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Preparation and Prodrug Studies of Quercetin Pentabenzensulfonate

You PENG,[a,b] Zeyuan DENG,*[,a] and Chunfeng WANG[b]

[a]*State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P. R. China, and* [b]*Department of Chemistry and Engineering, Jiujiang University, Jiujiang 332005, P. R. China*

(Received June 30, 2008; Accepted August 18, 2008)

Pentabenzensulfonate (QPBS), a potential prodrug for quercetin, was designed and synthesized in high yield. It possesses better physical properties such as solubility, lipid/water partition coefficient, LogP, and hydrolysis kinetics than its original form. The LogP value (2.04) and the half-life of the hydrolysis value (3.85 h) show that oral bioavailability is improved evidently compared with that of quercetin. These results indicate that QPBS can be considered as a potential prodrug for quercetin.

Key words——benzensulfonate; crystal structure; prodrug; hydrolysis; partition coefficient

INTRODUCTION

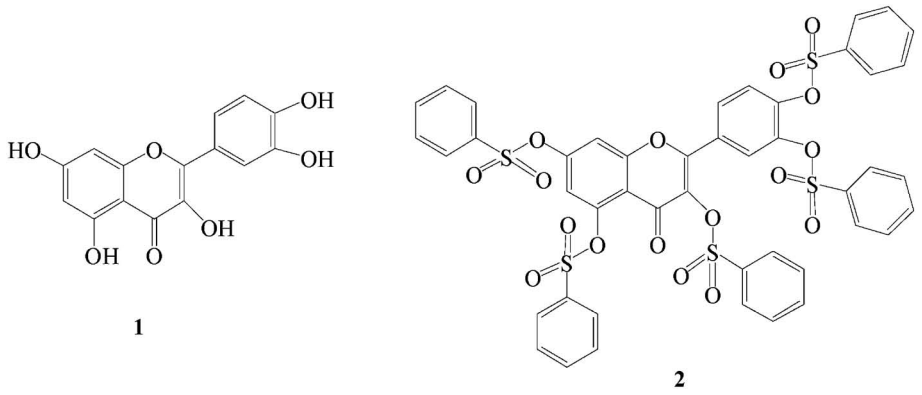
Flavonoids are a class of naturally occurring polyphenolic compounds that have been isolated from various vascular plants. They are a large constituent of the human diet and show a wide variety of biological properties in mammals, such as antioxidant, antiinflammatory, antiviral, antiproliferative, and anticarcinogenic effects.[1–3] One of the more abundant flavonoids, quercetin 1(3,3′,4′,5,7-pentahydroxyflavone), has been found to exert antiproliferative effects on human cells derived from breast, ovarian, leukemic, and colon cancers[4–7] (Scheme 1). Despite its *in vitro* biological activity, quercetin has not been employed widely in therapeutic medicine because it is practically insoluble in water or oil.[8] To further improve the bioavailability, and biological activity of quercetin, we report the first chemical synthesis, crystal structural and prodrug studies of its sulfonic acid ester 2. We are preparing flavonoid analogues with enhanced solubility and bioavailability and previously reported the synthesis and crystal structure of a sulfonate analogue of flavonoid.[9] Here we report the synthesis and crystal structure of quercetin pentabenzensulfonate (QPBS) 2 (a=12.000(2) Å, b=13.796(3) Å, c=14.289(3) Å, α=70.31(3)°, β=77.74(3)°, γ=88.08(3)°, V=2174.5(8) Å^3, Z=2, space group P-1), in which five benzensulfonate moieties are attached to the five hydroxyl groups of the flavonoid. This conjugate possesses high oil solubility according to our experiments. The results of prodrug studies of QPBS shows that it can be considered a potential prodrug for quercetin.

*e-mail: dengzeyuanpaper@126.com

EXPERIMENTAL

Melting points were determined with an XPCI Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were recorded in d_6-$CHCl_3$ on a Varian INOVA 400 MHz spectrometer using TMS as an internal standard. FT-IR spectra were recorded on a Nicocet 5700 FT-IR spectrophotometer. MS spectra were recorded on a Waters ZQ4000/2695 micromass. Chromatographic procedure were performed an Agilent 1100 series HPLC.

Preparation of QPBS A solution of quercetin 0.120 g (0.4 mmol) and K_2CO_3 in dry CH_2ClCH_2Cl (15 ml) was stirred at −20℃ for 2.5 h under an argon atmosphere. Then the benzene sulfonic acid chloride (2.4 mmol) in CH_2Cl_2 (2 ml) was instilled in this solution for 20 min and the mixture was stirred for 0.5 h. It was then poured into ice water, followed by extraction with ether/ethyl acetate (v/v, 1/2), washing with aqueous $NaHCO_3$, drying, and removal of solvent under reduced pressure to give the crude product. Purification using flash chromatography (silica, $CHCl_3$/$(CH_3)_2CO$, 10/1) yielded white solid QPBS 2 (0.4 g; yield: 98%); m. p. 261–262℃; IR (KBr, disc): ν1672 (C=O), 1620 cm^{-1} (C=C); ^{1}H-NMR ($CDCl_3$) δ: 6.94(d, 1H, H_6); 7.19 (d, 1H, H_8), 7.26 (d, ,1H, $H_{5'}$), 7.33 (m, 5H, phenylsulfonyl-4-H), 7.54 (t, 10H, phenylsulfonyl-3,5-H), 7.87–7.96 (m, 2H, $H_{2'}$, $H_{6'}$), 8.13 (d, 10H, phenylsulfonyl-2,6-



1 2

Scheme 1.

H). MS, m/z: 1003.46 ($M^+ + 1$). Anal.: calcd. for $C_{45}H_{30}O_{17}S_5$: C, 53.88; H, 3.01; S, 15.98. Found: C, 53.74; H, 3.00; S, 15.94.

X-Ray Analysis Crystals of QPBS **2** of suitable quality for single crystal X-ray diffraction were obtained by slow evaporation from a CH_2Cl_2 solution. Diffraction intensities of the compound were collected at 298 (2) K using a Bruker SMART CCD area detector with Mo-$K\alpha$ radiation ($\lambda = 0.71073$ Å). The collected data were reduced using the SAINT program,[10] and empirical absorption corrections were performed using the SADABS program.[11] The structure was solved by direct methods and refined against F^2 using the full-matrix least-squares methods with the SHELXTL program.[12] All of the nonhydrogen atoms were refined anisotropically. All hydrogen atoms were set in idealized positions and refined using the riding model. Final values of agreement factors were $R = 0.0336$ and $R_w = 0.0707$ for 1688 independent reflections with $I > 2\sigma(I)$. Crystallographic data for **2** have been deposited with the Cambridge Crystallographic Data Center (CCDC No. 675678). Copies of available materials can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 IEZ, UK (fax: (44) 01223 336033); e-mail: deposit@ccdc.ac.uk).

Solubility and Lipid/Water Partition Coefficient Determination

Chromatographic Procedure Analysis was performed on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, a vacuum degasser, an automatic injector, and a variable wavelength detector. Separation was carried on a 5-μl Venusil XBP-C18 column (250 mm × 4.6 mm, Agela Technologies, China) using acetonitrile : methanol (25 : 75, v/v) as the mobile phase at a flow rate of 1.0 ml/min. The effluent was monitored at 260 nm with detector sensitivity of 2.00 AUFS, and the maximum absorbance waves for 1 and 2 all were determined at 260 nm with a DAD detector. Calibration curves established that excellent linearity existed over the entire concentration range from 0.123 μg/ml to 6.15 μg/ml for QPBS and 0.054 μg/ml to 7.56 μg/ml for quercetin, respectively. The column temperature was 20°C. The injected volume of the analyzed samples was 10 μl.

Solubility Determination The solutions of quercetin and QPBS in water, methanol, hexane, and ethyl acetate were obtained at 37°C by equilibrating excess amounts of each of the compounds with these solvents. The solubility was determined according to the reported method.[13] Sample was added to the test tube containing 5 ml of solvent with continuous shaking for 30 min to prepare saturated solution. This mixture was allowed to equilibrate at room temperature (37 ± 0.1°C) for 24 h. The upper clear solution was diluted and the corresponding solubility was determined using HPLC.

Lipid/Water Partition Coefficient Determination Solutions of each compound in this study were prepared with aqueous saturated octanol and transferred to assay tubes containing equal volumes of octanol-saturated water. These mixtures were agitated for 2 h at 37°C after centrifugation at 3000 rpm for 10 min, and the water phase were analyzed using HPLC. Partition coefficients were calculated according to the ratio of drug concentration in the noctanol phase and the buffer phase.

Studies on Hydrolysis Kinetics The reaction solution of QPBS was prepared by dissolving 1.1 mg of QPBS in 30 ml of Clark-Lubs water buffer solution (pH 7.4) and a quantitative DMF to yield a 22 μg/ml

of concentration in a 50-ml volumetric flask. Twenty-five milliliters of the above solution was transferred to a flask and stirred at the same temperature (37℃). The concentrations of QPBS and quercetin in buffer solution at different time intervals were determined using HPLC.

RESULTS AND DISCUSSION

Solubility and Lipid/Water Partition Coefficient The solubility of the prodrug QPBS increased in all solvents examined compared with quercetin (Table 1). Quercetin cannot be detected in water, hexane, and ethyl acetate, although it dissolves readily in methanol. Table 1 summarizes the solubility and partition coefficients of quercetin and its analogue QPBS. The solubility of quercetin is 37.6 μg/ml in methanol at 37℃ and that of its derivative QPBS is up to 847.9 μg/ml under the same conditions. Table 1 shows that the lipophilicity of the derivative QPBS is enhanced markedly due to esterification. Surprisingly, the hydrophilicity of QPBS is also enhanced markedly. A possible reason is that esterification of OH in quercetin breaks the H-bond of intermolecules, resulting in lower lattice energy and consequently improves water solubility. The apparent lipid/water partition coefficient LogP (2.04) of the prodrug indicates the oral bioavailability is improved compared with that of quercetin, which is less than 1%.[14]

Hydrolysis Kinetics QPBS can be hydrolyzed to produce quercetin and benzene sulfonic acid in aqueous solution. This study found that the reaction rate of hydrolysis is dependent only on the concentration of QPBS. The hydrogen ion concentration remained essentially constant throughout the experiment. Thus QPBS hydrolysis is modeled as a pseudo first-order reaction. If the volume is constant, which it was in this experiment, and the concentration of the reacting species QPBS is represented by A, then we can write the first-order rate law as 15):

$$ln(A_0/A) = kt \quad (1)$$

The hydrolysis half-lives of 2 are determined for first-order or pseudo first-order reactions by Eq. (2):

$$T_{1/2} = ln2/k = 0.693/k \quad (2)$$

By plotting $ln\ (A_0/A)$ against reaction time (T) according to Eq. (1) at constant temperature and pH, a straight line passing through the Microsoft Office Excel 2003 was obtained (Fig. 1). The results indicate that in buffer aqueous solution QPBS hydrolysis follows pseudo first-order kinetics under constant conditions of pH and temperature. The slope of the straight line corresponds to the reaction rate constant (k). The equation of the straight line is $Y = 5\times10^{-5}$ X + 0.2189 (R = 0.9996). The slope k is $5\times10^{-5}\ s^{-1}$, and then $T_{1/2}$ = 3.85 h calculated using Eq. (2).

There are four possible intermediates in the hydrolysis process (tetra-, tri-, di-, and monobenzenesulfate of quercetin), and we determined the concentrations of quercetin in buffer solution at different time intervals. The plot conversion K *vs.* t gives a curve (Fig. 2) indicating that it is about 16 h when 90% of the rate of quercetin formation from QPBS is achieved.

X-Ray Structure The molecular structure and molecular packing of QPBS 2 are shown in Fig. 3 and Fig. 4, respectively. The C28–C33 benzene ring and the C7–C9/O16/ C10–C15 chromen ring are not coplanar, with the dihedral angle of 31.4(3)°, which

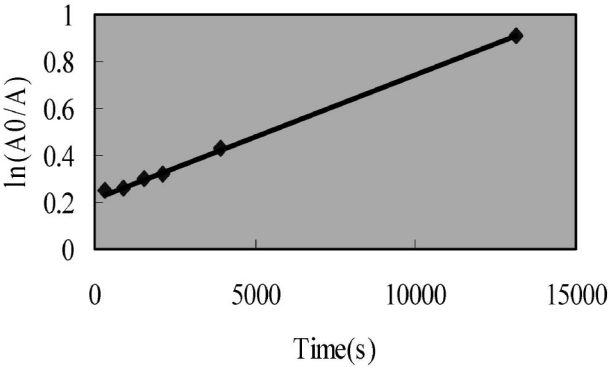


Fig. 1. Plots of ln (A_0/A) *vs* t for **2** Hydrolysis Reactions in Buffer Solution (pH = 7.4) at 37℃

Table 1. Solubility[a] and Partition Coefficients of Quercetin and Its Analogs

Compound	Solubility				Log P
	water	methanol	hexane	Ethyl ethanoate	
quercetin	—[b]	37.6±1.13	—[b]	—[b]	—[b]
QPBS	0.33±0.10	847.9±9.98	1.36±0.36	1940±30	2.04±0.65

a: unit of solubility is μg/ml. b: cannot be detected.

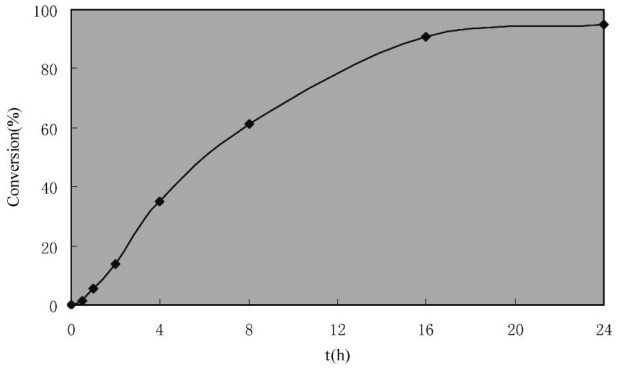


Fig. 2. Plots of Conversion (%) *vs* t of Quercetin in Buffer Solution (pH=7.4) at 37℃

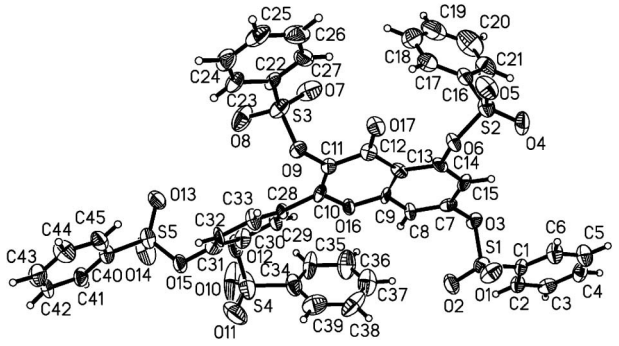


Fig. 3. Molecular Structure of Quercetin Pentabenzensulfonate **2**.

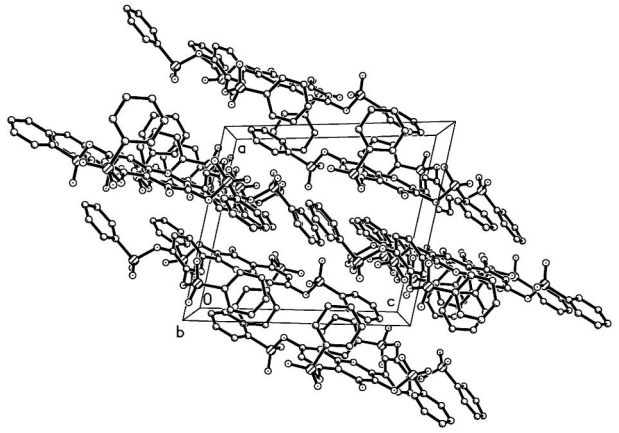


Fig. 4. Molecular Packing of **2**, Viewed along the *b* Axis
Hydrogen atoms have been omitted for clarity.

can decrease the steric effects between them. The dihedral angles between the C7–C9/O16/C10–C15 chromen ring and the adjacent benzene rings C1–C6, C16–C21, and C22–C27 are 9.4(3)°, 74.2(3)°, and 15.8(3)°, respectively. The dihedral angles between the C28–C33 benzene ring and the adjacent benzene rings C34–C39 and C40–C45 are 51.6(3)° and 13.1(3)°, respectively. As expected, each S atom locates at the center of the tetrahedral geometry. The bond angles subtended at the S1, S2, S3, S4, and S5 atoms range from 97.6(3)°–118.5(3)°, 102.4(3)°–120.2(3)°, 99.2(3)°–120.6(3)°, 101.5(4)°–122.9(5)°, and 100.3(3)°–120.4(3)°, respectively, indicating that the tetrahedral geometries deviate from the ideal tetrahedral configurations.

Acknowledgments The authors acknowledge support from the Science and Technology Item of the Education Department of Jiangxi Province (No. GJJ08440), Opening Foundation of the State Key Laboratory of Food Science and Technology in Nanchang University (No. NCU200508), and the Program for Changjiang Scholars and Innovative Research Team of the Ministry of Education (No. IRTO540).

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