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^{1}H AND ^{13}C NMR SPECTRAL ASSIGNMENTS OF AN AMINO ACID-COUMARIN HYBRID[†]

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Vidoslav Dekić[1], Biljana Dekić[1], Niko Radulović[2*]

[1]Department of Chemistry, Faculty of Science and Mathematics, University of Priština, Lole Ribara 29, 38220 Kosovska Mitrovica, Serbia

[2]Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

Abstract. *Synthesis and detailed spectral analysis of a new 4-substituted coumarin-amino acid derivate are presented in this paper. A new glycine derivate of (3-nitro-2-oxo-2H-chromen-4-yl)amino]acetate was prepared by condensation of 4-chloro-3-nitrocoumarin and ethyl glycinate hydrochloride. The complete assignment of ^{1}H and ^{13}C NMR chemical shifts of the synthesized compound was carried out by the aid of a combination of 1D (^{1}H and ^{13}C NMR) and 2D (^{1}H-^{1}H-COSY, NOESY, HSQC and HMBC) NMR experiments.*

Key words: *4-chloro-3-nitrocoumarin, glycine, synthesis, spectral analysis, ^{1}H NMR, ^{13}C NMR, 2D NMR*

1. INTRODUCTION

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine since ancient times. Coumarin derivatives are widely used in the field of medicines and drugs for many years [1-6] as for example coumarin and its derivatives exhibited pronounced anticancer and antimicrobial activities as revealed from literature. They have been applied for treatment of various diseases [1-6] due to their antibacterial, antithrombotic and vasodilatory, antimutagenic, lipoxygenase and cyclooxygenase inhibition, scavenging of reactive

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[*] Corresponding author. Tel.: +381 18 533 015; fax: +381 18 533 014. E-mail address: nikoradulovic@yahoo.com (N.S. Radulović).

oxygen species, and antitumorigenic properties. Also, they are used as additives in foods, perfumes, cigarettes, cosmetics, for optical brighteners, in fluorescent and laser dyes [2, 7-11].

Coumarin derivatives are very variable in structure, due to the various types of substitution possible in their basic structure, which can influence their biological activity. The interesting biological activities of coumarins have made them attractive targets in organic synthesis.

In continuation of our efforts to design new coumarin derivatives [12, 13], herein we report the synthesis of an amino acid-coumarin hybrid (a new coumarin derivate with ethyl glycinate as a substituent on the coumarin moiety) and complete assignments of their ^{1}H and ^{13}C NMR spectral data based on a combination of 1D and 2D NMR experiments including COSY, NOESY, HSQC and HMBC.

2. MATERIALS AND METHODS

3.1. General remarks

Melting points were determined on a Kofler hot-plate apparatus and are uncorrected. HRMS(EI) spectra were recorded on a Finnigan-MAT 8230 BE mass spectrometer. The IR measurements (attenuated total reflectance, ATR) were carried out with a Thermo Nicolet 6700 FTIR instrument. For TLC, silica gel plates (Kiesel 60 F_{254}, Merck) were used. Visualization was affected by spraying the plates with 1:1 (v/v) aqueous sulfuric acid and then heating. All reagents and solvents were obtained from commercial sources (Aldrich, USA; Merck, Germany; Fluka, Germany) and used as supplied, except that the solvents were additionally purified by distillation.

3.2. NMR spectra

All NMR spectra were recorded at 25 °C in DMSO-d_6 with TMS as an internal standard. Chemical shifts are reported in ppm (δ) and referenced to TMS (δ_H = 0 ppm) in ^{1}H NMR spectra or to residual DMSO-d_5/$^{13}CD_3SOCD_3$ (δ_H = 2.50 ppm, δ_C = 39.52 ppm) in heteronuclear 2D spectra. Scalar couplings are reported in Hertz. 20 mg of sample was dissolved in 1 ml of DMSO-d_6, and 0.7 ml of the solution transferred into a 5 mm Wilmad, 528-TR-7 NMR tube.

The ^{1}H and ^{13}C NMR spectra of compound **3** were recorded on a Bruker Avance III 400 MHz NMR spectrometer (^{1}H at 400 MHz, ^{13}C at 100 MHz), equipped with a 5-mm dual ^{13}C/^{1}H probe head. The ^{1}H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 0.125 Hz digital FID resolution, 51 280 FID size, with 6410 Hz spectral width, and an overall data point resolution of 0.0003 ppm. The ^{13}C spectra were recorded with Waltz 161H broadband decoupling, 1024 scans, 0.5 s relaxation delay, 1 s acquisition time, 0.5 Hz digital FID resolution, 65 536 FID size, 31 850 Hz spectral width, and an overall data point resolution of 0.005 ppm.

Standard pulse sequences were used for 2D spectra. COSY and NOESY spectra were recorded at spectral widths of 5 kHz in both *F*2 and *F*1 domains; 1 K × 512 data points were acquired with 32 scans per increment and the relaxation delays of 2.0 s. The mixing time in NOESY experiments was 1 s. Data processing was performed on a 1K × 1K data matrix. Inverse-detected 2D heteronuclear correlated spectra were measured over 512

complex points in *F*2 and 256 increments in *F*1, collecting 128 (HSQC) or 256 (HMBC) scans per increment with a relaxation delay of 1.0 s. The spectral widths were 5 and 27 kHz in *F*2 and *F*1 dimensions, respectively. The HSQC experiments were optimized for C–H couplings of 145 Hz; the HMBC experiments were optimized for long-range C–H couplings of 10 Hz. Fourier transforms were performed on a 512 × 512 data matrix. $\pi/2$ Shifted sine-squared window functions were used along *F*1 and *F*2 axes for all 2D spectra.

3.3. Synthesis

3.3.1. Synthesis of 4-chloro-3-nitrocoumarin (3)

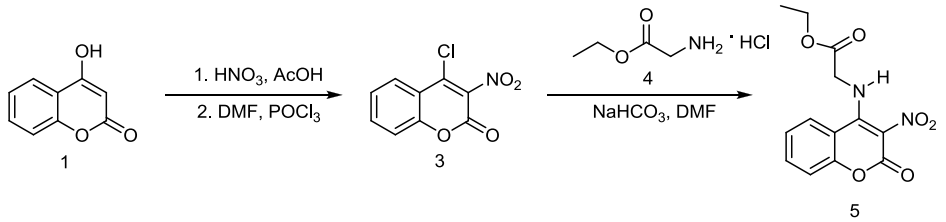
4-Chloro-3-nitrocoumarin, was obtained from 4-hydroxycoumarin, in two reaction steps according to a published procedure [14]. In the first step 4-hydroxycoumarin (1) was nitrated using 72% HNO_3 (w/w) in glacial AcOH to afford 4-hydroxy-3-nitrocoumarin (2). The starting compound 4-chloro-3-nitrocoumarin (3) was prepared from 4-hydroxy-3-nitrocoumarin (2) in a reaction with $POCl_3$ in *N*,*N*-dimethylformamide in the yield of 72%.

3.3.2. Synthesis of [(3-nitro-2-oxo-2H-chromen-4-yl)amino]acetate (5)

The solution of 4-chloro-3-nitrocoumarin (3) (1 g, 4.4 mmol) and glycine ethyl ester hydrochloride (4) (0.61 g, 4.4 mmol) in *N*,*N*-dimethylformamide (10 ml), in the presence of sodium bicarbonate (0.84g, 0.01 mol), was stirred at room temperature for 3 h. The reaction was quenched by adding cold water (20 ml). The precipitated solid was collected by filtration and washed with water. The purity of the synthesized compound was assessed by TLC. The target product, [(3-nitro-2-oxo-2*H*-chromen-4-yl)amino]acetate (5), was obtained as yellow crystals, m.p. 181-183 °C, in good yield - 89%. HRMS(EI): M^+ ($C_{13}H_{12}N_2O_6$) 292.0684, requires 292.0695 (Δ= - 1.1 mmu). IR (neat): 3328 (N-H), 3015 (Ar-H), 2990 (C-H), 1746 (C=O, ester), 1689 (C=O, lactone), 1610 (C=C), 1520 and 1327 (NO_2), 1197, 1068, 915, 860, 751 cm^{-1}.

3. Results and Discussion

The reaction of 4-chloro-3-nitrocoumarin [14] (3) with glycine ethyl ester hydrochloride (4) in *N*,*N*-dimethylformamide gave ethyl [(3-nitro-2-oxo-2*H*-chromen-4-yl)amino]acetate (5) as a yellow crystalline solid in high yield (89%) (scheme 1).



Scheme 1 Synthesis of ethyl [(3-nitro-2-oxo-2*H*-chromen-4-yl)amino]acetate

High-resolution electron impact mass spectrometry (HR-EIMS) of the synthesized compound indicated a molecular formula of $C_{13}H_{12}N_2O_6$ ($[M]^+$ at *m/z* 292.0684, Δ= - 1.1 mmu). The IR spectra of the synthesized compound showed characteristic vibration of N-H (3328 cm^{-1}) and Ar-H bonds (3015 cm^{-1}). Absorptions corresponding to two carbonyl groups appeared at 1746 (ester), 1689 cm^{-1} (lactone). The presence of the NO_2 group was evident from IR absorptions that appeared at 1520 and 1327 cm^{-1}.

The ^{1}H NMR spectrum of this compound contained seven signals. Four of them, corresponding to aromatic protons, appeared as two sets of doublet of doublets at 8.24 and 7.46 ppm, and two sets of doublet of doublet of doublets at 7.78 and 7.50 ppm. Integration of the ^{1}H NMR spectrum confirmed that each of these signals corresponded to one proton.

The ^{1}H NMR spectrum also exhibited two upfield signals, corresponding to more shielded protons of one methyl and two methylene groups. Integration and spin-spin coupling analysis allowed the assignation of these signals. The multiplet signal centered at 4.16 ppm, corresponding to four protons, was found to originate from the overlapping two methylene groups, whereas the three methyl protons appeared as a triplet at 1.23 ppm. Based on the value of the chemical shift, the remaining broad singlet at 8.95 ppm was readily assigned to the more downfield proton of the secondary amino group which connected the coumarin part of molecule and the amino acid substituent.

The assignment of aromatic methine signals was inferred from the observed HSQC and HMBC correlations (Fig. 1). In the HMBC spectrum a non-protonated carbon signal at 147.5 ppm showed correlations (corresponding to three-bond C-H couplings) with two proton signals, one doublet of doublets at 8.24 ppm, corresponding to an aromatic methine proton from the coumarin part of molecule, and methylene multiplets at 4.10-4.20 ppm from the substituent side-chain. Thus, this carbon should occupy the position C-4 of the coumarin moiety, while the HMBC interaction of this carbon allowed the assignation of the aromatic proton H-5 whose signal appeared as a doublet of doublets, as expected due to the two significantly different coupling constants between H-5 and H-6 (J = 8.4 Hz) and H-5 and H-7 (J = 1.2 Hz).

The rest of the aromatic protons were easily assigned based on their mutual NOESY interactions (Fig. 2, Table 1). The chemical shift of carbon atoms to which these protons were bonded to was subsequently determined from the HSQC spectrum (Table 1). The assignment of these carbons was additionally supported through HMBC correlations between H-5 and C-7, H-6 and C-8, H-7 and C-5 and H-8 and C-6.

Carbon atoms C-4a and C-8a were assigned according to the existence of simultaneous HMBC interactions of protons H-6 and H-8, as well as H-5 and H-7 with the carbon signals at 114.2 and 151.4 ppm, respectively. In a similar manner to the previously studied compounds [13] a characteristic interaction through two bonds between H-8 and the quaternary C-8a were observed in the HMBC spectrum. The assignment of substituent carbon atoms was performed starting from the correlations of methyl protons. These protons showed an HSQC correlation with the carbon at 14.4 ppm and were thereby assigned to the methyl-carbon.

Also, the methyl protons in the HMBC correlated with the carbon at 61.9 ppm. Since correlations through three bonds do not exist, it is clear that the methyl protons coupled with the methylene carbon C-5` through two bonds. This assumption is further corroborated by HSQC data. Additionally, the multiplet of overlapping signals at 4.10-4.20 ppm has a HSQC interaction with the carbon at 45.6 ppm which was assigned to C-2'.

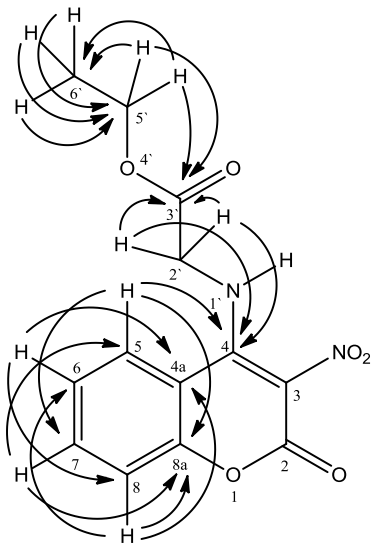


Fig. 1 The observed HMBC correlations of the synthesized compound.

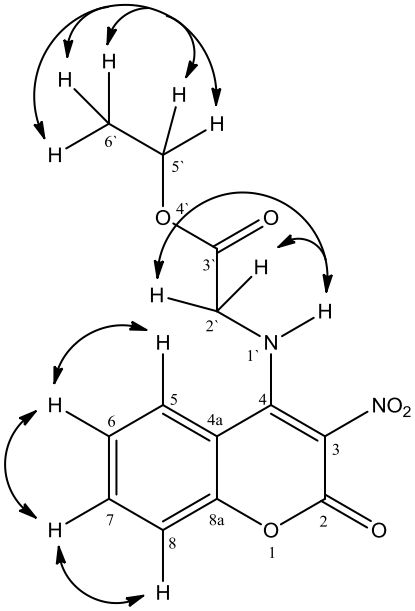


Fig. 2 The observed NOESY correlations of the synthesized compound.

Finally, the last two carbon signals at 155.5 and 117.1 ppm were attributed to the lactone carbonyl (C-2) and nitro-bearing carbons (C-3), respectively, based on their chemical shifts, since no H interactions were observed in any 2D spectra and by comparison with the analogous signals in related compounds [13].

Table 1 NMR data of the synthesized compound in DMSO-d_6 recorded at 400 (^{1}H) and 100 MHz (^{13}C)

Position	δ_H, m (J, Hz)	δ_C	NOESY[a]	HMBC[b]
2	-	155.5	-	-
3	-	117.1	-	-
4	-	147.5	-	-
4a	-	114.2	-	-
5	8.24, dd (8.4, 1.2)	124.6	6	7, 8a, 4
6	7.50, ddd (8.4, 7.6, 1.2)	125.3	5, 7	4a, 8
7	7.78, ddd (8.4, 7.6, 1.2)	134.7	6, 8	5, 8a
8	7.46, dd (8.4, 1.2)	118.1	7	4a, 6, 8a
8a	-	151.4	-	-
NH	8.95 s	-	2`	-
2'	4.10-4.20, m[c]	45.6	N-H	4, 3`
3'	-	168.4	-	-
5'	4.10-4.20, m[c]	61.9	6`	6`, 3`
6'	1.23, t (7.0)	14.4	5`	5`

[a] NOESY interactions of the hydrogen from the column "Position" with the corresponding hydrogen from the column "NOESY".
[b] HMBC interactions of the hydrogen from the column "Position" with the corresponding carbons from the column "HMBC".
[c] Overlapping signals.

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ASIGNACIJA ^{1}H I ^{13}C NMR SPEKTARA AMINOKISELINSKOG DERIVATA KUMARINA

U ovom radu su opisani sinteza i spektralna analiza novog aminokiselinskog derivata kumarina. Glicinski derivat kumarina [(3-nitro-2-oxo-2H-chromen-4-yl)amino]acetate dobijen je kondenzovanjem 3-hlor-4-nitrokumarina i etil-glicinat hidrohlorida. Kompletna asignacija signala u ^{1}H i ^{13}C NMR spektrima sintetisanog jedinjenja izvršena je kombinovanjem ^{1}H i ^{13}C NMR sa ^{1}H-^{1}H- COSY, NOESY, HSQC i HMBC eksperimentima.

Ključne reči: *4-hlor-3-nitrokumarin, glicin, sinteza, spektralna analiza, ^{1}H NMR, ^{13}C NMR, 2D NMR*