Synthesis and Characterization of a Flavonoid Chrysin Derivative

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Keywords: Chrysin; Synthesis; Crystal structure; ct-DNA

Abstract: Chrysin, was also known as 5,7-dihydroxyl flavone, with many different biological activities such as antioxidant, antiviral, antidiabetic, and anti-anxiety, etc. A chrysin derivative, 5,7-dihydroxyflavone-8-sulfonate potassium, was synthesized by sulfonation reaction under room temperature to improve its water-solubility and bioavailability. And the derivative was investigated by elemental analysis, nuclear magnetic resonance (NMR) and X-ray single-crystal diffraction, respectively. The crystal structure analysis shows that 5,7-dihydroxyflavone- 8-sulfonate potassium belongs to orthorhombic system. In its crystal structure, sulfonate oxygen atoms and flavone carbonyl oxygen atoms coordinate with potassium ions, and K-O coordination forms a one-dimensional polyionic chain. There's π···π stacking interaction between the adjacent flavonoid skeletons. The self-assembly of the compound into a three-dimensional supramolecular structure was performed by π···π stacking and K-O coordination. Meanwhile, the interaction between the compound and calf thymus DNA (ct-DNA) was investigated by fluorescence spectrometry, and the results showed that the interaction between the derivative and DNA was stronger than that of the chrysin.

1. Introduction

Chrysin, also named 5,7-dihydroxyl - flavone, is a kind of natural flavonoid compound widely existing in nature. It has a variety of physiological activities, such as anti-diabetes, anti-anxiety, anti-oxidation, anti-virus and anti-cancer activity, and etc [1-4]. In order to improve the physiological activity of chrysin, many of its derivatives have been synthesized, such as a series of 5,7-dialkoxy flavones, 5-hydroxy-7-alkoxy flavones, 8-amino-5,7-dihydroxy flavone and 6-amino-5,7-dihydroxy flavone, etc [5]. Many researchers investigated the bioactivity of its derivatives [6-9]. Other studies have been carried out on the interactions between chrysin and its derivatives and biomacromolecules [10-12]. The researchers found that the introduction of sulfonic acid group into flavones molecular could improve the water solubility and biological activity. Daidzein-3'-sulfonate sodium was synthesized and studied anti-hypoxic- ischemic activity, which proved that the water-solubility and anti-hypoxic-ischemic activity of the compound were superior to daidzein [13]. Pusz sulfonated chrysin to obtain chrysin-4'-sulfonate sodium and synthesized complexes with chrysin-4'-sulfonate ligand acting with different metal ions, respectively [14-16]. In this paper, a new type of chrysin derivative, 5,7- dihydroxyflavone-8- sulfonate potassium (shown in Figure 1) was synthesized by sulfonation reaction at room temperature. It was characterized by NMR, FT-IR, and its crystal structure was determined by X-ray single crystal diffraction. The effect of the derivative on ct-DNA was investigated by fluorescence spectrometry.

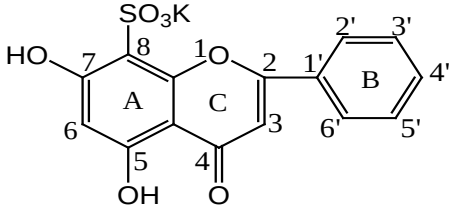

Figure 1 Simple chemical structure of 5,7- dihydroxyflavone-8- sulfonate potassium.

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2. Experiment

2.1 Instruments and reagents

Bruker-300 NMR spectrometer (TMS as internal standard, DMSO-d_6 as solvent), PE-2400 element analyzer, Bruker Smart-1000 CCD type single crystal diffraction, Sartorius electronic balance, Hitachi F-4600 fluorescence spectrometer, Bruker Tensor-27 Fourier transform infrared spectrometer (FT-IR), Agilent Cary60 UV visible spectrophotometer.

Chrysin (Xi'an Ruisaen bio-tech Co. LTD, the purity is more than 98 percent), Ethidium bromide (EB, Sigma), three hydroxy-methyl amino-methane (Tris, Sigma), calf thymus DNA (ct-DNA, Beijing Solaibao Technology Co. LTD) was determined by the UV spectrum of A_{260}/A_{280}>1.8, showed its purity conforms to the requirement. 50mmol·L^{-1} Tris-50 mmol·L^{-1}NaCl buffer solution of pH7.2 and acetic acid- sodium acetate buffer solution with pH5.8 were prepared in accordance with the regular method. The experimental used reagents were of analytical grade.

2.2 Synthesis and characterization

2.0 g chrysin was weighed and placed in 25 ml round bottom flask, then 10 ml concentrated H_2SO_4 was added into and reaction for 15 h at room temperature. Next 40 ml saturated KCl solution was poured into the flask. When yellow precipitation was precipitated, standing for 5 h, and then pumped filtration, the filter cake was washed to neutral with a saturated KCl solution. The filter cake was recrystallized in ethanol-water (volume ratio of 1:1) as the solvent, and then the derivative was obtained. Lastly the derivative was dried in vacuum at 105degrees celsius for 10 h and the yield was 78 percent. The melting point of this compound is 293 degrees celsius (decomposition).

With TMS as internal standard and DMSO-d_6 as solvent, the obtained derivative was characterized by NMR. Then using KBr as diluent, by pressing tablet, the infrared spectrogram of the derivative was determined by Fourier transform infrared spectrometer. Next, an appropriate amount of the derivative saturated solution was prepared using ethanol-water (volume ratio of 2:1) as the solvent, let stand at room temperature, and the light yellow block-shaped single crystal was obtained after 3 days for structural testing.

3. Results and discussion

3.1 Synthesis of 5,7- dihydroxyflavone-8- sulfonate potassium

As early as 10 years ago, some researchers used concentrated sulfuric acid H_2SO_4 as sulfonating agent to obtain the sulfonated products of chrysin. Some researchers sulfonated chrysin at 140 degrees celsius and obtained only B loop sulfonated product chrysin-4'-sulfonic acid [14]. And the A ring sulfonated product of chrysin-6-sulfonic acid was obtained at 80 degrees Celsius [12]. In this paper, with the concentrated H_2SO_4 as sulfonating agent, a new chrysin A loop sulfonated product chrysin -8- sulfonic acid was synthesized at room temperature (r.t). It was also the velocity control product obtained at low temperature. The reaction has the advantages of mild conditions and easy treatment of products. Their synthesis routes were shown in Figure 2. And three products are isomers, which are sulfonated products of concentrated H_2SO_4 and chrysin at different temperatures.

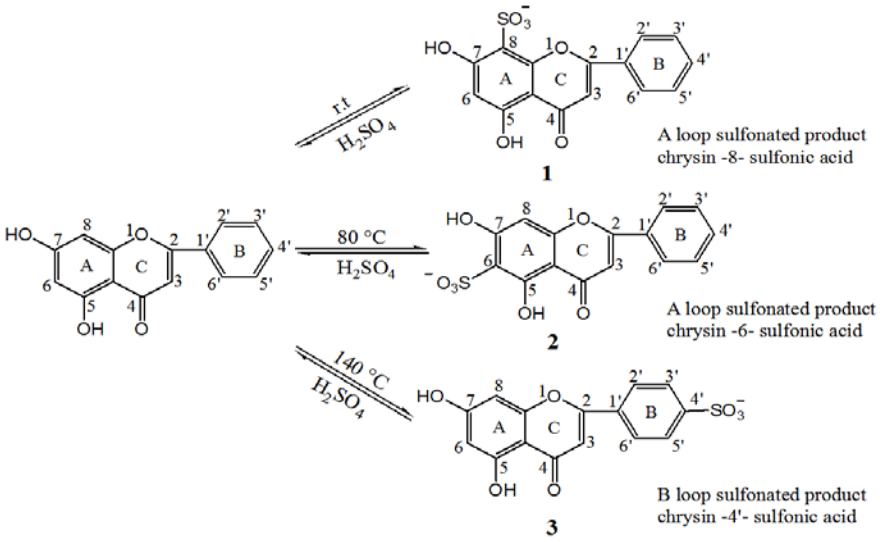

Figure 2 The synthesis routes of three sulfonated products.

3.2 Spectral data analysis

The infrared spectral data of the compound are as follows: IR (cm^{-1}, KBr) V: 3458, 1637, 1601, 1571, 1453, 1230, 1185, 1092, 1047, 837, 766, 691. In the IR spectrum, the wide absorption peak at 3458 cm^{-1} was the hydroxyl absorption peak, while the vibration absorption peak of carbonyl appeared at 1637 cm^{-1}.Absorption peaks at 1092 cm^{-1} and 1047 cm^{-1} were characteristic absorption of sulfonic acid groups, indicating that sulfonic acid groups were introduced into the molecules of chrysin.

NMR data are as follows: ^{1}H-NMR (ppm, DMSO-d_6, 300 MHz) δ: 13.24 (s, 1H, H-C5-OH), 12.64 (s, 1H, H-C7-OH), 8.52 (s, 2H, H-C2', C6'), 7.73 (s, 3H, H-C3', C4', C5'), 7.27 (s, 1H, H-C3), 6.36 (s, 1H, H-C6); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 182.5 (4-C), 163.8 (5-C), 161.7 (2-C), 160.7 (7-C), 152.7 (8a-C), 132.4 (1' -C), 130.7 (4'-C), 129.3 (3' -C, 5'-C), 127.4 (2' -C, 6'-C), 110.7 (8-C), 104.9 (4a-C), 104.3 (3-C), 99.1 (6-C). In ^{1}H-NMR spectra, the chemical shift δ in the absorption peak of 13.24 ppm and 12.64 ppm were absorption peaks of hydroxyl hydrogen at 5-C and 7-C, respectively. Their chemical shift data are high than normal phenolic hydroxyl hydrogen because of the two intramolecular hydrogen bonds O(1)-H(1)···O(7) and O(2)-H(2)···O(3). Compared with ^{1}H-NMR spectrum of chrysin, absorption peak of hydrogen atom at 8-C in chrysin A loop disappear. This indicates that the sulfonate group was introduced into the 8-C position of the chrysin molecule. In the ^{13}C-NMR spectrum, the chemical shift of carbonyl carbon atoms was 182.5 ppm and that of 8-C was 110.7 ppm.

3.3 Crystal structure

The size of single crystals was selected as 0.37 mm × 0.20 mm × 0.13 mm, a diffraction experiment was performed by the Bruker Smart-1000 CCD type single crystal diffractometer. Ray of Mo-K α diffraction was used at 0.71073 Å wavelength to scan in omega (ω) with graphite monochromator. At 293K 13998 diffraction point was collected, of which 2367 independent diffraction intensity data. All calculations were completed by SHELXS97 program. The crystallographic data and structure analysis of parameters are shown in Table 1.

Table 1 Crystallographic parameter.

Empirical formula	$C_{15}H_9O_7SK$
Formula weight	372.38
Crystal system	Orthorhombic
Space group	*Pc*
a, *b*, *c* (Å)	*a*=19.0846(19), *b*=20.6555(19), *c*=7.5148(7)
V ($Å^3$)	2962.3(5)
Z, *Dc* (mg/m^3)	8, 1.670

μ (mm^{-1})	0.537
F (000)	1520
h, k, l	-21≤h≤22, -24≤k≤22, -7≤l≤8
θ range (°)	1.45 ~ 25.10
Total reflections/Unique [R_{int}]	13998/2637 [R_{int} = 0.0492]
Final R Indices [$I>2\sigma(I)$]	R_1 = 0.0423, wR_2 = 0.1080
R Indices (all data)	R_1 = 0.0713, wR_2 = 0.1287
GOF(S)	1.043
$\Delta\rho\times10^{-3}/(e\bullet nm^{-3})$	0.205, -0.262

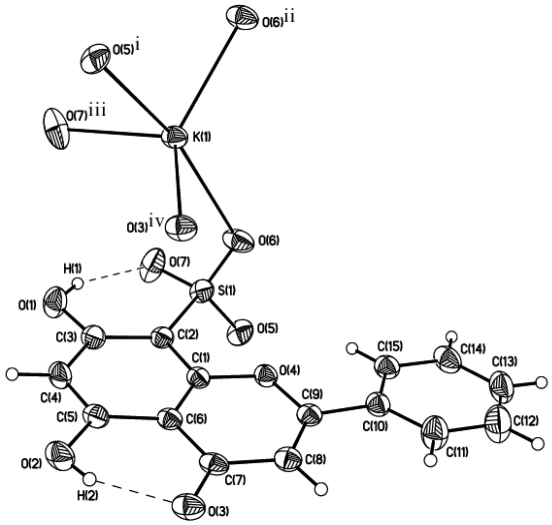

Figure 3 The coordination environment of the K(I) in molecular $C_{15}H_9O_4SO_3K$.

Atomic ellipsoids are drawn at 30 percent probability level. The dashed lines indicate hydrogen bonds. Symmetry codes are as follows, (i) *x*, *y*, 1+*z*. (ii) *x*, 1/2−*y*, 1/2+*z*. (iii) 1/2−*x*, *y*, 1/2+*z*. (iv) −*x*, 1−*y*, 1−*z*.

Molecular formula $C_{15}H_9O_4SO_3K$, molecular structure consists of a potassium ion K (I) and a 5, 7- dihydroxyflavone -8- sulfonate anion (seen Figure 3). The bond lengths and bond angles of 5, 7- dihydroxyflavone -8- sulfonate anion are basically the same as 5, 7- dihydroxyflavone -6- sulfonate anion [12]. The dihedral angle of A loop [C(1) -C(6)] and C loop [O(4) /C(1) /C(6) -C(9)] is 1.27(14)°, and the dihedral angle of loop B [C(10) -C(15)] and benzoapyrene ring A/C [O(4) /C(1) -C(9)] is 7.26 (15)°. The whole flavonoid skeleton is basically coplanar, and the average offset distance of each atom relative to the least square plane is 0.051 Å. K (I) coordination number is 5, coordination atoms are from sulfo oxygen atom O(5)^{i} [symmetric code (i): *x*, *y*, 1+*z*.], O(6), O(6)^{ii} [symmetric code (ii): *x*, 1/2-*y*, 1/2+*z*.] and O(7)^{iii} [symmetric code (iii): 1/2-*x*, *y*, 1/2+*z*.] and carbonyl oxygen atom O(3)^{iv} [symmetric code (iv): -*x*, 1-*y*, 1-*z*.], respectively. The bond length of K–O bond are as follows: 2.755(3) Å for K–O(6), 2.753(2) Å for K–O(6)^{ii}, 2.643(2) Å for K–O(3)^{iv}, 2.675(2) Å for K–O(5)^{i}, 2.654(3) Å for K–O(7)^{iii}, respectively. In addition, the two intramolecular hydrogen bonds exists in the molecular, O(1)-H(1)···O(7) and O(2)-H(2)···O(3), respectively. They are formed by the hydroxyl hydrogen atom and the sulfonic oxygen atom and the carbonyl oxygen atom respectively, and their bond lengths and bond angles are 2.588 Å, 2.604 Å and 152°, 148°, respectively. In addition, there may be π···π accumulation in the flavonoid skeletons. Thus π···π stacking action and K-O coordination make it possible to self-assemble into three-dimensional supramolecular structures. This needs further study.

3.4 Determination of interaction with ct-DNA

Using methanol as solvent, chrysin and its derivative 5,7- dihydroxyflavone-8- sulfonate potassium solution were prepared, respectively. Their concentrations were all 3.0×10^{-4} mol·L^{-1}. Firstly, two solutions 3.0×10^{-5} mol·L^{-1} ethidium bromide (EB) for 1.0 ml and 3.12×10^{-5} mol·L^{-1} ct-DNA solution for 1.0 ml were added respectively into 10 ml scale test tube, then shake the tube and keep reaction for 30 min at room temperature. Secondly, different amounts of compounds to be

tested were added, respectively. After 30 min, dilute with the buffer solution to scale. Finally, their fluorescence emission spectra were measured at 550 nm ~ 750 nm with the excitation wavelength of 525 nm and slit Ex and Em 10 nm as scanning conditions, respectively.

Ethidium bromide (EB) as a fluorescence probe with a high sensitivity, good selectivity. Its own fluorescence is very weak, but its flat molecule structure can have a single insertion with double-stranded DNA, which greatly enhances its fluorescence [12]. If the addition of small organic molecule M to the EB-DNA system can have an embedding effect similar to that of EB occurs on DNA, this small molecule will compete with EB for the same DNA binding site. Furthermore, if the interaction between the small molecule M and DNA molecule is stronger, EB will be squeezed out of the DNA double helix structure to form a new M-DNA system, thus significantly reducing the fluorescence intensity of EB-DNA. The results of the effects of chrysin and its derivative 5,7-dihydroxyflavone-8- sulfonate potassium on the fluorescence intensity of EB-DNA system are shown in Figure 4. With the increase of the concentration of chrysin, the fluorescence spectrum of the system hardly changed (seen in Figure 4A). As the concentration of the derivative increased, the fluorescence intensity of the system gradually decreased (seen in Figure 4B), indicating that the interaction between the derivative and DNA was stronger than that of chrysin. This was probably because the introduction of sulfonic acid group enhanced the interaction between the derivative and DNA, which would provide certain experimental basis for the development of new drugs derived from chrysin.

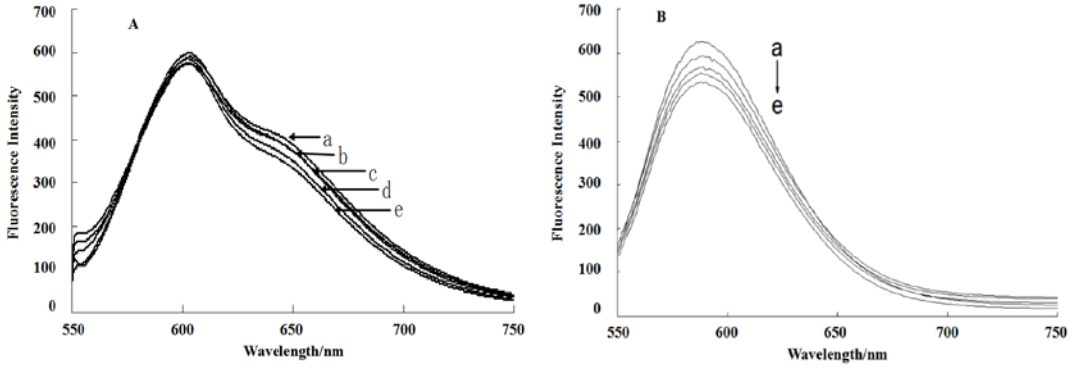

Figure 4 Effect of chrysin(A) and 5,7- dihydroxyflavone-8- sulfonate potassium (B) on the emission spectra of EB-DNA system.

a~e: absence (a) and presence (b, c, d, e) of increasing, (0, 1.5, 3.0, 4.5, 6.0)×10^{-5} mol·L^{-1} chrysin and 5,7- dihydroxyflavone-8- sulfonate potassium, respectively.

4. Conclusion

A derivative of chrysin, 5,7-dihydroxyflavone-8-sulfonate potassium, was synthesized by sulfonation reaction under room temperature to improve its water-solubility and bioavailability. And the derivative was investigated by elemental analysis, FT-IR, NMR and X-ray single-crystal diffraction, respectively. The results indicate 5,7-dihydroxyflavone- 8-sulfonate potassium belongs to orthorhombic system. In its crystal structure, sulfonate oxygen atoms and flavone carbonyl oxygen atoms coordinate with potassium ions, and K-O coordination forms a one-dimensional polyionic chain. There's π···π stacking interaction between the adjacent flavonoid skeletons. The self-assembly of the compound into a three-dimensional supramolecular structure was performed by π···π stacking and K-O coordination. Meanwhile, the interaction between the compound and ct-DNA was investigated by fluorescence spectrometry, and the results showed that the interaction between the derivative and DNA was stronger than that of the chrysin. It would provide certain experimental basis for the development of new drugs derived from chrysin.

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