Synthesis, Crystal Structure and Antitumor Activity of Tectochrysin-6-sulfonate (Postprint)

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Abstract

In order to enhance water-solubility and biological utilization rate of tectochrysin, sodium 5-hydroxyl-7-methoxyflavone-6-sulfonate (1) was synthesized and its structure was identified on the basis of 1H NMR, FT-IR and elemental analysis. 5-Hydroxyl-7-methoxyflavone-6-sulfonate was assembled with Ni(II) or Mn(II), hexaquanickel(II) bis(5-hydroxyl-7-methoxyflavone-6-sulfonate) tetrahydrate (2) and hexaquamanganese(II) bis(5-hydroxyl-7-methoxyflavone-6-sulfonate) tetrahydrate (3) were obtained and characterized by IR spectroscopy. The crystal structures of 2 and 3 were determined by X-ray single-crystal diffraction analysis. The results showed that 2 and 3 are isomorphous crystals and crystallize in monoclinic crystal system, space group C2/c. In 2 and 3, the supramolecular structures are organized into hydrophilic and hydrophobic regions. Hydrophilic regions are generated by O–H×××O hydrogen bonds among sulfonate groups, latticed water molecules and coordinated water molecules. The - stacking interactions assemble the flavone skeletons into columns and these columns form hydrophobic regions. The sulfonate groups play an important role as a bridge of the hydrophilic and hydrophobic regions as well as the inorganic and organic components. Three-dimensional networks of 2 and 3 are furnished by extensive array of hydrogen bonds, - stacking interactions and electrostatic interactions. The anti-proliferative activities of 1–3 in vitro against human leukemia cells K562 and human lung cancer cells A549 were evaluated by the standard MTT assay. The pharmacological activity results showed that the introduction of sulfonic acid groups enhanced the antitumor activity of tectochrysin.

Full Text

Preamble

Synthesis, Crystal Structure and Antitumor Activity of Tectochrysin-6-sulfonate

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ABSTRACT

To enhance the water solubility and biological utilization rate of tectochrysin, sodium 5-hydroxy-7-methoxyflavone-6-sulfonate (1) was synthesized and its structure was identified by ^{1}H NMR, FT-IR, and elemental analysis. Compound 1 was then assembled with Ni(II) or Mn(II) to obtain hexaquanickel(II) bis(5-hydroxy-7-methoxyflavone-6-sulfonate) tetrahydrate (2) and hexaquamanganese(II) bis(5-hydroxy-7-methoxyflavone-6-sulfonate) tetrahydrate (3), which were characterized by IR spectroscopy. The crystal structures of 2 and 3 were determined by X-ray single-crystal diffraction analysis. The results showed that 2 and 3 are isomorphous crystals that crystallize in the monoclinic crystal system, space group C2/c. In both complexes, the supramolecular structures are organized into hydrophilic and hydrophobic regions. The hydrophilic regions are generated by O–H $\cdots$ O hydrogen bonds among sulfonate groups, lattice water molecules, and coordinated water molecules. The – stacking interactions assemble the flavone skeletons into columns, which form hydrophobic regions. The sulfonate groups play an important role as a bridge connecting the hydrophilic and hydrophobic regions as well as the inorganic and organic components. Three-dimensional networks of 2 and 3 are furnished by extensive arrays of hydrogen bonds, – stacking interactions, and electrostatic interactions. The anti-proliferative activities of 1–3 against human leukemia cells K562 and human lung cancer cells A549 were evaluated by the standard MTT assay. The pharmacological results showed that the introduction of sulfonic acid groups enhanced the antitumor activity of tectochrysin.

Keywords: 5-hydroxy-7-methoxyflavone-6-sulfonate; spectroscopic property; crystal structure; antitumor activity

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1 INTRODUCTION

Tectochrysin (5-hydroxy-7-methoxyflavone) is a naturally widespread flavonoid and one of the effective components of propolis [1], exhibiting various biological activities such as anti-cancer [2], anti-oxidant [3], and trypanocidal activity [4]. However, flavonoids generally have poor solubility and low biological utilization rates, making it necessary to synthesize water-soluble flavonoid derivatives to improve their potential biological activities. The introduction of a sulfonate group into flavonoid molecules can enhance their water solubility and biological

activities [5, 6]. We have previously synthesized several flavonoid sulfonates [7, 8] and studied the biological activities of sodium 7,4 -dihydroxyisoflavone-3 -sulfonate [9] and sodium 4 -hydroxy-7-methoxyisoflavone-3 -sulfonate [10]. The results showed that the biological activities of flavonoid sulfonates are superior compared with the corresponding parent flavonoids. In this paper, sodium 5-hydroxy-7-methoxyflavone-6-sulfonate (1) was synthesized and subsequently assembled with Ni(II) or Mn(II) to obtain hexaquanickel(II) bis(5-hydroxy-7-methoxyflavone-6-sulfonate) tetrahydrate Ni(H O) · 4H O (2) and hexaquamanganese(II) bis(5-hydroxy-7-methoxyflavone-6-sulfonate) tetrahydrate Mn(H O) · 4H O (3). The structure of 1 was established by ^{1}H NMR, FT-IR, and elemental analysis. Compounds 2 and 3 were characterized by IR spectroscopy, and their crystal structures were determined by X-ray single-crystal diffraction analysis. The antitumor activities of 1–3 against human leukemia cells K562 and human lung cancer cells A549 were evaluated by the standard MTT assay [11, 12].

Scheme 1. Chemical structure of 1

2 EXPERIMENTAL

2.1 Materials and Physical Measurements

All chemicals and solvents used for synthesis were of analytical grade, commercially available, and used without further purification. Samples were dried using a 2K-82B vacuum drying oven. The infrared spectrum was recorded on a Nicolet 170SX FT-IR spectrophotometer with KBr pellets in the 4000–500 cm ^{1} region. The ^{1}H NMR spectrum was recorded on a Bruker AM-300 spectrometer with TMS as internal reference and DMSO-d as solvent. Carbon and hydrogen contents were analyzed using a PE-2400 elemental analyzer. Crystal structures were determined with a Bruker Smart-1000 CCD diffractometer.

2.2 Synthesis of 1

Tectochrysin (2.0 g) was slowly added to concentrated sulfuric acid (10 mL) with stirring. The reaction was maintained at room temperature for 15 hours, then poured into saturated NaCl aqueous solution (50 mL), resulting in the formation of a yellow precipitate. After 5 hours, the precipitate was filtered and washed with saturated NaCl aqueous solution until the filtrate reached pH 7. The precipitate was recrystallized from an ethanol-water solution (V:V = 1:1) to afford compound 1, which was dried at 105 °C for 10 hours under vacuum. Yield: 75%. IR (cm ^{1}, KBr) : 3467, 1644, 1606, 1486, 1447, 1206, 1145, 1098, 1046, 897, 809, 773, 686. ^{1}H NMR (DMSO-d , 300 MHz) : 13.13 (s, 1H), 7.56–7.87 (m, 5H), 6.78 (s, 1H), 6.52 (s, 1H), 3.89 (s, 3H). Anal. Calcd. for C H NaO S (%): C, 51.89; H, 2.99. Found (%): C, 51.15; H, 3.21.

2.3 Syntheses of 2 and 3

An aqueous solution of Ni(NO) ·6H O (10%, 5 mL) was mixed with an aqueous solution of 1 (5%, 10 mL), yielding compound 2 after 24 hours. Compound 2 was recrystallized from an ethanol-water solution (V:V = 3:1). Green block crystals suitable for X-ray analysis were obtained by slow solvent evaporation for approximately 5 days at room temperature. Yield: 81%. IR (cm ^{1}, KBr) : 3461, 1646, 1608, 1483, 1445, 1208, 1176, 1099, 1042, 898, 805, 770, 678.

The synthesis of 3 was similar to that of 2, except that Ni(NO) ·6H O was replaced with MnSO ·H O. Yield: 85%. IR (cm ^{1}, KBr) : 3460, 1643, 1596, 1486, 1446, 1201, 1182, 1099, 1043, 898, 811, 761.

2.4 X-ray Crystallography of 2 and 3

A single crystal of 2 or 3 was mounted on a Bruker SMART-1000 CCD diffractometer equipped with graphite-monochromated MoK radiation (= 0.71073 Å) for intensity data collection at 296(2) K. The structures were solved by direct methods using the SHELXTL software package [13] and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located at geometrically calculated positions and refined using a riding model. Final refinement converged to R = 0.0407, wR = 0.1077 for 2 and R = 0.0322, wR = 0.0717 for 3. Details of crystal data collection and refinement are given in Table 1 , and selected bond lengths and bond angles are listed in Table 2 . Additional crystallographic data have been deposited as supplementary materials.

2.5 In Vitro Anti-proliferative Activities

2.5.1 Cell Culture Leukemic K562 cells and human lung cancer A549 cells were incubated in RPMI-1640 medium. Tumor cells were seeded at 5×10^{3} cells per well in 96-well plates and incubated for 24 hours at 37 °C in humidified air containing 5% CO .

2.5.2 Antitumor Activity Evaluation DMSO solutions of compounds 1–3 were prepared as stock solutions. After cells were incubated for 24 hours, each compound solution was added to the 96-well plates at concentrations of 5, 10, 20, 40, and 80 mol/L. Following 48 hours of incubation, 10 L of 5 mg/mL MTT solution was added to each well. Tumor cells were further incubated for 4 hours at 37 °C in humidified air containing 5% CO . Then, 150 L DMSO was added to each well, and the solution was clarified by shaking for 10 minutes. The absorbance of each well was measured using a microplate reader, and IC values were analyzed using IBM-SPSS (19.0) software.

3 RESULTS AND DISCUSSION

3.1 Spectroscopic Properties of 1-3

In the IR spectrum of 1, the broad absorption band at 3465 cm ^{1} is assigned to hydroxyl groups. The carbonyl stretching vibration appears as a strong band at 1646 cm ^{1}. Bands arising from the aromatic system occur near 1607, 1487, and 1447 cm ^{1}. Strong absorption bands at 1095 and 1052 cm ^{1} are attributed to the sulfonate group, consistent with literature reports [14]. Out-of-plane wagging vibrations for the aromatic system are observed at 899, 870, 771, and 687 cm ^{1}. C-O stretching vibrations appear as strong bands at 1205 and 1144 cm ^{1}. These IR results confirm that the sulfonate group was introduced into the tectochrysin molecule. The IR spectra of 2 and 3 are similar to that of 1 because they share the same flavone skeleton.

In the ^{1}H NMR spectrum of 1, the chemical shift at 13.15 ppm corresponds to the hydroxyl proton (C5-OH), which appears at a higher chemical shift than normal due to the formation of an O-H $\cdots$ O hydrogen bond between the carbonyl oxygen (C(4)=O) and the hydroxyl hydrogen (C(5)-OH). Compared with the ^{1}H NMR spectra of tectochrysin [15] and chrysin [16], the peak corresponding to the proton on ring A of tectochrysin (C(6)-H) is absent, indicating that the sulfonate group was introduced at the C(6) position of ring A.

3.2 Description of the Molecular Structures of 2 and 3

Compounds 2 and 3 consist of a metal cation [Ni(H O)]^{2} or [Mn(H O)]^{2} , two 5-hydroxy-7-methoxyflavone-6-sulfonate anions, six coordinated water molecules, and four lattice water molecules. The molecular structures of 2 and 3 are shown in Fig. 1 [Figure 1: see original paper]. The metal cation [Ni(H O)]^{2} or [Mn(H O)]^{2} , located on an inversion center, has a slightly distorted octahedral geometry and is coordinated by six oxygen atoms from water molecules. Four coordinated water molecules occupy the equatorial plane, while the remaining two occupy axial positions. The Ni-O distances (Table 2) are nearly identical to equivalent Ni-O distances in Ni(H O) $\cdot$10H O [17]. The Mn-O distances (Table 2) are consistent with literature values [18]. Bond distances and angles of the flavone skeleton agree with those of tectochrysin [19]. In the flavone skeleton of 2 or 3, the benzopyran ring consists of rings A (C(1)-C(6)) and C (C(3)-C(4)/O(5)/C(8)-C(10)). The dihedral angle between rings A and C is 1.99° in 2 and 1.89° in 3, while that between the benzopyran ring and ring B (C(11)-C(16)) is 1.39° in 2 and 1.41° in 3, similar to values found in tectochrysin [19]. The flavone skeleton is essentially planar, with mean deviations from the mean plane of 0.021 Å for 2 and 0.020 Å for 3. The similar S-O distances involving O(9), O(10), and O(11) indicate that the negative charge is delocalized over the three oxygen atoms in 2 and 3 (Table 2).

3.3 Supramolecular Interactions in 2 and 3

3.3.1 Hydrogen Bonds Sulfonate groups, coordinated water molecules, and lattice water molecules in 2 or 3 are linked by O-H · · · O hydrogen bonds (Fig. 1 [Figure 1: see original paper]). Hydrogen atoms from coordinated water molecules form O(3)-H(3C) ··· O(10) and O(2)-H(2A) ··· O(11) hydrogen bonds with oxygen atoms of the sulfonate group. A notable structural feature of 2 and 3 is the formation of a hydrogen-bonding chain between the sulfonate group and coordinated water molecules, mediated by a lattice water molecule, containing O(13)-H(13B) · · · O(10) and O(1)-H(1C) · · · O(13) interactions. O(10) is involved in a three-centered hydrogen bond with H(13B) and H(3C), comprising O(13)-H(13B) · · · O(10) and O(3)-H(3C) · · · O(10). A hydrogen bond O(3)-H(3B) ··· O(12) exists between a coordinated water molecule and a lattice water molecule. An independent O(7)-H(7) · · · O(6) intramolecular hydrogen bond forms an intramolecular S(6) motif (Fig. 1 [Figure 1: see original paper]). In addition to these hydrogen bonds, six other types of classic O-H ··· O hydrogen bonds exist in 2 and 3. Details of the hydrogen bonds and their geometries are given in Table 3 . The extensive hydrogen-bonding network makes this region hydrophilic (Fig. 3 [Figure 3: see original paper]).

3.3.2 - Stacking Interactions The flavone skeletons of 2 or 3 are arranged in an antiparallel fashion and stacked into columns through - stacking interactions. In 2 (Fig. 2 [Figure 2: see original paper]), the Cg1-Cg2# distance is 3.552 Å with a perpendicular distance (Cg1 on ring 2#) of 3.461 Å, where Cg1 is the centroid of the benzopyran ring of the flavone skeleton at (x, y, z) and Cg2# is the centroid of the benzene ring of the flavone skeleton at (1−x, −y, −z). The Cg2-Cg1* distance is 3.622 Å with a perpendicular distance (Cg2 on ring 1*) of 3.419 Å, where Cg2 is the centroid of the benzene ring of the flavone skeleton at (x, y, z) and Cg1* is the centroid of the benzopyran ring of the flavone skeleton at (1/2−x, 1/2−y, −z). The - stacking interaction in 3 is similar to that in 2: Cg1-Cg2# is 3.529 Å with a perpendicular distance (Cg1 on ring 2#) of 3.450 Å; Cg2-Cg1* is 3.608 Å with a perpendicular distance (Cg2 on ring 1*) of 3.410 Å. These values are close to those reported for typical aromatic - stacking interactions [20], confirming the presence of - stacking interactions in 2 and 3. The - stacking interactions pack along the [110] direction, linking flavone skeletons into columns and making this region hydrophobic (Fig. 3 [Figure 3: see original paper]).

3.4 Antitumor Activity

Cisplatin was used as the positive control. The in vitro anti-proliferative activities against human leukemia K562 cells and human lung cancer A549 cells were evaluated by the standard MTT assay, with tumor cell growth inhibition expressed as IC values (Table 4). The pharmacological results showed that compounds 1-3 exhibit anti-proliferative activity against human cancer cells K562 and A549. The antitumor activities of compounds 2 and 3 are stronger

than that of 1, indicating that the introduction of sulfonic acid groups enhances the antitumor activity of tectochrysin.

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Note: Figure translations are in progress. See original paper for figures.