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Unexpected role of 5-OH in DPPH radical-scavenging activity of 4-thiaflavans. Revealed by theoretical calculations

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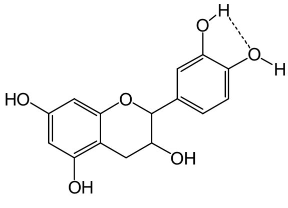
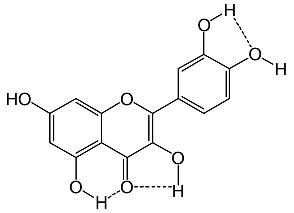
Abstract—The O–H bond dissociation enthalpies (BDEs) of 4-thiaflavans were calculated by a combined density functional theory method (RO)B3LYP/6-311 + G(2d,2p)//AM1/AM1. The calculated BDEs not only gave a reasonable explanation on the DPPH radical-scavenging activity difference of 4-thiaflavans, but also revealed the unexpected role of 5-OH in enhancing the antioxidant activity of A-ring.

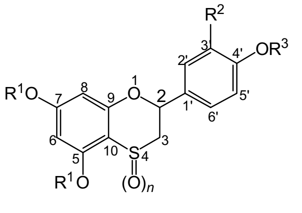
Flavonoids are the most abundant natural antioxidants (ArOH) and have received considerable attention in recent years.[1–6] Most of flavonoids contain a catecholic group in B-ring and a resorcinolic group in A-ring (Scheme 1). Since the radical derived from catechol can be well stabilized by the *ortho*-hydroxyl group and an intramolecular hydrogen bond,[7–10] the B-ring has been recognized as the active center of flavonoids to scavenge free radicals.[1–6] To improve the A-ring's activity, Capozzi et al. recently synthesized a series of 4-thiaflavans, which was inspired by the structure of α-tocopherol and took the advantage of electron-donating property of 4-sulfide sulfur.[11] The DPPH radical-scavenging experiment indicated that 4-thiaflavans (**1**, **2**, **3**, and **5**, Scheme 1) remain rather high antioxidant activity regardless of the existence of catecholic B-ring or not, suggesting that the 4-sulfide sulfur is similar to *ortho*-hydroxyl in reducing the O–H bond dissociation enthalpy (BDE), a theoretical parameter successfully used to measure the H-atom-donating ability of various antioxidants.[7–10,12–16] However, considering the fact that the O–H BDEs of catechol and *para*-SCH_3 substituted phenol have been estimated to be 7–10 and ~4 kcal/mol lower than that of phenol, respectively,[9,10,17,18] it seems *para*-SCH_3 effect cannot account for the similar activity between A-ring and B-ring of 4-thiaflavans.[19] To give an elucidation on the structure–activity relationship (SAR) of 4-thiaflavans, we attempt to calculate O–H BDEs for these antioxidants and investigate the substituent effect on the O–H BDEs thoroughly.



Catechin



Quercetin



4-thiaflavans

4-thia-flavans	R^1	R^2	R^3	*n*
1	H	OH	H	0
2	H	OH	H	1
3	H	H	H	0
4	Me	H	H	0
5	H	H	Me	0
6	H	H	H	1
7	H	H	Me	1

Scheme 1. Molecular structures of catechin, quercetin, and 4-thiaflavan.

In this paper, O–H BDEs were calculated by a combined density functional theory (DFT) method, labeled as (RO)B3LYP/6-311 + G(2d,2p)//AM1/AM1, which takes advantages of accuracy and economy.[9] The detailed

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Keywords: Thiaflavans; Flavonoids; DPPH radical; O–H bond dissociation enthalpy; DFT method; Substituent effect.

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calculation procedures are as follows. The geometry optimization and the determination of vibrational frequencies were performed using the semiempirical AM1 method.[20] Then, single-point electronic energies (SPEs) were obtained by DFT method using B3LYP functional on the 6-311 + G(2d,2p) level. Employing the molecular enthalpy in gas phase at 298 K, which consists of B3LYP/6-311 + G(2d,2p) calculated SPE, AM1 calculated zero point vibrational energy (scaled by a factor of 0.973), vibrational contribution to energy (scaled by a factor of 0.973), translational, rotational, and *PV*-work terms, O–H BDE = $H_r + H_h - H_p$, in which H_r is the enthalpy of radical generated after H-abstraction reaction, H_h is the enthalpy of hydrogen atom, −0.49765 hartree, and H_p is the enthalpy of parent molecule. Although AM1 fails to give accurate C–S bond length, the AM1-based O–H BDEs are very similar to those calculated by the pure DFT method (see below). While, although PM3 gives more accurate C–S bond length than AM1, the PM3-calculated O–H bond lengths in parent molecules and C–O· bond lengths in oxygen-centered radicals are much less accurate than those calculated by AM1. Hence, the PM3-based O–H BDEs are not applicable (data not shown). All of the calculations were performed using GAUSSIAN 98 program package.[21]

According to the DPPH radical-reducing activity (RA, Table 1), the seven 4-thiaflavans can be clearly separated into two groups, that is, **1**, **2**, **3**, and **5** in the first group with the RA higher than 80 and **4**, **6**, and **7** in the second group with the RA lower than 30. Interestingly, a similar partition can be achieved according to the O–H BDEs, namely, **1**, **2**, **3**, and **5**, which hold at least one O–H BDE lower than 80 kcal/mol is in the first part and **4**, **6**, and **7**, which contain O–H BDEs higher than 85 kcal/mol is in the second part. Considering the fact the N–H BDE of DPPH–H is just 80 kcal/mol,[22] it is reasonable to accept that the H-atom-donating reaction is preferred in the DPPH radical-reducing process and BDE is indeed a good theoretical parameter to characterize the DPPH radical-reducing activity of 4-thiaflavans.[23] In the viewpoint of physical organic chemistry, the differences in O–H BDEs of 4-thiaflavans can be elucidated by means of substituent effects, including electronic effect and intramolecular hydrogen bond effect.

Table 1. Single-point electronic energies (SPEs, in hartree), thermal corrections to energies (TCEs, in hartree, $T = 298.15$ K), O–H BDEs (in kcal/mol), and DPPH radical-reducing activity (RA) for 4-thiaflavans

	SPE[a]	TCE[b]	O–H BDE[c]	RA[d]
1	−1315.320303	0.264219		83
1-radical (4′)[e]	−1314.685067	0.250854	78.18	
1-radical (5)[e]	−1314.682411	0.250043	79.35	
1-radical (7)[e]	−1314.675706	0.249714	83.35	
2	−1390.534305	0.268441		83
2-radical (4′)[e]	−1389.898640	0.255167	78.50	
2-radical (5)[e]	−1389.873292	0.253068	93.13	
2-radical (7)[e]	−1389.877143	0.252114	90.13	
3	−1240.070282	0.258589		85
3-radical (4′)[e]	−1239.407136	0.242119	87.27	
3-radical (5)[e]	−1239.432531	0.244967	79.60	
3-radical (7)[e]	−1239.425789	0.244626	83.62	
4	−1318.688129	0.318976		—
4-radical (4′)[e]	−1318.038776	0.304959	86.64	
5	−1279.379081	0.288762		83
5-radical (5)[e]	−1278.741414	0.275139	79.54	
5-radical (7)[e]	−1278.734785	0.274801	83.50	
6	−1315.284327	0.262818		23
6-radical (4′)[e]	−1314.633335	0.248874	87.71	
6-radical (5)[e]	−1314.623421	0.248993	94.00	
6-radical (7)[e]	−1314.627282	0.248481	91.27	
7	−1354.593256	0.292983		6
7-radical (5)[e]	−1353.932384	0.279160	93.98	
7-radical (7)[e]	−1353.936352	0.278648	91.18	

[a] Calculated by (RO)B3LYP/6-311 + G(2d,2p).

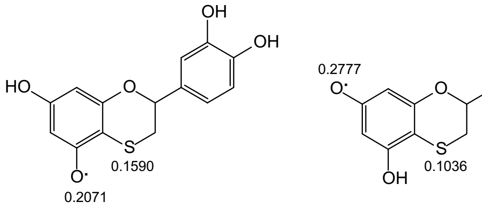
[b] Calculated by AM1.

[c] BDE = $[(SPE_r + TCE_r \times 0.973 + rt) - (SPE_p + TCE_p \times 0.973 + rt) + H_h] \times 627.5095$, in which H_h is the enthalpy of hydrogen atom, −0.49765 hartree, and subscripts p and r indicate the parent molecule and the corresponding radical generated after H-abstraction reaction, respectively.

[d] Data from Ref. 11.

[e] Positions for H-atom abstraction are in parentheses.

As indicated in Table 1, the BDEs for catecholic B-ring in **1** and **2** are very similar to each other, implying that the sulfide or sulfoxide sulfur has little effect on B-ring's O–H BDE. While, the BDEs for A-ring in **1** and **2** are much different. The former are considerably lower than the latter, which not only results from the contrary electronic effect of sulfide and sulfoxide sulfur[24] but also from the intramolecular hydrogen bond formed between 5-OH and sulfoxide oxygen. The more interesting finding is that the O–H BDE of 5-OH is unexpectedly 4 kcal/mol lower than that of 7-OH in **1**, which is contrary to the traditional idea that the electronic effect of *para*-substituents is stronger than that of *ortho*-groups.[25–27] Moreover, similar trends can be found in **3** and **5**, where 5-OH is more active than 7-OH. And the comparable BDEs between 5-OH and catecholic B-ring do account well for the similar DPPH radical-scavenging activity between **1**, **2**, **3**, and **5**. In fact, for the latter two 4-thiaflavans, there is no catecholic group at all. Consequently, it is reasonable to reach a conclusion that 5-OH rather than 7-OH is the active center for 4-thiaflavans with the absence of catecholic B-ring, which deviates from the original intention in the design of 4-thiaflavans that 7-OH should take more advantage of *para*-sulfide group than 5-OH and should be the active center.[11] The reason underlying the unexpected substituent effect is that the empty d orbitals in sulfur atom participate in the formation of p–d conjugation between the oxygen and the *ortho*-sulfur atom, which efficiently stabilizes the oxygen radical in 5-position. Whereas, there is no p–d conjugation between the oxygen and the *para*-sulfur atom at all. A straightforward elucidation on the interesting substituent effect can be achieved by employing the spin densities at sulfur and oxygen atoms in 4-thiaflavan radicals (Scheme 2). The (RO)B3LYP/6-31G(d)-calculated spin densities on O and S of 5-OH-derived radical of **1** are 0.2071 and 0.1590, respectively. While, the corresponding values for 7-OH-derived radical are 0.2777 and 0.1036, respectively. Apparently, *ortho*-sulfur atom is stronger than the *para*-counterpart to relieve



Scheme 2. Spin density distribution in **1**-derived radicals, calculated by pure DFT method (RO)B3LYP/6-31G(d).

the spin density on oxygen-centered radical. Although much effort has been devoted to determining O–H BDEs of a great number of phenolics and to investigating the substituent effect on O–H BDEs,[22,28,29] it seems the great potential of *ortho*-sulfur atom in reducing O–H BDE is missed in previous studies. We hope our theoretical calculation will arouse the interest of experimentalist to verify the prediction.

In brief, by means of DFT calculation, we obtained O–H BDEs of 4-thiaflavans. The BDEs not only gave a reasonable explanation on the DPPH radical-scavenging activity difference of 4-thiaflavans, but also offered deeper insight into the active site of the antioxidants, that is, 5-OH is more potent than 7-OH to scavenge DPPH radical, which is of great significance in rational design of better antioxidants.

Acknowledgements

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