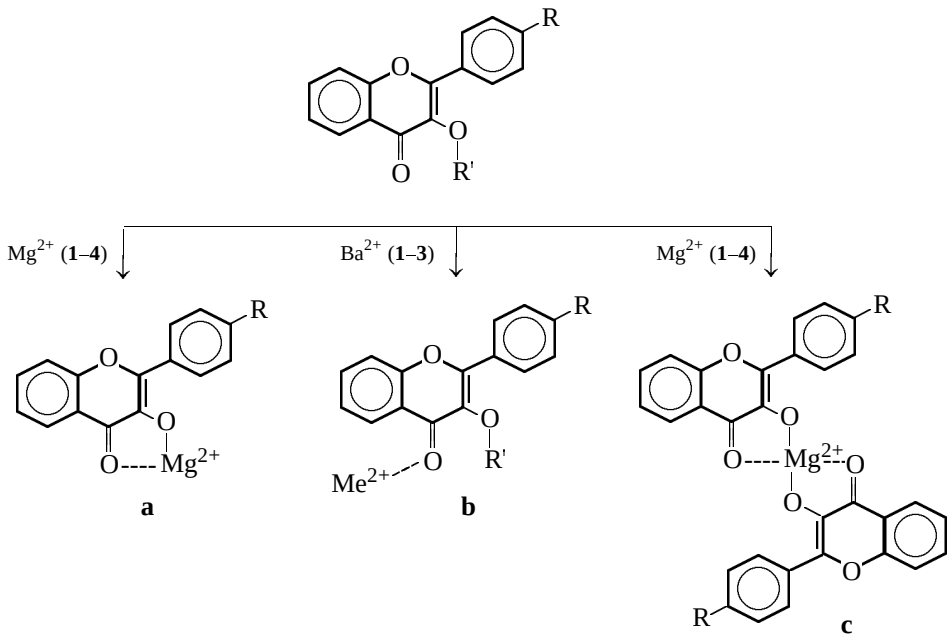



R' = H, $CH_3$.

of the calcium complexes (in particular, with crown-substituted flavonol) to any of types (**a**–**c**).

To refine the previously obtained results, we used fast atom bombardment (FAB) mass spectrometry. A matrix containing the complexes was bombarded with xenon atoms. This "nondestructive" mass-spectrometric method was already used previously for identifying some flavonols and their complexes [15–17] and for studying complexes of alkaline-earth metals with crown ethers [18–20], oxazolone metal complexes [21], and diacetonates [22, 23]. It should be noted that the stoichiometry of alkaline-earth metal complexes with crown rings and/or mixed ligands [21], determined by FAB mass spectrometry, was confirmed by other physicochemical methods. Furthermore, Johnstone *et al*. [18] suggested a procedure for estimating the stability constants by FAB mass spectrometry, namely, by determining the relative intensities of the peaks of the free ligand, complex, and a reference substance.

**Complexes of flavonols with $Mg^{2+}$ ions.** The mass spectra of flavonols **1**–**3** in the presence of low concentrations of $Mg^{2+}$ ions contain peaks of protonated ligand **I** and of ions **II** and **III** (Table 1). As the metal ion concentration is increased, the signals from the $[MgL_2 \cdot H^+]$ ion (**II**) rapidly disappear, and the signal of protonated flavonol gradually decreases in intensity. At the same time, the peak of ion **III**, $MgL^+$, grows in intensity. As the $Mg^{2+}$ concentration is increased further, a new peak of $[HL \cdot MgClO_4]^+$ (**IV**) appears, the $MgL^+$ peak somewhat grows in intensity, and the peak of flavonol **I** fully disappears.

We attribute the observed effects to two different possible processes. At high ligand concentrations and low concentrations of magnesium ions, favorable conditions are created for formation of the 1 : 2 complex $MgL_2$; peak of its protonated form **II** is observed in the spectrum. As the metal ion content is increased, the 1 : 2 complex dissociates, and the chelate $MgL^+$ is formed, giving peak **III** which grows in intensity.

$$2HL + Mg^{2+} + ClO_4 \longrightarrow [MgL_2 \cdot H]^+ + HClO_4, \quad (1)$$

$$[MgL_2 \cdot H]^+ + Mg^{2+} + ClO_4 \longrightarrow 2MgL^+ + HClO_4. \quad (2)$$

At high concentrations of magnesium perchlorate in solution, the concentration of $MgClO_4^+$ species grows. These species associate with the free ligand molecules remaining in the solution [reaction (3)]. We believe that, in this case, the $MgClO_4^+$ ions serve the same function as the hydrogen ions in solutions with low $Mg^{2+}$ concentrations [scheme (4)]:

$$HL + MgClO_4^+ \longrightarrow [HL \cdot MgClO_4]^+, \quad (3)$$

$$HL + H^+ \longrightarrow [HL \cdot H]^+. \quad (4)$$

The occurrence of reaction (3) is proved by similar behavior of the matrix peaks. At low $Mg(ClO_4)_2$ concentrations, peaks of protonated *m*-nitrobenzyl alcohol $[MNBA \cdot H]^+$ are observed, and at high concentrations, peaks of the associate $[MNBA \cdot MgClO_4]^+$ are ob-

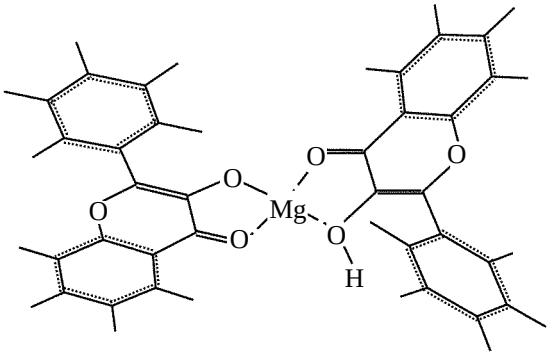
**Table 1.** Peak assignment in the mass spectra of flavonols **1**–**3** and **5** in the presence of metal ions

| Flavonol | Ion | *m/z* | Peak (structure) | Assignment |
|---|---|---|---|---|
| **1** | – | 239 | **I** | $[HL \cdot H]^+$ |
| | $MgL^+$ | 261 | **III, V** | $MgL^+$, $[HL \cdot MgClO_4 - HClO_4]^+$ |
| | | 499 | **II** | $[MgL_2 \cdot H]^+$ |
| | | 361 | **IV** | $[HL \cdot MgClO_4]^+$ |
| | $Ca^{2+}$ | 377 | **IV** | $[CaHL \cdot ClO_4]^+$ or $[HL \cdot CaClO_4]^+$ |
| | | 277 | **V** | $[CaHL \cdot ClO_4 - HClO_4]^+$ |
| | $Ba^{2+}$ | 475 | **IV** | $[BaHL \cdot ClO_4]^+$ or $[HL \cdot BaClO_4]^+$ |
| | | 375 | **V** | $[BaHL \cdot ClO_4 - HClO_4]^+$ |
| **2** | – | 269 | **I** | $[HL \cdot H]^+$ |
| | $Mg^{2+}$ | 291 | **III, V** | $MgL^+$, $[HL \cdot MgClO_4 - HClO_4]^+$ |
| | | 559 | **II** | $[MgL_2 \cdot H]^+$ |
| | | 391 | **IV** | $[HL \cdot MgClO_4]^+$ |
| | $Ca^{2+}$ | 407 | **IV** | $[CaHL \cdot ClO_4]^+$ or $[HL \cdot CaClO_4]^+$ |
| | | 307 | **V** | $[CaHL \cdot ClO_4 - HClO_4]^+$ |
| | $Ba^{2+}$ | 505 | **IV** | $[BaHL \cdot ClO_4]^+$ or $[HL \cdot BaClO_4]^+$ |
| | | 405 | **V** | $[BaHL \cdot ClO_4 - HClO_4]^+$ |
| **3** | – | 282 | **I** | $[HL \cdot H]^+$ |
| | $Mg^{2+}$ | 304 | **III, V** | $MgL^+$, $[HL \cdot MgClO_4 - HClO_4]^+$ |
| | | 585 | **II** | $[MgL_2 \cdot H]^+$ |
| | | 404 | **IV** | $[HL \cdot MgClO_4]^+$ |
| | $Ca^{2+}$ | 420 | **V** | $[CaHL \cdot ClO_4]^+$ or $[HL \cdot CaClO_4]^+$ |
| | | 320 | **V** | $[CaHL \cdot ClO_4 - HClO_4]^+$ |
| | $Ba^{2+}$ | 518 | **IV** | $[BaHL \cdot ClO_4]^+$ or $[HL \cdot BaClO_4]^+$ |
| | | 418 | **V** | $[BaHL \cdot ClO_4 - HClO_4]^+$ |
| **5** | – | 253 | **I** | $[L \cdot H]^+$ |
| | $Mg^{2+}$ | 375 | **IV** | $[L \cdot MgClO_4]^+$ |
| | $Ca^{2+}$ | 390 | **IV** | $[L \cdot CaClO_4]^+$ |
| | $Ba^{2+}$ | 518 | **IV** | $[L \cdot BaClO_4]^+$ |

served. The associates of both flavonol and the matrix are unstable and, as will be shown below, lose $HClO_4$ molecules in the course of desorption. This fact accounts for appearance of the matrix peak $[MNBA \cdot MgClO_4 - HClO_4]^+$ and also for additional growth of the intensity of the $MgL^+$ peak as the metal ion concentration is increased [reaction (5)]. Since the $MgL^+$ ion in this case originates from dissociation of a more complex species rather than from direct complexation, we will hereinafter denote this ion as $[HL \cdot MgClO_4 - HClO_4]^+$ (peak **V**).

$$[HL \cdot MgClO_4]^+ \longrightarrow [HL \cdot MgClO_4 - HClO_4]^+ + HClO_4. \quad (5)$$

In the mass spectra of flavonol–magnesium perchlorate mixtures, assignment of peak **II** to the species $[MgL_2 \cdot H]^+$ is ambiguous, with $MgL^+ \cdot HL$ being an alternative structure. Quantum-chemical calculations of associates of different structures show that the protonated 1 : 2 chelate




has the lowest enthalpy of formation. The calculations also show that, in the most stable complexes, the hydrogen atom of the 3-OH group is involved in the complexation. If this atom is replaced by an organic radical, formation of the chelates $[MgL_2 \cdot H]^+$ and $MgL^+$ is impossible.

To check our hypothesis, we studied the mass spectra of 3-methoxyflavone **5** in which the hydrogen atom of the 3-OH group is replaced by the methyl group. At low concentrations of the magnesium ion, we observed a single peak assigned to the protonated flavone, $[L^5 \cdot H]^+$ (**I**), and at high $Mg^{2+}$ concentrations, the $[L^5 \cdot MgClO_4]^+$ peak (**IV**). Peaks **II** and **III** were never observed, irrespective of the Mg : L ratio, which indirectly confirms formation of the complexes $MgL^+$ and $MgL_2$ by flavonols **1**–**3**.

**Complexes of flavonols with $Ca^{2+}$ and $Ba^{2+}$ ions.** The complexes formed by $Ca^{2+}$ and $Ba^{2+}$ with flavonols **1**–**3** differ significantly from the $Mg^{2+}$ complexes both in the concentration dependence of the signal intensities in the mass spectra and in the stoichiometry of the complex species.

At low concentrations of $Ca^{2+}$ and $Ba^{2+}$, there are no peaks in the mass spectra assignable to any complex species. The only revealed peaks are those of protonated flavonols **I** (Table 1). As the metal ion concentrations are increased, the $[HL \cdot MClO_4]^+$ and $ML^+$ peaks appear in the spectra.

The $[HL \cdot MClO_4]^+$ species may be associates similar to species **IV** detected in the spectra of the $Mg^{2+}$ complexes [Eq. (3)], or, which is more probable, spectrophotometrically detectable ions of "external" complexes of the stoichiometry CaHL and BaHL. The latter species are formed at high concentrations of the metal ions. The structures of the species detected in the mass spectra should be given in this case as $[CaHL \cdot ClO_4]^+$ and $[BaHL \cdot ClO_4]^+$.

Although the species $CaL^+$ and $BaL^+$ detected in the mass spectra are similar in the stoichiometry to the chelates $MgL^+$, we believe that the formed complexes are not chelates. Firstly, the large size of the $Ca^{2+}$ and $Ba^{2+}$ ions do not allow them to be accommodated in the "cavity" between the electron-donor oxygen atoms of the carboxy and 3-hydroxy groups in the chelating moiety of the flavonol molecules. This conclusion is confirmed by the absorption and fluorescence spectra of the complexes: The spectral characteristics of the magnesium chelates $MgL^+$ differ dramatically from those of the complexes formed by the $Ca^{2+}$ and $Ba^{2+}$ ions. Secondly, the calcium and barium ions are incapable of covalent bonding with the oxygen atoms of the flavonol reaction center. Therefore, formation of the complexes $CaL^+$ and $BaL^+$ requires the occurrence of the flavonol molecule in solution in the ionized state. It is known, however, that all flavonols are weak acids and do not dissociate in organic solvents (except dimethylformamide) [24]. In our mass spectra, we also have not detected the peaks of flavonolate anions, irrespective of the presence of alkaline-earth metal ions.

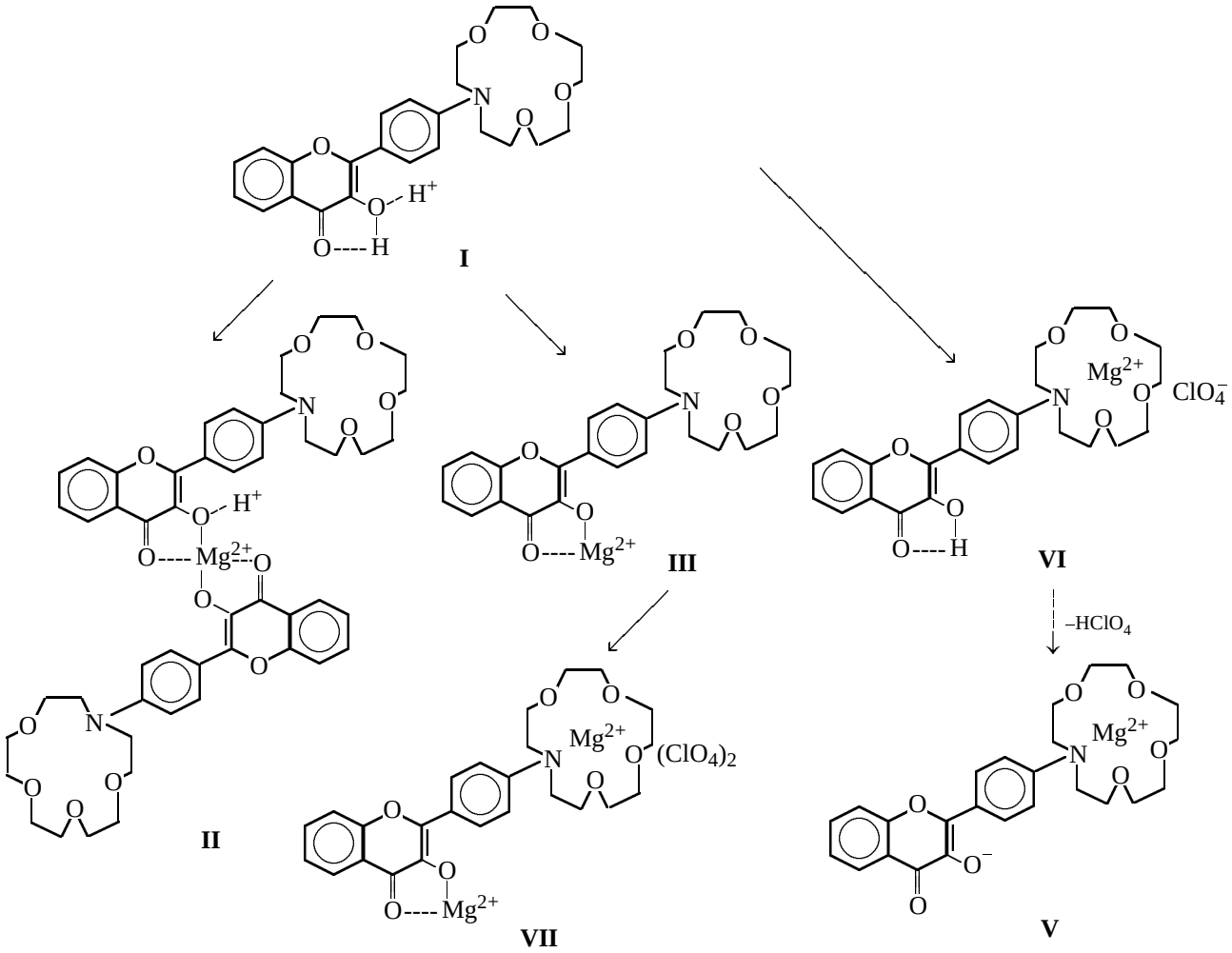
Therefore, we suggest that the $CaL^+$ and $BaL^+$ ions originate from decomposition of the more complex species $[HL \cdot MClO_4]^+$ in the course of desorption [Eq. (5)]. To examine the possibility of such fragmentation, we used the MS/MS technique. In the mass spectra of the isolated beam of ions with *m/z* 475 ($[HL \cdot BaClO_4]^+$), along with the base peak, we also detected the fragment peak with *m/z* 375. The ion giving this peak is formed by elimination of the $HClO_4$ molecule from the parent ion: $[HL \cdot BaClO_4 - HClO_4]^+$. Thus, the results confirm our suggestion that the $CaL^+$ and $BaL^+$ ions detected in the mass spectra do not have the chelate structure.

**Complexes of the crown-substituted flavonol with $Mg^{2+}$ ions.** Crown-substituted flavonol **4** has two complexation centers. Therefore, it was logical to expect formation of complexes with two metal ions. Spectrophotometric studies of solutions containing the crown-substituted flavonol and magnesium perchlorate revealed formation of two complexes, $MgL^+$ and $Mg_2L^{3+}$, with the former being a chelate and the latter, a crown complex.

**Table 2.** Peak assignment in the mass spectra of crown-substituted flavonol **4**

| Ion | *m/z* | Peak (structure) | Assignment |
|---|---|---|---|
| – | 456 | **I** | $[HL \cdot H]^+$ |
| $Mg^{2+}$ | 478 | **III** | $MgL^+$ |
| | 933 | **II** | $[MgL2 \cdot H]^+$ |
| | 578 | **VI** | $[MgHL \cdot ClO_4]^+$ |
| | 478 | **V** | $[MgHL \cdot ClO_4 - HClO_4]^+$ |
| | 702 | **VII** | $[Mg_2L \cdot 2ClO_4]^+$ |
| $Ca^{2+}$ | 594 | **VI** | $[CaHL \cdot ClO_4]^+$ |
| | 494 | **V** | $[CaHL \cdot ClO_4 - HClO_4]^+$ |
| | 832 | **VIIa** | $[Ca_2HL \cdot 3ClO_4]^+$ |
| | 732 | **VIII** | $[Ca_2HL \cdot 3ClO_4 - HClO_4]^+$ |
| | 949 | **X** | $[Ca(HL)_2 \cdot ClO_4 - HClO_4]^+$ |
| $Ba^{2+}$ | 692 | **VI** | $[BaHL \cdot ClO_4]^+$ |
| | 592 | **V** | $[BaHL \cdot ClO_4 - HClO_4]^+$ |
| | 1028 | **VIIa** | $[Ba_2HL \cdot 3ClO_4]^+$ |
| | 928 | **VIII** | $[Ba_2HL \cdot 3ClO_4 - HClO_4]^+$ |
| | 1147 | **IX** | $[Ba(HL)_2 \cdot ClO_4]^+$ |
| | 1047 | **X** | $[Ba(HL)_2 \cdot ClO_4 - HClO_4]^+$ |
| | 1483 | **XI** | $Ba_2(HL)_2 \cdot 3ClO_4]^+$ |
| | 1383 | **XII** | $[Ba_2(HL)_2 \cdot 3ClO_4 - HClO_4]^+$ |
| | 1283 | **XIII** | $[Ba_2(HL)_2 \cdot 3ClO_4 - 2HClO_4]^+$ |

The mass spectra of solutions of flavonol **4** with a low magnesium concentration contain five peaks (Table 2). One of them is the peak of protonated crown-substituted flavonol (species **I**). Two other peaks were assigned to the 1 : 1 ($MgL^+$) and protonated 1 : 2 ($[MgL_2 \cdot H]^+$) chelates (species **III** and **II**, respectively). The *m/z* value for the fourth peak allows its assignment to $[HL \cdot MgClO_4]^+$. In contrast to the associate of similar stoichiometry detected in the mass spectra of the other flavonols, this new peak appears at considerably lower metal concentration in the solution. This fact suggests that the corresponding species is the "nonchelate" crown complex $[MgHL \cdot ClO_4]^+$ (**VI**, see below; hereinafter, the ion numbering in the schemes corresponds to the peak numbering in the tables). The *m/z* value of the fifth peak is by 100 units lower than that of peak **VI**. We believe that the corresponding species is formed by fragmentation of $[MgHL \cdot ClO_4]^+$. Thus, this peak should be presumably assigned to species **V**.

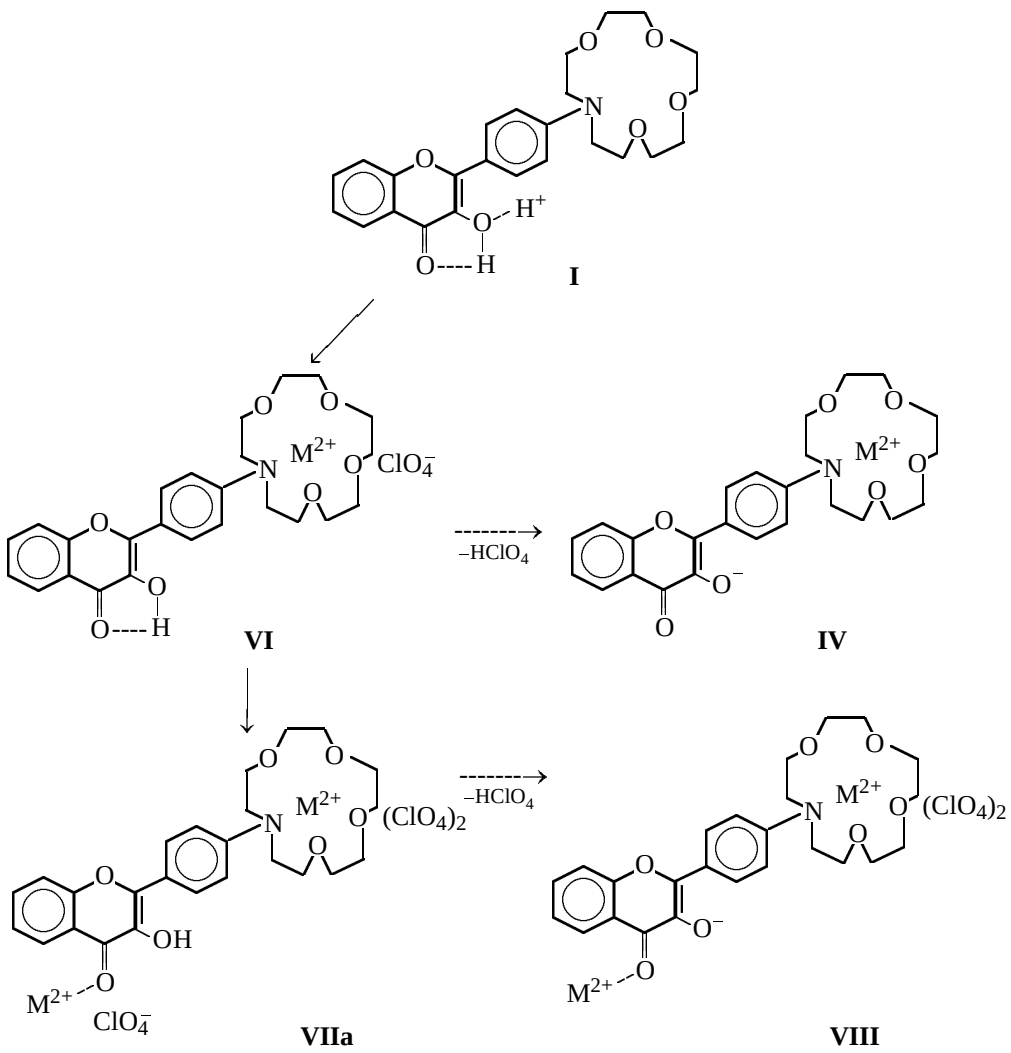



The 1 : 2 complex $MgL_2$ and the crown complex MgHL with the crown-substituted flavonol are formed at low $Mg^{2+}$ concentrations and high ligand concentrations; therefore, they cannot be detected spectrophotometrically because of strong absorption of the ligand.

As the magnesium concentration is increased, a peak assignable to a 2 : 1 complex, $[Mg_2L \cdot 2ClO_4]^+$ (**VII**), appears in the spectra. Ions of a more complex stoichiometry such as $Mg_2L_2$ (expected peak $[Mg_2L_2 \cdot 2HClO_4]^+$) or $Mg_3L_2$ ($[Mg_3L_2 \cdot 3HClO_4]^+$) were not detected in the mass spectra of the crown-substituted flavonol.

**Complexes of the crown-substituted flavonol with $Ba^{2+}$ ions.** At low concentrations of the Ba ions, we detected in the mass spectra peak **I** and peaks of the $[HL \cdot BaClO_4]^+$ and $BaL^+$ ions. Since the $Ba^{2+}$ ions form stable complexes with monoaza-15-crown-5 ether [10, 25], it was logical to assign the first of these peaks to the crown complex $[BaHL \cdot ClO_4]^+$ (peak of type **VI**, see below). As already noted, formation of chelates is untypical of barium ions because of their large size and incapability of flavonol for ionization. Therefore, we believe that, as in the case of the other flavonols, the $BaL^+$ ion is formed by fragmentation of the crown complex $[HL \cdot BaClO_4]^+$ (peak **IV**) in the course of desorption, and its formula should be therefore given as $[HL \cdot BaClO_4 - HClO_4]^+$ (peak of type **V**).

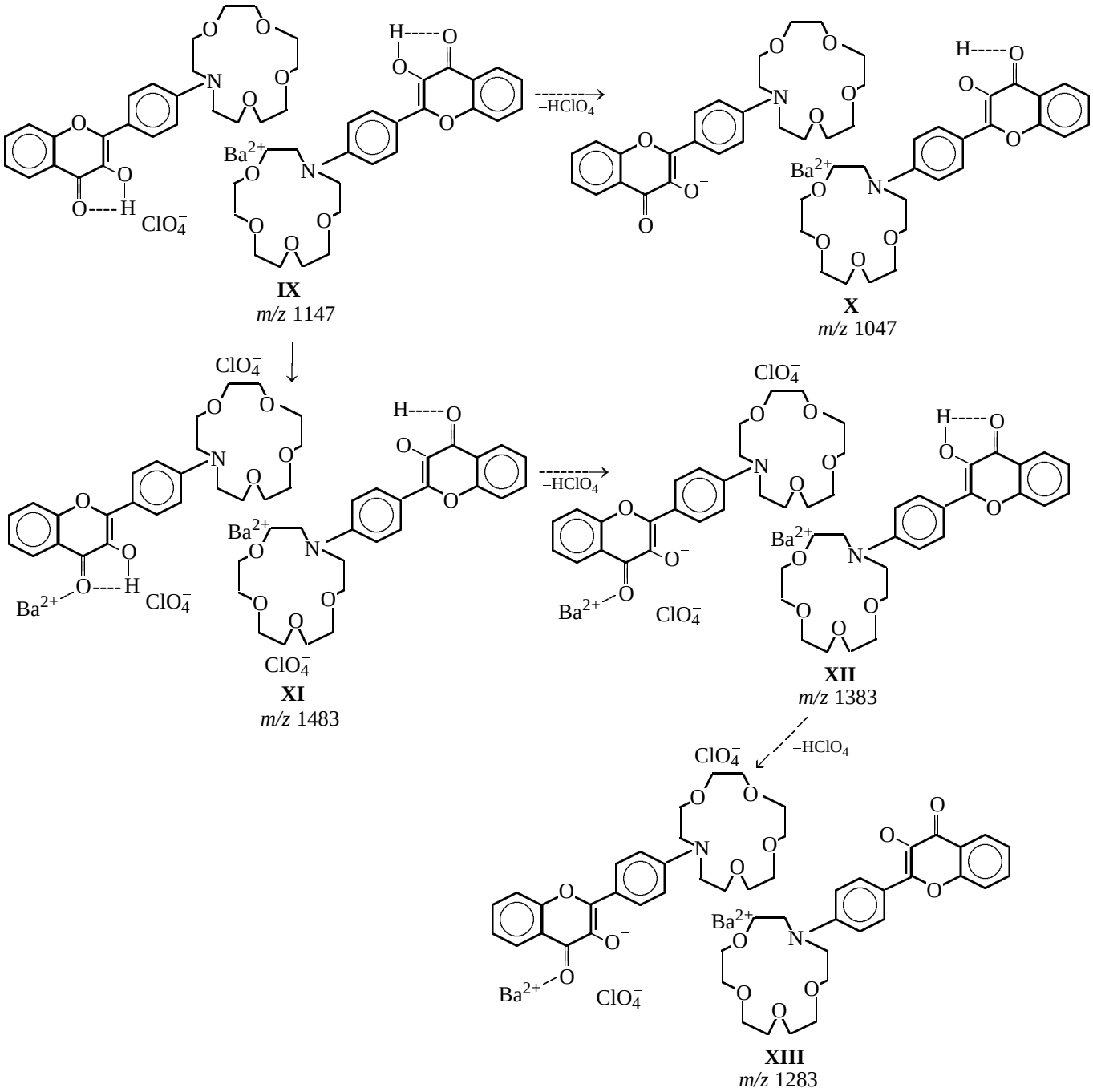
At high concentrations of the $Ba^{2+}$ ions, two more peaks appear in the mass spectra: weak peak **VIIa**, $[HL \cdot BaClO_4 \cdot Ba(ClO_4)_2]^+$, and strong peak **VIII**, $[HL \cdot BaClO_4 \cdot Ba(ClO_4)_2 - HClO_4]$ (**VIII**). The former peak, in our opinion, corresponds to the spectrophotometrically detected complex $Ba_2HL$ in which the first of the metal ions coordinates with the crown ring, and the second one forms an "external" complex with the carbonyl oxygen atom. The corresponding species is unstable, since it is associated with three perchlorate ions simultaneously; in the course of desorption, it eliminates a perchloric acid molecule, transforming into species **VIII**.




Along with the above-described peaks, the mass spectra of the crown-substituted flavonol in the presence of barium ions contain five weak signals more: *m/z* 1147 (**IX**), 1047 (**X**), 1483 (**XI**), 1383 (**XII**), and 1283 (**XIII**). We tentatively assign these peaks to the so-called "sandwich" complexes (see below). Formation of such complexes is characteristic of ions with a large radius, including $Ba^{2+}$; such species were described in numerous papers (see, e.g., [16, 25, 26]). The complex $Ba(HL)_2$ gives rise to peaks **IX** and **X**, with species **X** being formed from **IX** by elimination of a perchloric acid molecule. Peaks **XI**, **XII**, and **XIII** can be assigned to a more complex "sandwich" species $Ba_2(HL)_2$ in which one of the ligands is bidentate and forms an "external" complex with barium ions. The species giving peaks **XII** and **XIII** are formed from the initial species $[Ba_2(HL)_2 \cdot 3ClO_4]^-$ (**XI**) by elimination of one and two $HClO_4$ molecules, respectively.

**Complexes of the crown-substituted flavonol with $Ca^{2+}$ ions.** The mass spectra of solutions of the crown-substituted flavonol in the presence of potassium perchlorate show that the stoichiometry of the monoligand complexes is fully identical to that of the respective $Ba^{2+}$ complexes. For example, at low concentrations of calcium ions we observed peak **VI** and its fragmentation peak **V**, and at high concentrations, peak **VIIa** and fragmentation peak **VIII**.

It is known that, as the ionic radius decreases in the alkaline-earth series, the probability of formation of "sandwich" crown complexes decreases also. This is confirmed by our results. The mass spectra of a mixture of the crown-substituted flavonol with calcium perchlorate contain only one weak peak (*m/z* 949) assignable to the "sandwich" complex. Comparison of the spectra of the barium and calcium complexes suggests that this peak belongs to a species of type **X**, formed by elimination of a perchloric acid molecule from the $[Ca(HL)_2 \cdot ClO_4]^+$ ion which was not detected.




Comparison of the data obtained with the results of similar studies performed by electronic spectroscopy [9–11] shows that nondestructive mass spectrometry gives a more complete information on the structure of flavonol complexes. Mass spectrometry allows the measurements to be performed at the component concentrations at which photometric or fluorimetric measurements are impossible. Furthermore, using mass spectrometry, it is possible to distinguish species giving similar electronic absorption and emission spectra.

## EXPERIMENTAL

The mass spectra in the modes of detection of positive and negative ions were taken with a NERMAG R10-10 spectrometer. As matrix we used *m*-nitrobenzyl alcohol. The matrix was bombarded with a beam of xenon atoms (9 keV) at a current of 0.2–0.4 A.

Flavonols were prepared by the Algar–Flynn–Oyamada reaction [27, 28]; their purity was checked by thin-layer chromatography. The complexes were pre-

pared by adding commercial alkaline-earth metal perchlorates to solutions of flavonols in acetone. The molar ratios of flavonols and metal ions in acetone solutions were ~35 : 1, 5 : 1, 1 : 1, 1 : 10, and 1 : 20, at flavonol concentrations of $5 \times 10^{-3}$ M. To make the measurements, 1-ml portions of acetone solutions were mixed with 5 ml of *m*-nitrobenzyl alcohol. In the resulting solvent mixture, the complexes were less stable than in acetone. However, spectrophotometric studies of similar flavonol–metal systems in methanol and acetonitrile showed that variation of the solvent composition does not result in appearance of new complexes or disappearance of the existing ones; only the ratio of the species varies.

To optimize the geometry of associates of the $Mg^{2+}$ ion with two flavonol ligands and calculate the enthalpy of formation of the associates, we used the PM3 method with the MOPAC 6.0 software [29].

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