UDC: 547.587.51:543.42
Review article

CHARACTERIZATION OF THIAZOLO-CHROMANDIONE BY MOLECULAR SPECTROSCOPY AND SPECTRAL BEHAVIOR IN A WIDE pH RANGE

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Abstract

In this study, the derivatives of coumarin with a complex organic structure were analyzed using spectroscopic methods through UV/Vis and IR spectroscopy. UV/Vis measurements were conducted using different pH conditions, varying from pH 1-12, to observe the impact of pH on the electronic behavior and structure of the substance. In the UV/Vis spectra, measurements were made recording the light absorbance in the region of (200-800 nm). Three main absorption bands have been identified. The first notable shift was observed in the band of (212 nm) in a highly acidic environment (pH = 1), suggesting the protonation of the substance. Second shift was observed in (190-210 nm) corresponding to $\pi\rightarrow\pi^*$ and third shift to $n\rightarrow\sigma^*$ (250 nm) electronic transitions, with the lone pairs of heteroatoms such as oxygen and nitrogen playing a key role in stabilizing these transitions. Structural changes and spectral shifts were observed through the changes in pH, especially in basic pH, where coumarin structure breakdown and regeneration occurred. Using IR spectroscopy, the characteristic vibrations of functional groups were analyzed. Benzene deformations and the presence of a diazo group (N=N) were observed in the infrared spectrum. Using a combination of these methods helped to identify the characteristic groups and determine the structure of the substance, displaying the connection between its structure and behavior under a wide pH range.

Keywords: IR spectroscopy, UV/Vis spectroscopy, protonation, pH, electronic transitions

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1. Introduction

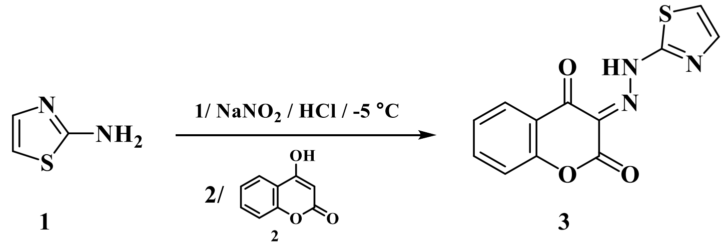
Complex organic compounds synthesis and analysis are significant in organic chemistry and biochemistry because of their characteristic structures and diverse applications [1]. Plant extracts that are derivatives of coumarin are often referred to as herbal remedies. The hydroxy-phenolic group of coumarins, are highly active compound known for its ability to scavenge free radicals. [2], especially against reactive oxygen species [3]. Research has demonstrated that the consumption of coumarin-containing products can protect aflatoxin-induced hepatocarcinogenesis [4]. Also, coumarins are widely used in cancer treatment and management of post-radiotherapy effects [5], as well as in therapies for kidney cancer and melanoma [6]. Spectroscopic methods, such as UV/Vis and IR spectroscopy, provide powerful analyzing tool for their structural changes and identifying key functional groups that influence their chemical and electronic behavior, especially under the influence of external factors, such as pH variations, giving insight for the chemical and biological properties of these molecules, and understanding of the mechanisms of action and reactivity of the substance [7]. Furthermore, these methods are non-invasive and non-destructive, as electromagnetic radiation doesn't damage or alter the sample`s composition [8]. UV/Vis spectroscopy is typically used in two main ways. The first involves scanning the entire UV/Vis radiation region, ranging from 190 to 800 nm, to obtain the compound`s characteristic absorption bands. The second application focuses on determining the concentration of an unknown substance by measuring the absorption of light by a liquid sample within a specific wavelength range [9]. In addition to UV/Vis

https://doi.org/10.62792/ut.jnsm.v10.i19-20.p3081

spectroscopy, molecular spectroscopy that excites other types of molecular motions is Infrared spectroscopy (IR), a widely employed technique in both industry and scientific research. Infrared spectroscopy is a reliable and non-destructive method used for measurements, quality control, and dynamic analysis [10].

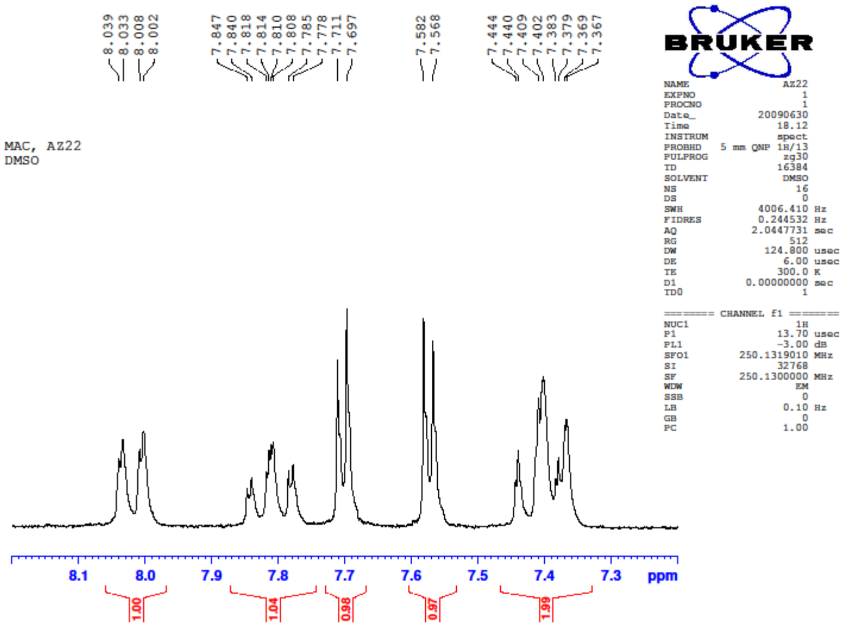
2. Results and Discussion

The coumarin derivative, which is the subject of this study, (*E*)-3-[2-(thiazol-2-yl)hydrazinylidene]chroman-2,4-dione (**3**, Scheme 1), was initially synthesized as a very potent anticancer drug [11]. The synthetic road was through the reaction of diazotation of 2-aminothiazole (**1**), which gave the corresponding diazonium salt as an intermediate. Those salts are very unstable, and they are rarely isolated but rather used *in situ* for the next reactional step. Thus, the diazonium salt of **1** was treated with an alkaline solution of 4-hydroxycoumarin (**2**), which immediately produced a heavy precipitate with an intense orange to red color of product **3** (Scheme 1). After purification by crystallization from ethanol, NMR spectra were recorded. In the ^{1}H-NMR spectra of the compound **3** (Figure 1) clearly the characteristic pattern of coumarin protons can be observed: a doublet at 8 ppm with integral 1, a triplet at 7.81 ppm with integral 1, and a multiple at 7.38 ppm with integral 2, for the four aromatic protons of the coumarin moiety. On the other hand, two new signals appear, one doublet at 7.7 and one doublet at 7.57 ppm, both with integral 1. Those two doublets without any doubt originate from two protons at the thiazole ring, which is now coupled with the coumarin ring through the diazo bridge.



Scheme 1. Synthesis of the coumarin derivative **3**

Also, ^{13}C-NMR was measured and in the region from 118 – 158 ppm, twelve carbons were observed, nine of the coumarin and three of the thiazole ring. Mass spectra were also in accordance with the supposed molecular weight, since the molecular pick was observed at 274 *m/z* from the ionic species $[M+H]^+$, and at 296 *m/z* from $[M+Na]^+$ species. After the verification of the structure of the obtained product, it was noticed that in the ^{1}H-NMR spectra, one signal from the hydroxy group at position 4 of the coumarin moiety is missing, but this was not very significant at this point because it is very common for the phenolic proton not to appear because of the rapid proton exchange. But with detailed investigation by IR and UV/Vis spectroscopy, it was concluded that this is not the case only in ^{1}H-NMR spectra, but it has a deep influence on the other spectroscopic means. Namely, it is obvious that this structure is very dynamic, existing in a few tautomeric forms, and yet the equilibrium is strongly shifted towards the (*E*)-hydrazinylidene form (scheme 2), as it was revealed by the detailed diffractometric study [12]. Thus, the proton of the hydroxy group at position 4 of the coumarin ring easily migrates to the second nitrogen of the diazinyl group at position 3 of the coumarin core. However, in our understanding, this proton is actually “sitting” in-between the two orbitals of the oxygen and nitrogen atoms, without forming a real covalent bond, which can be the explanation for the absence of the characteristic bands in the IR spectrum in the region from 3550 – 3200 cm^{-1}, neither for OH and nether for the NH group [21]. The same occurrence was noticed by missing signals for both groups in the ^{1}H-NMR spectrum of this compound (**3**).



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Time               18.12
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PULPROG             zg30
TD                 16384
SOLVENT             DMSO
NS                    16
DS                     0
SWH             4006.410 Hz
FIDRES          0.244532 Hz
AQ             2.0447731 sec
RG                   512
DW               124.800 usec
DE                  6.00 usec
TE                 300.0 K
D1            0.00000000 sec
TD0                    1

======= CHANNEL f1 =======
NUC1                  1H
P1                 13.70 usec
PL1                -3.00 dB
SFO1         250.1319010 MHz
SI                 32768
SF           250.1300000 MHz
WDW                   EM
SSB                    0
LB                  0.10 Hz
GB                     0
PC                  1.00
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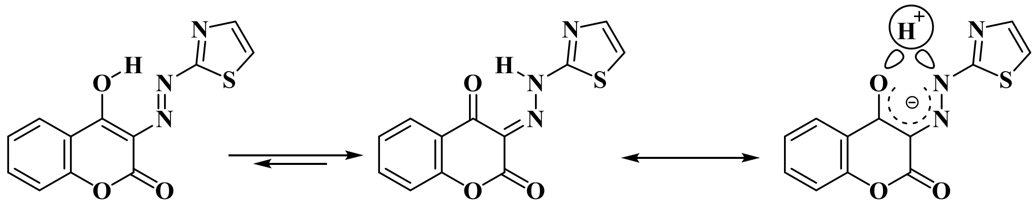
Figure 1. 1H-NMR spectra of 3


(*E*) - diazenyl form **(*E*) - hydrazinylidene form** **resonant form**

Scheme 2. Some of the possible tautomeric forms of 3

IR spectroscopy confirmed the presence of several important functional groups through characteristic vibrations [4]. The band at 3650-3450 cm^{-1} (Figure 2, circle 1) should indicate the presence of the OH group through oxygen and hydrogen vibrations, but the vibrational band of the phenolic group is usually of high intensity because of the significant changes in the dipole moment of this group. In this case, the intensity is very low, which is very unusual, suggesting that this is not an “actual” phenolic group. On the other hand, the band at 3100-3050 cm^{-1} (Figure 2, circle 2) was characteristic of aromatic CH bonds (both from the coumarin and thiazole rings). Furthermore, the band at 1750 cm^{-1} (Figure 2, circle 3), suggested the presence of a keto-group in the molecule, associated with the vibrations of the carbonyl group C=O. The IR spectrum also included characteristic benzene deformations at 1600-1450 cm^{-1} (Figure 2, circles 4 and 5), typical of an aromatic structure, and out-of-plane benzene deformations at 800 cm^{-1} (Figure 2, circle 7) and 700 cm^{-1} (Figure 2, circle 8). The vibration at 1400 cm^{-1} (Figure 2, circle 6) suggested the presence of a diazo group (N=N), confirming a specific interaction of this group with the complex structure of the substance.

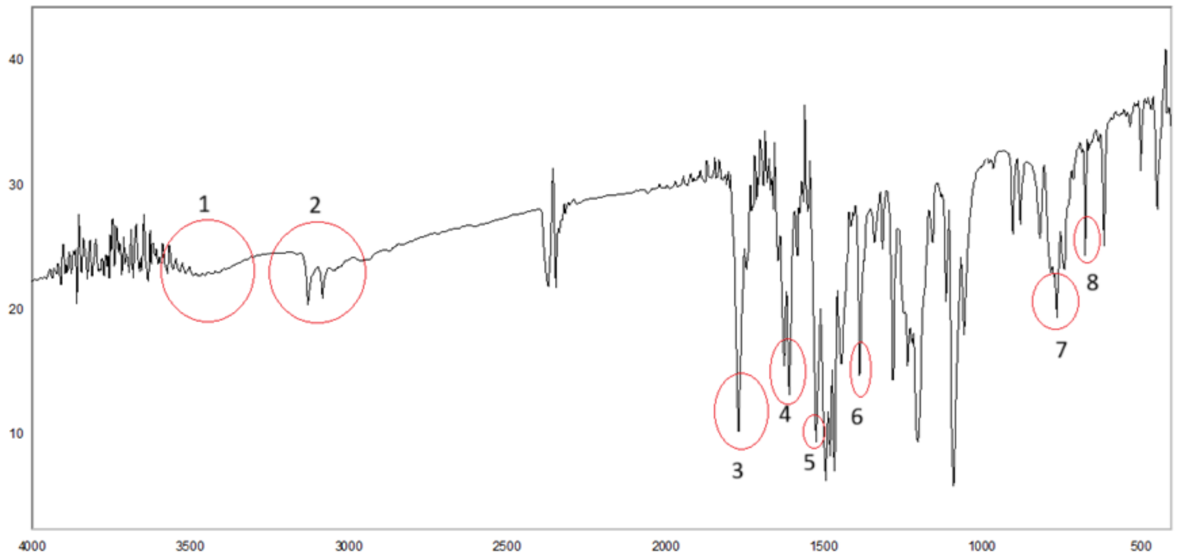


Figure 2. The IR spectrum of 3

Preparation of Buffers (pH 1–12) - For the UV/Vis spectroscopic analysis, the substance was prepared in solutions using various acids and bases, covering a wide range of pH, from 1 to 12. These solutions were mixed and diluted to a fixed volume to obtain the specified pH levels. Strong acids were used for lower pH values, while alkaline buffers were prepared by combining basic solutions with other components to maintain pH stability. All buffer solutions were prepared at the same final volume to ensure consistent conditions for spectroscopic analysis.

UV/Vis spectroscopy was used to analyze the structural and electronic behavior of the substance across varying pH conditions. This technique includes the explanation of the electronic transitions, spectral shifts, and functional group dynamics of the compound in response to environmental changes.

This technique allowed the identification of pH-dependent electronic transitions and shifts in absorbance bands. Using a UV/Vis spectrophotometer the measurements were transmitted keeping the recording light absorbance in the wavelength range of 200 nm to 800 nm. Detailed spectral data were obtained by systematically varying the pH of the solutions, highlighting changes in the electronic configuration of the molecule, particularly its $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. These measurements provided essential information on the stability and reactivity of the substance. In the first absorption band, spectral measurements showed a notable shift at 212 nm, compared to the following bands at 268 nm and 329 nm. Notable structural variations were suggested in response to the pH of the environment, highlighting the sensitivity of the coumarin derivative's electronic configuration to external conditions. The spectral shift becomes marked in highly acidic conditions (pH = 1), indicating protonation of the compound. This variation indicates changes in the $\pi \rightarrow \pi^*$ electronic transitions, where protonation redistributes the electronic density within the molecule. Electrons actively participating in the protonation process resulted from these changes in the observed spectral behavior. The fact that the first band undergoes a notable shift, while the second and third remain relatively unchanged, highlights that protonation mostly impacts specific structural regions without crucially affecting the entire molecular framework. Thus, in highly acidic environments (pH = 1-2), announced spectral shifts were observed. This shift, illustrated in Figure 3, indicated significant protonation of hydroxyl (OH) and imine (NH) groups within the molecular structure. Surprisingly, the second absorption band, located at 269 nm, demonstrated greater stability across different pH levels. However, under basic conditions (pH = 9), a noticeable shift was detected, corresponding to the structural breakdown of the coumarin lactone ring. This ring-opening event at pH = 9 reflects the sensitivity of the lactone group to deprotonation in alkaline environments. Further to more strongly basic conditions (pH = 10-12), the UV/Vis data revealed a regeneration of the lactone ring structure, restoring the cyclic configuration of the molecule, as shown in Figure 3. This structural recovery suggests that the molecule undergoes a dynamic equilibrium between open and closed conformations depending on the basicity of the environment, with pH = 10 maintaining an optimal point for the stabilization of the cyclized form. These spectral observations collectively illustrate the serious impact of pH on the electronic and structural behavior of the substance. The potential of the molecule to adapt its configuration and electronic properties in response to protonation and deprotonation processes provides valuable insights into its chemical utility. These findings enhance the understanding of the substance`s pH-dependent behavior but also certify a foundation for exploring the potential of the substance's applications in environments where electronic and structural sensitivity to pH is crucial.

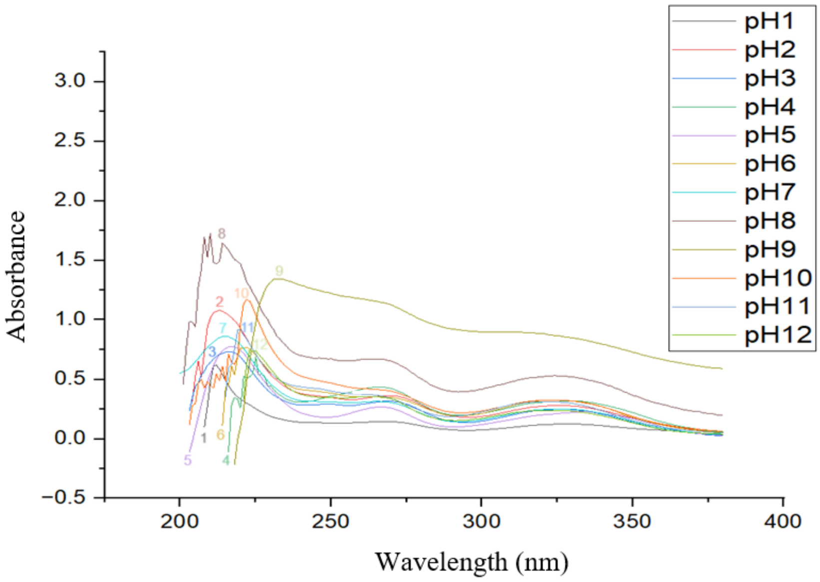


Figure 3. UV/Vis spectra of the substance at varying pH values

The increase in absorbance with pH may indicate that the analyzed substance is more active in pH = 8-9 and more moderate in pH = 5-7 (Figure 4) This can be noticed clearly in the correlation diagram between the absorbance and pH.

3. Experimental Methods

Solvents and reagents of “puriss” grade for synthesis and spectroscopic measurements were purchased from Sigma-Aldrich and were used without further purification. UV/Vis spectra were measured using Ultraspec 1600 spectrophotometer, in the range from 190-800 nm. Infrared spectra were recorded from 4000 to 400 cm^{-1} on Perkin-Elmer 1000 Spectrophotometer, using the KBr pellet technique and ATR. The NMR spectra were run on a Bruker-250 DRX Spectrometer using standard Bruker Topspin software. DMSO-d_6 was used as a solvent, and the chemical shifts were referenced to the residual solvent signal (2.5 ppm for ^{1}H and 39.5 ppm for ^{13}C spectra). Mass spectra were measured with Q-TOF premier (MICROMASS) spectrometer (ESI+ mode) in combination with a WATERS Acquity UPLC system. The melting point was determined on a Reichert heating plate and was uncorrected.

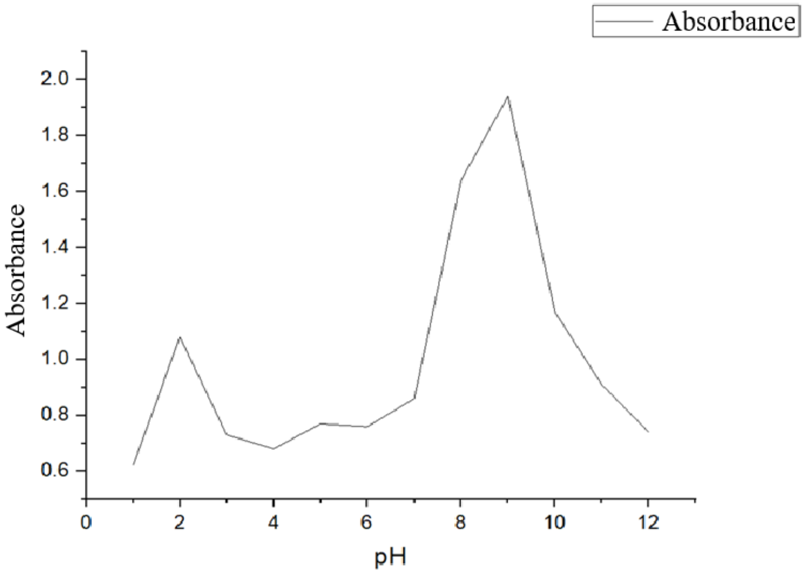


Figure 4. The dependence of the absorbance form pH.

3.1 Synthesis of (E)-3-[2-(thiazol-2-yl)hydrazinylidene]chroman-2,4-dione (1): The coumarin derivative: (*E*)-3-[2-(thiazol-2-yl)hydrazinylidene]chroman-2,4-dione (**1**), was synthesized according literature [11], with minor modifications. 2-Aminothiazole (10 mmol, 1 g) was suspended in 10 mL of water, followed by adding 40 mL of HCl (6M), obtaining clear solution which was then cooled in the ice-salt bath down to -10 °C. Afterwards, an aqueous solution of $NaNO_2$ (10 mmol, 0.7g/5 mL H_2O) was added dropwise and stirred vigorously on a magnetic stirrer. After 45 minutes, this solution was added dropwise to the fresh solution of 4-hydroxycoumarin (10 mmol, 1.62 g) in 10 mL NaOH (10 wt.). An intensively colored and voluminous precipitate of the product was obtained immediately, which was stirred for 15 minutes in the bath and 30 minutes at room temperature. Finally, the precipitate was filtered by vacuum, washed 3 times with distilled water, and dried in air. The purification was carried out by the technique of recrystallization using ethanol as solvent. Orange-red crystals (82 %), mp 209-211 ^{0}C. FTIR (KBr, ν/cm-1): 3125, 3080 (CH, aromatic stretching), 1765 (C=O, stretching), 1623, 1606, 1521 (aromatic deformations). ^{1}H-NMR (DMSO-d_6, δ/ppm, J/Hz): 8.12 – 7.45 (six aromatic protons, four at the coumarin and two at the thiazole ring). ^{13}C-NMR (DMSO-d_6, δ/ppm): 158 – 118 (twelve carbons, nine of the coumarin and three of the thiazole ring). TOF-MS-ES+ (*m/z*): 274 $[M+H]^+$, 296 $[M+Na]^+$, $C_{12}H_7N_3O_3S$.

4. Conclusions

The combination of UV/Vis and IR spectroscopy has proven to be the key functional groups of the coumarin derivative and explaining its structural and electronic behavior under varying pH conditions. These techniques identified spectral shifts and electronic transitions, giving insight into the stability and reactivity of the substance's molecular structure from the impact of protonation and deprotonation processes.

The dynamic behavior of the lactone ring was one of the most important findings, which went through structural breakdown in mildly basic conditions (pH= 9) and regenerates at higher pH levels (pH=10–12).

This flexibility of the ring-opening and cyclization explains the compound's adaptability and stability in different chemical environments. Moreover, the shifts observed in the UV/Vis spectrum, particularly at 212 nm and 269 nm, underline the sensitivity of the electronic structure to changes in acidity and basicity, offering insights into the influence of pH on the electronic configuration of π-bonds and lone-pair orbitals.

Overall, this study provides a comprehensive understanding of the structural and electronic behavior of the coumarin derivative, emphasizing the relationship between its molecular structure and environmental factors. The findings contribute to the broader knowledge of stability mechanisms and electronic transitions in complex organic compounds. These insights cover the way for further research, particularly in fields such as organic chemistry and pharmaceutical development, where understanding pH-sensitive behavior is critical for designing stable and functional compounds.

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