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Note

Quinone Hemiacetal Formation from Protocatechuic Acid during the DPPH Radical Scavenging Reaction

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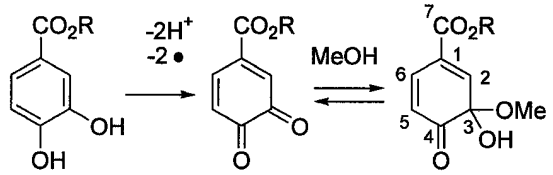
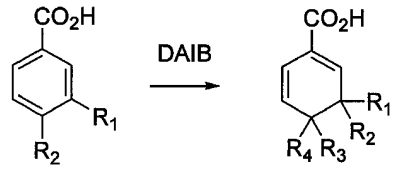
Protocatechuic acid was rapidly converted to protocatechuquinone 3-methyl hemiacetal and protocatechuquinone during the reaction with DPPH radical in methanol. The structure of the acetal was determined by comparing the NMR data with those of an authentic compound prepared by (diacetoxy)iodobenzene oxidation of protocatechuic acid.

Key words: protocatechuic acid; radical scavenging reaction; DPPH radical; quinone hemiacetal

Protocatechuic acid (**1**) is readily oxidized by two molar equivalents of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and quantitatively converted to protocatechuquinone (**2**) in acetone.[1)] However, in an alcoholic solvent such as methanol, an additional oxidation product (**3**) was detected in the solution together with **2** from **1**, although the molar consumption equivalence of DPPH radical in an alcoholic solvent was nearly the same as that in an acetone solution.[1,2)] A ^{1}H-NMR analysis of the mixture of **1** (0.3 mg) and DPPH radical (5.6 mg, 7.3 eq) after 8 min showed characteristic signals of H-5 (δ_H 6.41, d, J=10.3 Hz), H-2 (δ_H 6.92, d, J=2.0 Hz), and H-6 (δ_H 7.53, dd, J=10.3 and 2.0 Hz), which were similar to those of protocatechuquinone methyl ester (**5**) produced in the reaction of methyl protocatechuate (**4**) with DPPH radical,[1)] indicating the formation of **2** in the solution. In addition, another set of 1,2,4-trisubstituted benzene protons of H-5 (δ_H 6.09, J=10.1 Hz), H-2 (δ_H 7.21, d, J=2.0 Hz), and H-6 (δ_H 7.44, J=10.1 and 2.0 Hz) of a quinone-like product (**3**) appeared in the spectrum. *In situ* HMQC and HMBC measurements of the reaction mixture containing **1** (1.2 mg) and DPPH radical (14 mg, 4.5 eq) in 0.4 ml of methanol-d_4-acetone-d_6 (3:1) were carried out within 60 min to identify the reaction products, in which acetone-d_6 was added to enhance the solubility of DPPH radical as a co-solvent and had little influence on the reaction. The HMBC correlations for **2** were clearly consistent with protocatechuquinone, and all protons and carbons could be assigned (Table 1). On the other hand, H-6 (δ_H 7.44) of **3** showed HMBC correlation peaks with carbonyl (δ_C 198.0) and sp_2 methine (δ_C 145.1) carbons. These cross peaks correspond to those found in **2**, although the chemical shifts of the correlated carbons in **3** were shifted downfield compared to those



1: R=H **2**: R=H **3(a)**: R=H
4: R=Me **5**: R=Me **6**: R=Me



7: R_1=OMe, R_2=OH **8**: R_1=R_2=OMe, R_3,R_4=O
9: R_1=OH, R_2=OMe **10**: R_1,R_2=O, R_3=R_4=OMe

Table 1. NMR Data for Protocatechuquinone and Its Acetals[a]

	2	3	3a	8	10
H-2	6.92	7.21	7.22	7.26	6.54
H-5	6.41	6.09	6.10	6.07	6.48
H-6	7.53	7.44	7.43	7.42	6.76
C-1	140.9	128.6	128.6	130.9	—[b]
C-2	133.2	145.1	145.1	142.8	—
C-3	182.0	89.5	89.0	92.3	—
C-4	180.4	198.0	196.9	195.9	—
C-5	131.2	126.1	126.1	126.8	—
C-6	138.7	139.5	139.6	139.0	—
C-7	167.2	166.5	166.5	166.8	—

[a] δ (methanol-d_4) in ppm (^{1}H, 500 MHz; ^{13}C, 125 MHz), except δ_C for **2** and **3** (methanol-d_4-acetone-d_6 (3:1)).
[b] not measured.

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in **2** (δ_C 181.2 and 133.3, respectively). More noticeable was that H-5 (δ_H 6.10) of **3**, which resonated in a higher field than that of **2** (δ_H 6.43), showed characteristic HMBC correlation peaks with a considerably high field carbon of δ_C 89.5, while corresponding H-6 of **2** was correlated with a quinone carbonyl at δ_C 182.3, as well as with a quaternary carbon (δ_C 128.6). These differences between **2** and **3** suggest **3** to have been a 3-acetal derivative of **2**. In order to confirm the structure of **3**, protocatechuquinone 3- and 4-dimethyl acetals were prepared by (diacetoxy)iodobenzene (DAIB) oxidation of vanillic (**7**) and isovanillic (**9**) acids, respectively, in methanol-d_4.[3] The quinone acetals produced in the reaction mixture were directly analyzed by NMR, since they were not sufficiently stable to isolate from the solutions. As a result, the signals of **3** were found to be similar to those of the 3-acetal (**8**) derived from **7**, but distinctly different from those of the 4-acetal (**10**) from **9**, which indicates **3** having an acetal position at C-3 rather than C-4 as indicated in the HMBC results. However, the ^{1}H- and ^{13}C-NMR data for **3** and those for the protocatechuquinone 3-dimethyl acetal (**8**) seemed slightly but significantly different (Table 1), suggesting the possibility that **3** might have been a methyl hemiacetal at C-3 instead of the dimethyl acetal. Discrimination between the methyl hemiacetal and dimethyl acetal was problematic in the *in situ* NMR analyses, since methoxyl signals introduced from the solvent were completely deuterated and thus invisible in the ^{1}H-NMR spectrum. Protocatechuquinone 3-methyl hemiacetal was therefore prepared for a comparison by the DAIB oxidation of **1** in methanol-d_4 as already described. The resulting reaction mixture only showed the characteristic signals of 3-acetal, those of 4-acetal not being detected in the NMR spectra. The NMR signals from the 3-acetal (**3a**) derived from the hypervalent iodinane oxidation of **1** were slightly different from those of the dimethyl acetal (**8**) derived from **7** and were superimposable on those of **3**, which appeared during the reaction of **1** with DPPH radical. Thus, the structure of **3** was concluded to be protocatechuquinone 3-methyl hemiacetal. The preference for the formation of 3-hemiacetal formation over 4-hemiacetal from **2** could have been due to the lower electron density of 3-CO conjugated with the pendant carboxyl group than that of 4-CO.

In the reaction of protocatechuic acid and DPPH radical, protocatechuquinone (**2**) produced was thus rapidly equilibrated with its methyl hemiacetal (**3**) in a molar ratio of *ca.* 3:2 after 8 min and the mixture remaining unchanged after 60 min. The hemiacetal form seems to have been rather stable by the formation of an intramolecular hydrogen bond between 3-OH and 4-CO. In the case of methyl protocatechuate (**4**), the same reaction as that of the acid occurred to yield a mixture of protocatechuquinone methyl ester (**5**, H-5, δ_H 6.43; H-2, δ_H 6.93; H-6, δ_H 7.51) and its methyl hemiacetal (**6**, H-5, δ_H 6.11; H-6, δ_H 7.42), which could be identified by the similar NMR spectra to those of the reaction mixture with **1**, although H-2 of the latter was buried under strong peaks of 2,2-diphenyl-1-picrylhydrazine produced by the reduction of DPPH radical. However, the peak areas of the product derived from **4** were less than half of those from **1**. The smaller amounts of quinone and acetal produced from **4** might reflect rapid progress of a further oxidation reaction by consuming those intermediates, which resulted in the stronger radical scavenging activity of the ester than of the acid.[1,2] The reaction beyond the quinone or its equivalent acetal proceeding with DPPH radical in alcoholic solvents is still unclear, although the ester residue and an alcoholic solvent seem to be necessary for a rapid reaction. The tendency to form hydrates from quinones has been reported in the oxidation products of polyphenols.[4,5] In the case of **1**, signals for the quinone hydrate, which was tentatively identified by its spectral similarity to **3**, were detected in the reaction with DPPH radical when using acetone-d_6-deuterium oxide (4:1) as a solvent. Hence, this quinone-acetal or hydrate equilibrium could occur during polyphenol oxidation in an aqueous biological system, although the contribution of the hemiacetal produced from the quinone to the overall radical scavenging reaction remains unknown.

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