

SYNTHESIS AND CHARACTERIZATION OF A NEW COMPLEX OF OXOVANADIUM (IV) WITH NARINGENIN, AS POTENTIAL INSULINOMIMETIC AGENT

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Abstract

A new potentially biologically active complex of oxovanadium (IV) with naturally occurring flavanone naringenin has been synthesized and characterized in solid state by elemental analysis, molar conductance, UV-Vis spectra, FT-IR spectra, thermal analysis, and fluorescence spectra. From the experimental data, the complex was formulated as $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$.

Rezumat

A fost sintetizat un nou complex cu potențială acțiune biologică, format de oxovanadiu (IV) și o flavanonă naturală, naringenină. Complexul a fost caracterizat în stare solidă prin analiză elementală, conductanță molară, spectre UV-Vis și FT-IR, analiză termică și spectre de fluorescență. Pe baza datelor experimentale, complexul a fost formulat $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$.

Keywords: naringenin, oxovanadium (IV) complex

Introduction

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-chroman-4-one), is a naturally occurring flavanone found in citrus fruits [1] with a wide range of pharmacological properties (Figure 1).

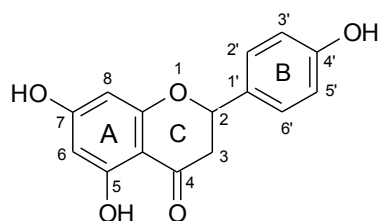


Figure 1.

The structure of naringenin

Unlike the flavones and flavonols [2, 3], naringenin has a weak antioxidant activity [4]. The antioxidant activity of flavonoids is generally associated with the following characteristics: (i) an *ortho*-dihydroxy structure in the B-ring, (ii) the presence of a 2,3 double bond in the C-ring, and/or (iii) the presence of a 4-oxo function in the C-ring [5]. Additionally, an OH group in position 3 of the C-ring was correlated with the antioxidant properties [6]. The

weak antioxidant activity of naringenin is explained by the absence of a C3 hydroxyl group from the flavonol basic moiety, the presence of a single 4' hydroxyl group and the saturated heterocyclic C ring, which led to the consequent lack of conjugation between the A and B rings. However, it was found that the metal chelation enhances the antioxidant activity of naringenin and that the solvent plays an important role in this enhancement [7]. Naringenin showed no significant cytotoxic effects *in vitro* on some human cancer cells [8], although *in vivo* was found to inhibit the lung metastasis induced by B16F10 melanoma cells in mice, as seen by the reduction in the number of lung tumour nodules [9]. Antiatherogenic [10] and anti-inflammatory effects [11, 12] have also been reported. Many studies reported the effects on lipid metabolism [13, 14] and plasma glucose levels [14-17]. Some mechanistic studies on hypoglycaemic effects of flavonoids showed that naringenin significantly increased AMP kinase (AMPK) activity and glucose uptake in muscle cells and liver [18, 19], but the hypoglycaemic activity of naringenin *in vivo* seems to be more complex. The multilevel effects on lipid metabolism lead to increased insulin sensitivity and glucose tolerance

as has been shown in animal models with metabolic syndrome [14].

Due to the presence of a chelating 5-hydroxy-4-keto group, naringenin can act as a bidentate ligand toward metal ions. Complexation of naringenin with Al (III) and Fe (II) has been examined by spectrophotometric and potentiometric methods and the results showed that complexes with 1:3 ratio (metal:naringenin) were formed with both metal ions [20]. The chelating ability is the most suitable explanation for the protective effect of naringenin against renal [21] or hepatic [22, 23] damages provoked by some toxic metals.

Some complexes obtained in solid state have been characterized and their biological activity was investigated. The cytotoxicity of copper (II) complex with naringenin of general composition $[\text{Cu}(\text{Nrg})_2(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$ has been estimated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay against three typical human tumour cell lines involving HepG-2, SGC-7901 and HeLa. The complex showed relatively significant inhibitory rate only against HepG2 cell line than that of Nrg [24]. The scavenging effects on OH^\cdot and O_2^\cdot of naringenin and their complexes of general formula $[\text{M}(\text{H}_2\text{L})_2 \cdot 2\text{H}_2\text{O}] \cdot \text{H}_2\text{O}$ ($\text{M} = \text{Cu}$ (II), Zn (II), Ni (II)) have been investigated. The results showed that the effect of the Cu (II) complex is the most remarkable, and the average scavenging ability of the complexes against OH^\cdot are higher than that of the ligand [25].

The present study aims to obtain a new complex of VO (IV) with naringenin that combines the well-known insulinomimetic activity of oxovanadium (IV) [26-28] with the hypoglycaemic effects reported for naringenin.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich, were reagent grade and were used without further purification.

The chemical analyses were performed on a Perkin Elmer PE 2400 analyser (for C, H, N, S) and a Shimadzu AA 6300 spectrometer (for V).

Electrical conductivity measurements were recorded at 20°C for a 10^{-3}M solution of the complex in dimethylsulfoxide (DMSO), using a Consort C830 (Turnhout, Belgium) conductometer with SK10T platinum electrode embedded in glass (cell constant 1.0 cm^{-1}).

IR spectra were recorded using KBr pellets on a FT-IR VERTEX 70 (Bruker) spectrometer in the range 400 - 4000 cm^{-1} . Electronic spectra by diffuse reflectance technique, with Spectralon as reference sample, were recorded in the range 200 - 900 nm, on a Jasco V 650 spectrophotometer.

Fluorescence spectra were recorded on a Jasco FP 6500 spectrofluorometer.

The thermogravimetric (TG) and differential thermal analysis (DTA) curves were recorded using a Labsys 1200 SETARAM instrument, with a sample mass of 16-31 mg over the temperature range of 20-900°C, using a heating rate of 10 K/min. The measurements were carried out in synthetic air atmosphere (flow rate 16.66 cm^3/min) by using alumina crucibles.

The X-ray powder diffraction patterns were collected on a DRON-3 diffractometer with a nickel filtered Cu K_α radiation ($\lambda = 1.5418 \text{ \AA}$) in a 2θ range of 5-70°, a step width of 0.05° and an acquisition time of 2 s on each step.

Synthesis of the complex

The complex was obtained by the following procedure: 2.00 g (7.3 mmol naringenin) were dissolved in 50 cm^3 distilled water containing a few NaOH pellets. To the resulted solution, a saturated solution of $\text{VO}\text{SO}_4 \cdot 5\text{H}_2\text{O}$ (molar ratio VO:ligand 1:2) was dropwise added, under continuous stirring. After the adjusting of pH to ~ 6 with 1M H_2SO_4 , a coloured solid precipitates immediately. The sparingly soluble product of khaki colour was filtered off through a fritted glass funnel, washed several times with water and dried in desiccator over CaCl_2 .

Results and Discussion

The complex is air stable and soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO), slightly soluble in MeOH, insoluble in water. The molar conductance of the 10^{-3}M solution of the complex in DMSO has the value $18.73 \text{ } \Omega^{-1}\text{cm}^2\text{mol}^{-1}$, showing that the complex is a non-electrolyte in DMSO [29]. The elemental analyses consist with the formula of the complex $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ as follows: *found*: V, 7.68%; C, 51.95%; H, 4.33 %; *calculated* for $\text{VC}_{30}\text{H}_{32}\text{O}_{16}$: V, 7.29%; C, 51.46%; H, 4.57%.

Infrared spectra

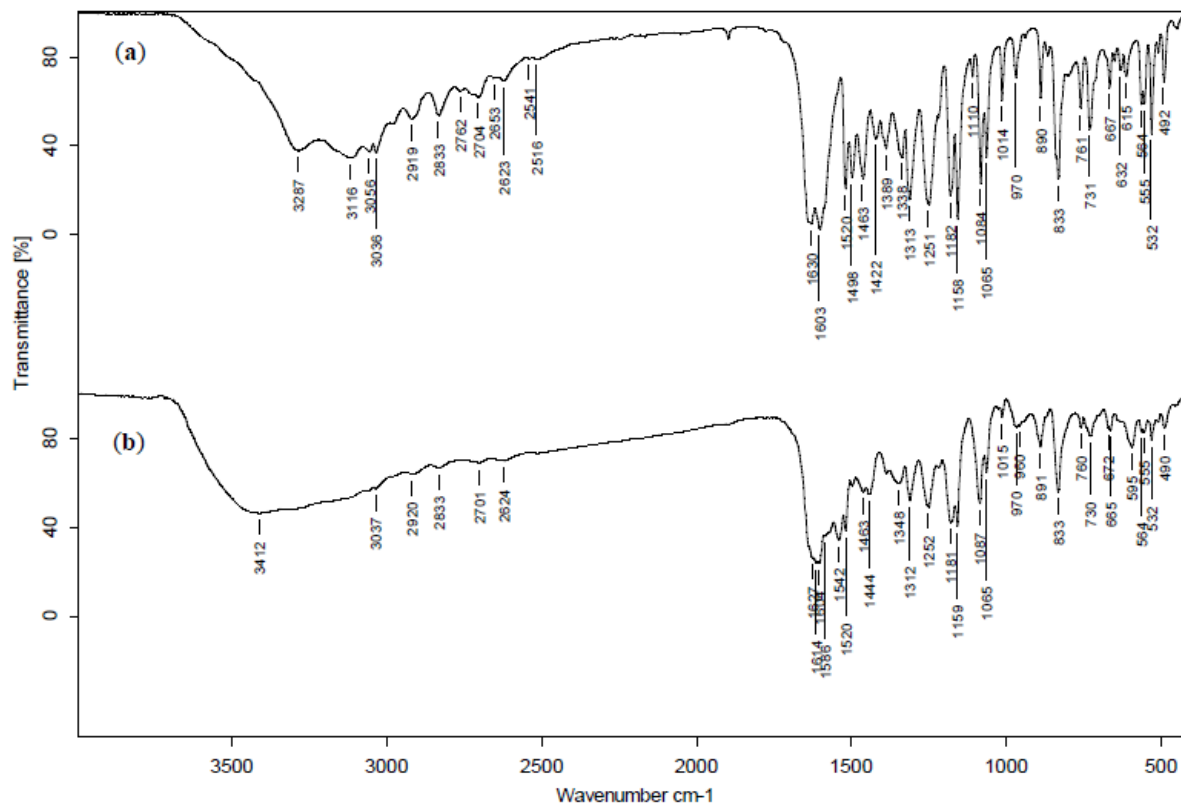
The main stretching frequencies (cm^{-1}) of the IR spectra of the ligand and its complex are given in Table I. The main difference in the IR spectra of the complex (Figure 2b), compared to the IR spectrum of the ligand (Figure 2a) is the position of the stretching frequency of C=O group. The $\nu(\text{C}=\text{O})$ vibration of the free ligand is at 1630 cm^{-1} , whereas for the complex, this peak shifts to 1614 cm^{-1} ($\Delta\nu$ (ligand-complexes) is equal to 19 cm^{-1}). This shift confirms that the group loses its original characteristics as consequence of forming a coordinative bond with the metal ion. Other differences are represented by the presence in the IR spectra of the complex, of a band placed at 970 cm^{-1} assigned to the $\nu(\text{V}=\text{O})$ stretching mode and a broad

band at 3412 cm^{-1} , assigned to $\nu(\text{OH})$ stretching vibrations from the water molecule [30].

Table I

Main IR data (cm^{-1}) for naringenin and its oxovanadium (IV) complex

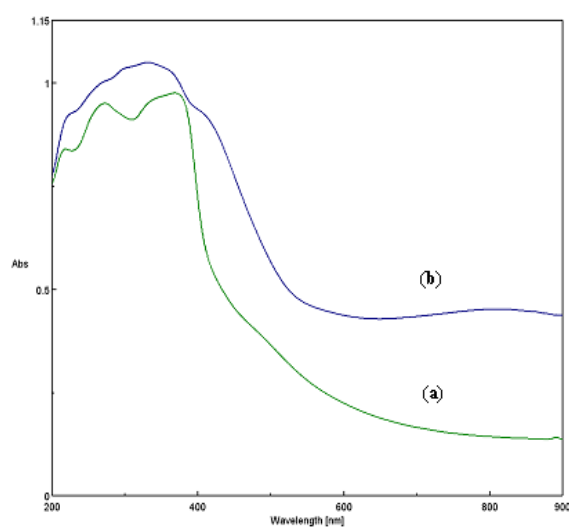
Compound	$\nu(\text{O-H})$	$\nu(\text{C=O})$	$\nu(\text{C=C})$	$\nu(\text{C-O-C})$	$\nu(\text{V=O})$
Nrg	3287 m	1630 s	1602 s	1251	-
$[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$	3412 m	1614 s	1604 s	1252	970

**Figure 2.**

IR spectra of naringenin (a) and $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ (b)

Electronic spectra

Naringenin exhibits two major ranges of absorption in UV-Vis region. The absorption bands in the range 330 - 370 nm correspond to the B ring portion (cinnamoyl system, band I), and the absorption band at 270 nm corresponds to the A ring portion (benzoyl system, band II) [31]. After the interaction with VO (IV) ion, a new band appears at 298.5 nm and this could be due to the formation of a new six-membered ring system between the metal ion and the oxygen atoms from 4-oxo and 5-OH sites. Band I remains relatively unchanged, indicating that there is no interaction of the cinnamoyl moiety with the metal centre (Figure 3, Table II). In the visible region, the absorption spectrum of the complex shows the characteristic bands of VO^{2+} in a square pyramidal environment. The absorptions at around 810 nm and the shoulders at about 558 are assigned to the spin allowed ${}^2\text{B}_2 \rightarrow {}^2\text{E}$, and ${}^2\text{B}_2 \rightarrow {}^2\text{B}_1$ transitions [32]. The shoulder at about 407 nm can be assigned to the ligand-to-metal charge transfer (LMCT) band.

**Figure 3.**

Diffuse reflectance electronic spectra of (a) naringenin and (b) $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$

Table II

UV-Vis data for naringenin and its oxovanadium (IV) complex

Compound	λ_{\max} (nm)						
	Nrg	272.5	-	337 (sh)	368	-	-
$[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$	272 (sh)	298.5 (sh)	330	362 (sh)	406.5 (sh)	557.5 (sh)	809.5

Thermal analysis

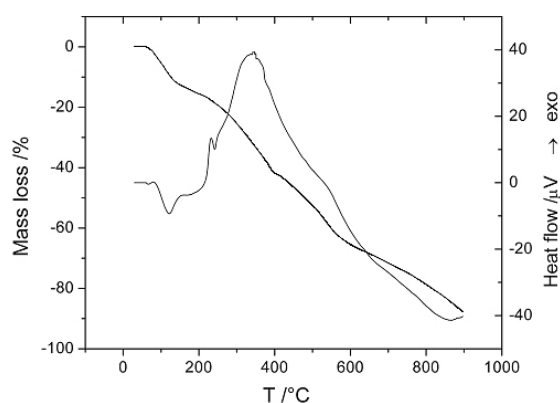
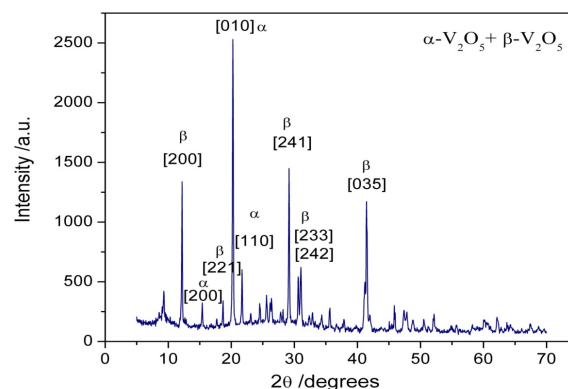
The results concerning the thermal decomposition/degradation of the synthesized complex are presented in Table III.

Table IIIThermal behaviour data (in synthetic air) for $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$

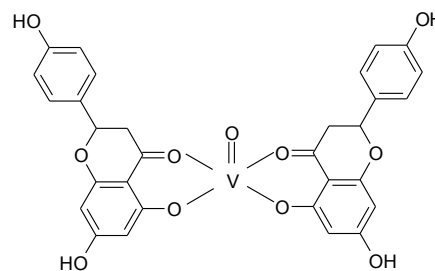
Compound	Step	Thermal effect	Temperature range / °C	$\Delta m_{\text{exp}} / \%$	$\Delta m_{\text{calc}} / \%$
$[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$	1.	Endothermic	55 - 151	13.41	13.45
	2.	Exothermic	210 - 460	28.19	28.11
	3.	Exothermic	460 - 900	50.43	50.52
	Residue (V_2O_5)				7.97

The TG and DTA curves corresponding to the heated complex in the 20 - 900°C temperature range are presented in Figure 4. The thermal decomposition of the complex occurs in three, well-defined steps. The first step, which is endothermic, corresponds to the loss of water molecules. Considering the low temperature corresponding to this transformation, we can appreciate the nature of water as for crystallization. The resulted anhydrous compound decomposed in two exothermic steps. The final product is a mixture of α (ASTM 72-0433) and β (ASTM 45-1074) modifications of V_2O_5 (Figure 5).

Taking into account all the above data, the proposed coordination for the complex is as follows (Figure 6).

**Figure 4.**TG and DTA curve of $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ **Figure 5.**

Powder X-ray diffraction pattern for residue

**Figure 6.**The possible structure of complex $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ *Fluorescence spectra*

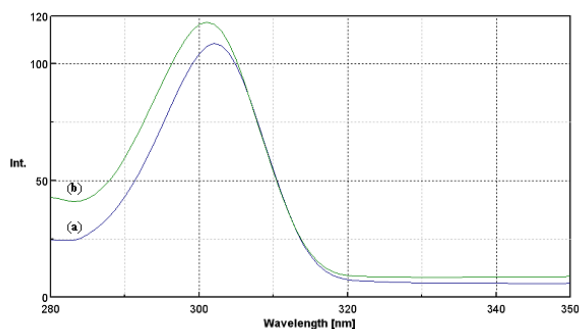
The fluorescence emission spectra were recorded at three excitation wavelengths, 260 nm (Figure 7), 330 nm (Figure 8) and 400 nm (Figure 9), respectively, both for ligand and complex. The data presented in Table IV revealed the following: (i) naringenin exhibits fluorescence for all excitation wavelengths used; (ii) the fluorescence of the complex is more intense than that of the ligand at $\lambda_{\text{exc}} = 260$ nm and it is quenched at $\lambda_{\text{exc}} = 330$ nm and 440 nm, and differences could be correlated with the coordination process.

Table IV

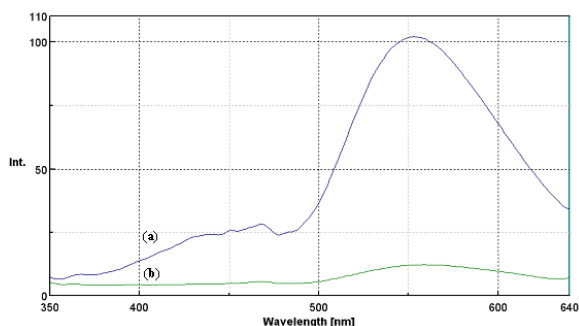
Fluorescence data for naringenin and its oxovanadium (IV) complex

Compound	$\lambda_{exc} = 260 \text{ nm}$		$\lambda_{exc} = 330 \text{ nm}$		$\lambda_{exc} = 400 \text{ nm}$	
	$\lambda_{em} \text{ (nm)}$	I (a.u.)	$\lambda_{em} \text{ (nm)}$	I (a.u.)	$\lambda_{em} \text{ (nm)}$	I (a.u.)
Nrg	302	109	553	102.16	534	173
$[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$	301	118	558	12.43	598	21

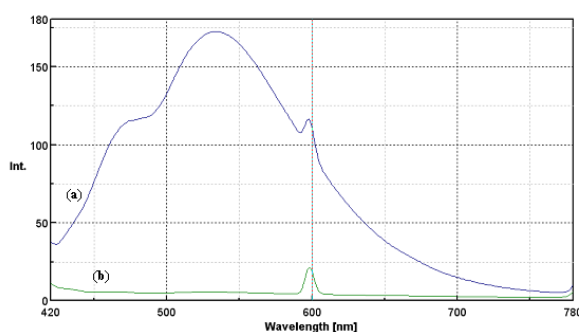
λ_{exc} = excitation wavelength; λ_{em} = emission wavelength; I = relative fluorescence intensity

**Figure 7.**

The emission spectra of (a) naringenin and (b) $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ at $\lambda_{exc} = 260 \text{ nm}$

**Figure 8.**

The emission spectra of (a) naringenin and (b) $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ at $\lambda_{exc} = 330 \text{ nm}$

**Figure 9.**

The emission spectra of (a) naringenin and (b) $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$ at $\lambda_{exc} = 400 \text{ nm}$

Conclusions

A new complex of VO (IV) with the naturally occurring flavanone naringenin has been synthesized and characterized by analytical and spectral investigations, as well as by thermal analysis. A square pyramidal stereochemistry for metallic ion

was proposed, based on the electronic spectra, while the IR spectra features indicated a coordination mode as bidentate chelate of the flavanone ligand. The thermal analysis (TG, DTA) elucidated the composition and also the number and nature of the water molecules. The results suggest that the complex has the general formula $[\text{VO}(\text{Nrg})_2] \cdot 5\text{H}_2\text{O}$. The obtained complex could be of interest as potential insulinomimetic agent.

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