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RESEARCH ARTICLE

Yan Xi, Wu Fuli, Liu Yun, Li Jiamian, Zheng Zebao,
Yan Huiqing, Si Shufeng

Synthesis, crystal structure and studies on properties of trisodium 5,3′,5′- trisulfonate-2,3,4,4′-tetrahydroxy-deoxybenzoin

Abstract A new water soluble compound trisodium 5,3′, 5′-trisulfonate-2,3,4,4′-tetrahydroxy-deoxybenzoin (TTDB) was synthesized and characterized by IR, UV, ^{1}H NMR, and elemental analysis. The single crystal of TTDB was determined by X-ray single crystal diffraction. The scavenging effect of compounds on hydroxy radicals was detected by fluorescent spectrophotometry. The electrochemical behavior of compounds in nonaqueous solution DMF was carried out by means of cyclic voltammetry. The experimental result shows that the crystal [$C_{14}H_{17}Na_3O_{18}S_3$] belongs to monoclinic, space group $C2/c$ with unit cell constants a = 1.4223(4) nm, b = 2.4327(8) nm, c = 1.3596(4) nm, $\alpha = 90°$, $\beta = 113.044(5)°$, $\gamma = 90°$, $Z = 8$, V = 4.329(2) nm^3, D_c = 1.925 mg/m^3, $F(000) = 2568$, F_w = 638.43, $R_1 = 0.0950$, $wR_2 = 0.2648$. The half effective concentration EC_{50} of scavenging hydroxy radicals of compound THDB is 53.1 μmol/L, while that of scavenging hydroxy radicals of compound TTDB is 47.3μmol/L. The electrochemistry redox processes of THDB and TTDB are different from each other.

Keywords antioxidation, crystal structure, cyclic voltammetry, isoflavone, trisodium-5,3′,5′-trisulfonate-2,3,4,4′-tetrahydroxy-deoxybenzoin

Isoflavones are among the most ubiquitous phenolic compounds found in nature. They have diverse physiological and pharmacological properties such as anticardiotach disorders [1], antiosteoporosis [2], estrogenic [3], antitumor [4], antimicrobial, antiallergic, and antioxidation [5]. Due to their structural constrains, they do not have satisfactory water and lipid solubility, which result in their poor performance of their biological effects, such as insufficient utilization and difficult absorption.

Inorder to overcome the deficiencies mentioned above, a new water soluble compound trisodium-5,3′,5′-trisulfonate-2,3,4,4′-tetrahydroxy-deoxybenzoin (TTDB) was synthesized based on 2,3,4,4′-tetrahydroxy- deoxybenzoin (THDB). The compounds were characterized by IR, UV, ^{1}H NMR, and elemental analysis. Their electrochemical behavior was determined by cyclic voltammetry. The scavenging effect of the compouds on hydroxy radicals was detected by fluorescent spectrophotometry for the first time.

Hydroxy radicals are one of the most important activated oxygen resulting in the hurt of tissue cells. They contribute to aging and many severe human diseases. Therefore, how to restrain and scavenge excess free radicals in human body, so as to enhance health health, extend longevity, and improve standard of living became a promising research.

1 Experimental

1.1 Instruments and chemicals

Nicolet 170SX FR-IR spectrometer, Cintra 10e UV-visible spectrophotometer, RF-5301PC fluorescence spectroscopy, Elementar Vario EL elemental analyzer, 500 Brüker nuclear magnetic resonance spectrometer, SMART 1000 CCD X-ray diffractomer, X-6 Micro melting point detector, SZ-93 automatic double fold water distiller, CHI705a electrochemistry analyzer, 213 platinum electrode, 232 saturated calomel electrode were employed.

2,3,4,4′-tetrahydroxydeoxybenzoin (THDB) was synthesized by us, and other chemicals of AR grade were purchased, and further purified if required.

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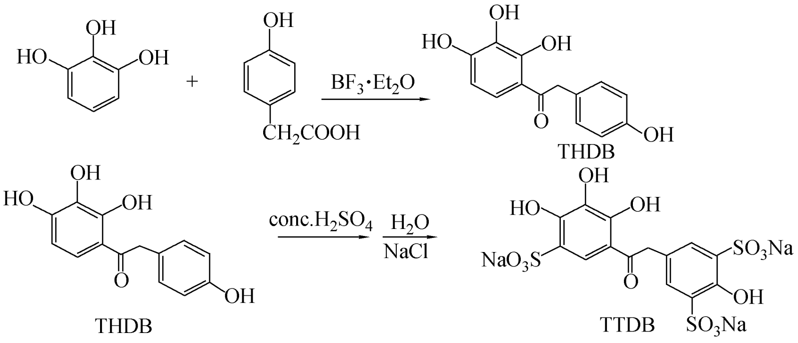
Yan Xi (✉), Wu Fuli, Liu Yun, Li Jiamian, Zheng Zebao,
Yan Huiqing, Si Shufeng
Department of Chemistry, Beijing Normal University,
Beijing 100875, China
E-mail: yanxi@bnu.edu.cn



Scheme 1 Synthetic route of TTDB

1.2 Experiment

1.2.1 *Synthesis of trisodium-5,3',5'-trisulfonate-2,3,4,4'-tetrahydroxy-deoxybenzoin* (*TTDB*)

The synthesis process is shown in Scheme 1. A stirred mixture of para-hydroxyphenyl acetic acid 7.6 g(0.05 mol), pyrogallic acid 6.3 g (0.05 mol) and newly distilled BF_3·Et_2O solution (25 mL) was heated to 80℃ under nitrogen protection, kept at this temperature under reflux for 3 h [6–8]. Most of the BF_3·Et_2O solution was reclaimed through distillation under reduced pressure, the residue poured into 500ml ice-water, the insoluble solid collected by filtration and washed with dilute sodium bicarbonate to give the brown raw product THDB. Yield: 92.4%. It was recrystallized with ethanol/water. The precursor compound THDB was obtained as a pale yellow solid. Yield: 73.5%. *m.p.* 190.9–192.2℃; UV(alkali solution), λ/nm: 320($\pi \rightarrow \pi^*$, B band), 412($\pi \rightarrow \pi^*$, R band); IR(KBr) (ν/cm): 3433.6(–OH), 1643.5(–CO–), 1610.8(Ph–); ^{1}H NMR(DMSO-d$_6$, 500 MHz, δ/ppm), δ:4.13(s, 2H, –COCH$_2$–), 6.41(d, 1H, J = 8.85 Hz, H5), 6.70(d, 2H, J = 8.05 Hz, H3'and H5'), 7.07(d, 2H, J = 8.30 Hz, H2' and H6'), 9.49(d, 1H, J = 8.90 Hz, H6), 8.65(s, 1H, –OH), 9.30(s, 1H, –OH), 10.14(s, 1H, –OH), 12.60(s, 1H, –OH); ESI(-)-MS, m/z: 261[M+1], 260[M$^+$].

THDB 2.6 g (10 mmol) was added to 10ml concentrated sulfuric acid, stirred under room temperature for 30 min, the solid dissolved gradually while the solution turned yellow. The reaction stopped only when there were no insoluble substances appear when a small amount of reactant liquid was added to water. The reactant was carefully poured to 40 ml ice-water, stirred, and solid NaCl added to the liquid. The solution was vigorously stirred until NaCl could dissolve no more. A floccular substance then appeared, and the insoluble solid was collected by filtration, washed with saturated NaCl solution, and recrystallized with 5% NaCl solution to give a white acicular crystal (TTDB) of 4.3g. Yield: 78%. *m.p.*>260℃ (decomposed); UV(H_2O, λ/nm): 224($\pi \rightarrow \pi^*$, E_2 band), 287($\pi \rightarrow \pi^*$, B band), 416($\pi \rightarrow \pi^*$, R band); IR(KBr) (ν/cm): 3441.4(–OH), 1642.5(–CO–); ^{1}H-NMR(D_2O, 500 MHz, δ/ppm), δ: 4.03(s, 2H, –COCH$_2$–), 6.82(d, 1H, J=8.80 Hz, H5), 7.10(d, 1H, J=7.45 Hz, H2'), 7.24(d, 1H, J=8.96 Hz, H6'); element analysis(%): $C_{14}H_9Na_3O_{14}S_3 \cdot 4H_2O$, observed: C 26.59, H 2.73, O 37.41; calculated: C 26.33, H 2.68, O 37.59.

1.2.2 *Crystal structure mensuration*

A saturated water solution of TTDB was prepared, volatilized under room temperature for about 42 days, appropriate crystal suit for X-ray single crystal analysis deposited in the solution, a crystal measuring 0.18 mm × 0.16 mm × 0.12 mm separated out, and analyzed on BRUCKER SMART 1000 CCD X-ray diffractomer. Mo Kα (λ=0.071073 nm) was used as the diffract headstream, measured with a graphite homochrometer, and diffraction data by means of $\phi-\omega$ scan in room temperature. The molecular structure was analyzed and optimized with the help of SHELXS-97 [9] program. The coordinates of non-hydrogen atoms were calculated by direct approach and corrected by repeating the whole at least twice and anisotropism. The TTDB crystal belongs to monoclinic space group $C2/c$ with unit cell constants a = 1.4223(4) nm, b = 2.4327(8) nm, c = 1.3596(4) nm, α = 90°, β = 113.044(5)°, γ = 90°, Z = 8, V = 4.329(2) nm^3, D_c = 1.925 mg/m^3, $F(000)$ = 2568, M_r = 627.43, Fw = 638.43, R_1 = 0.0950, wR_2 = 0.2648.

1.2.3 *Scavenging study of THDB and TTDB on hydroxy free radicals*

H_2O_2 decomposed under UV irradiation produced hydroxy free radical(·OH)[10], which had high activity to attack aryl compounds like *p*-Phthalic acid (TA) to produce fluorescent hydroxyl, i.e. *p*-phthalic acid (see reaction 1). But under the experiment conditions, the fluorescence of TA was too weak to be noticed. As a result, the relative concentration of ·OH in the system could be determined through fluorescent measurement of the products. When there was sample exsit in the system, the ·OH produced by H_2O_2 reacted with the sample, non-fluorescent substance was produced (see reaction 2). Then, the concentration of ·OH and TA reduced. The Scavenging ability of the sample on ·OH could be detected indirectly through the change in the intensity of fluorescence. Scavenging rate (%) equals to

$$\frac{F_0 - F}{F_0} \times 100\%$$

$$\text{TA} + \cdot\text{OH} \xrightarrow{k_1} \text{Products} \quad \text{(fluorescent)} \qquad (1)$$

$$\text{Sample} + \cdot\text{OH} \xrightarrow{k_2} \text{Products (non-fluorescent)} \qquad (2)$$

Reaction 1 and 2 were competition reactions, and Eq.1 can be deduced according to the competition dynamics:

$$F = F_0 \frac{k_1[TA]}{k_1[TA] + k_2[Sample]}$$

Appropriate transmogrification resulted in Eq.2:

$$\frac{F_0}{F} = 1 + \frac{k_2}{k_1} \cdot \frac{[Sample]}{[TA]}$$

F_0 and F denote to the fluorescent intensity of the system without or with the sample respectively. [*Sample*], [*TA*] denote the original concentration of the sample and TA, respectively. The minute change in [*Sample*], [*TA*] can be neglected due to short reaction time. In the plot of F_0/F versus [*Sample*]/[*TA*], a beeline could be obtained after linear conformity of the experiment data, whose slope is k_2/k_1. As k_1 is already known, the rate constant k_2 can be easily calculated.

Experimental method: 0.1 ml H_2O_2 aqueous solution (20 mmol/L), 0.1 mL TA aqueous solution (2 mmols/L), and a certain volume of sample aqueous solution (0.3 mmol/L) were added to 5mL sample pool, constrain the volume of the solution to 5mL, quickly placed it under UV light, and expose it to 254nm UV light for 20 min. The excited wavelength was set to 312 nm, detected under 427 nm, high sensitivity. Split width: EX=3 nm, EM=3 nm.

1.2.4 Study of electrochemistry properties

Isoflavone compounds perform well as an anti-oxidant. They can inhibit the oxidation of ascorbic acid in biosystems. Although the redox process is rather complicated, it exhibit much similarity to that of electrochemical systems. We studied the electrochemical properties of THDB and TTDB by means of three- electrode system.

The experiment was performed in three-electrode system under nitrogen protection. The platinum electrode was the sample to be worked on, and the saturated calomel electrode was used as reference. Another olatinum electrode was the counterpart. The sustain electrolyte tetrabutylam monium perchlorate was synthesized[11]. The platinum electrode used was washed ultrasonicly by concentrate nitric acid, acetone, and distilled water one after other. It was then rinsed and dried in the air for later use. The oxygen of the system was removed by high-purity nitrogen for 20 min before the experiments. A negative scan was used. The experiment temperature was 25℃.

2 Results and discussion

2.1 Crystal structure description

Figure 1 is the molecule structure of compound TTDB. Sulfonate groups inside the TTDB crystal form a hydrophilic hole. As can be seen in Fig. 2, the TTDB molecules piled up along *c* axis, O(1), O(3), O(10), O(11), O(12) and O(13) formed a hydrophilic hole measuring 4.9876 Å × 4.7306 Å.

The intermolecular force in TTDB among sulfonic O atom, hydroxy O atom and water molecules is the hydrogen bond. In the porous crystal structure, solvent molecules are guest molecules. They connect to the main frame by hydrogen bonds. Many of them are in flowing or disorder status, affecting the quality of diffraction point, resulting to the enlargement of R_{int}.

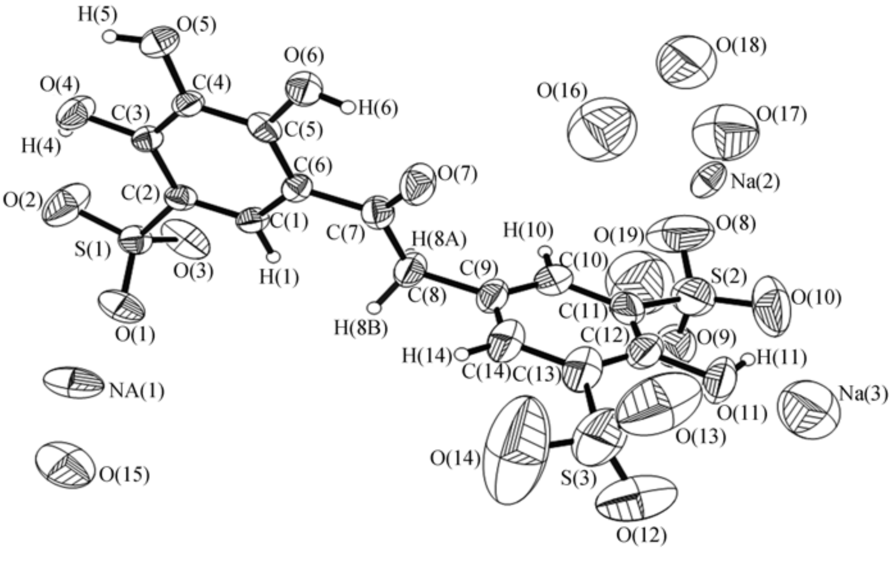


Fig. 1 The molecular structre of compound TTDB

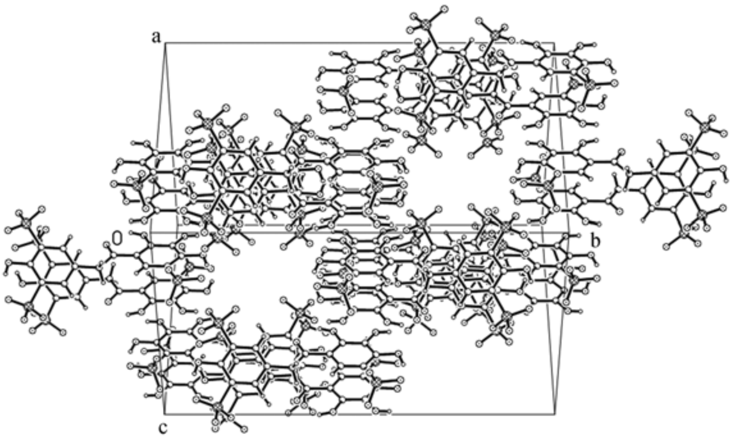


Fig. 2 The hydrophilic hole of TTDB

2.2 Scavenging study on hydroxy radicals

Figure 3 shows the comparison of the scavenging rate [13, 14] of THDB and TTDB on hydroxy radicals, with EC_{50}(THDB) = 53.1 μmol/L, and EC_{50}(TTDB) = 47.3 μmol/L. (EC_{50} is the half effective concentration of scavenging hydroxy radicals).

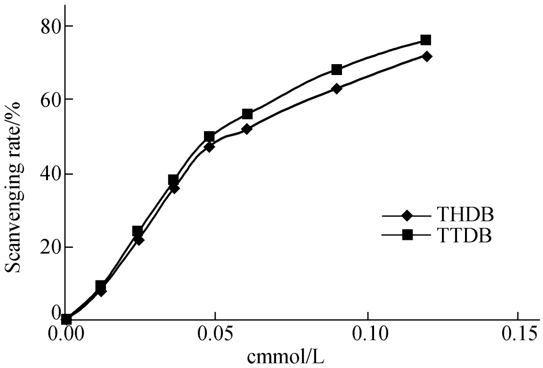


Fig. 3 Scavenging rate of varying THDB and TTDB on hydroxy radicals

Plot of F_0/F versus [*sample*]/[*TA*] (see Fig. 4) gave a straight line with the constant R = 0.99285. As k_1 was already known to have a value of 3.3×10^9 L/mol·s [15], the relative reaction rate k_2 could be calculated. The relative rate of THDB reacting with ·OH was 1.34×10^9 L/mol·s. The rate of TTDB reacting with ·OH was 1.51×10^9 L/mol·s. It can be seen from the result that the scavenging effect of TTDB on ·OH excelled that of THDB. To our knowledge no relative research has been reported so far.

2.3 Result of cyclic voltammetry

The redox process of the compounds synthesized in nonaqueous DMF solution was studied. The electric-activate group in THDB and TTDB was carbonyl group [16, 17].As can be observed in Fig. 5, there was one oxidation peak and two reduction peaks in THDB. The two reducton peaks appeared at −1.016V and −1.228V with negative scan, and the sole oxidation peak appeared at 0.219 V with positive scan. It was concluded that the reduction of carbonyl was a stepwise process. The peak at −1.016V corresponded to the first electron's reduction. The peak at 0.219V denoted the oxidation of the product and the peak at −1.228V represented the

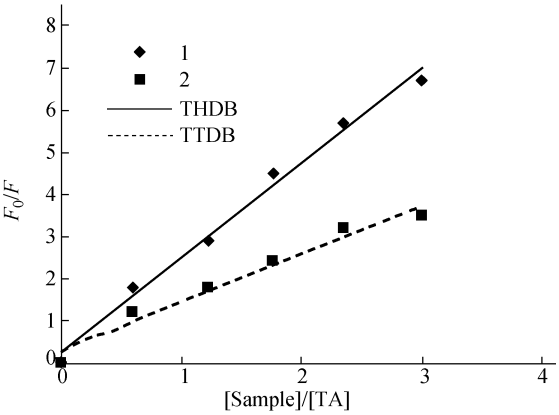


Fig. 4 Plot of F_0/F versus [*sample*]/[*TA*]

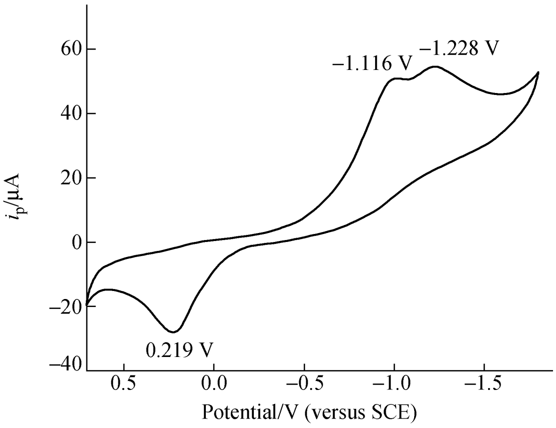


Fig. 5 Cyclic voltammograms of THDB in DMF

second electron's reduction. It was an irreversible process because there was no corresponding oxidation peak.

In Fig. 6, an oxidation peak and a reduction peak could be observed in TTDB. The intensity of the reduction peak was twice that of the oxidation peak. The reduction peak appeared at –0.999V with negative scan. The oxidize peak appeared at 0.193V with positive scan. Unlike THDB, only one reduction peak appeared in TTDB. It was deduced that two electrons were reduced at –0.999V simultaneously, only one corresponded to the oxidation reaction. Thus, the intensity of the oxidation peak at 0.193V with positive scan was nearly half that of the reduction peak.

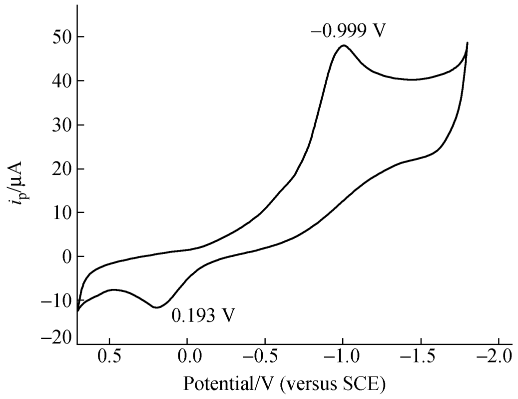


Fig. 6 Cyclic voltammograms of TTDB in DMF

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