Selection of Theoretical Parameter Characterizing Scavenging Activity of Antioxidants on Free Radicals

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ABSTRACT: Semiempirical quantum chemistry method Austin Model 1 (AM1) was employed to calculate the theoretical parameters of 15 antioxidants. Such parameters as bond order, bond length, and the difference of heat of formation (ΔHOF) between the antioxidant and its phenolic free radicals were obtained. Through correlating with previous experimental results ΔHOF was proven to be a proper parameter (r = 0.9491) to characterize the free radical-scavenging activity of antioxidants, but bond order and bond length were not able to represent the antioxidant's ability to scavenge free radicals. By comparing the results calculated by different semiempirical methods, including AM1, Modified Neglect of Diatomic Overlap, and Parametric Method 3, AM1 was found to be the best for calculating ΔHOF.

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KEY WORDS: AM1, antioxidant, bond length, bond order, free radical, ΔHOF, MNDO, PM3, quantum chemistry.

Antioxidants have attracted more and more attention owing to their potency in the pharmaceutical industry and their applications in food preservation and chemical engineering (1,2).

Up to now, many antioxidants have been found, including natural products such as flavonoids and vitamin E, or synthesized chemicals such as 2,6-di-*tert*-butyl-4-methylphenol (BHT).

Recent studies on antioxidant mechanisms indicated that the chain reaction was controlled mainly through free radical-scavenging by phenolic hydroxyls of antioxidants (3,4). So if a proper theoretical parameter to characterize the ability of antioxidants to scavenge free radicals can be found, it will be possible to predict their antioxidant activity, which will undoubtedly improve the selection of new antioxidants.

A simple analysis shows that the strength of the O–H bond in phenolic hydroxyl represents its ability to scavenge free radicals (5). The weaker the O–H bond, the more active the antioxidant. Therefore, indexes characterizing O–H bond strength may be used as prediction parameters. There are many indexes to measure the bond strength, such as bond order, bond length (6), and the difference of heat of formation (HOF) between an antioxidant and its phenolic free radicals (ΔHOF) (7). Van Acker *et al.* (5) considered ΔHOF to be probably the best parameter to predict antioxidant activity. But further verification is required, especially for molecules having many differences in structure. As it was difficult to remove other antioxidant mechanisms, such as chelating metals to reduce Fenton reaction (8,9), there were no appropriate rate constants for free radical scavenging available until Roginsky *et al.* (10) determined the relative rate constants of 15 antioxidants with structures: **I**, quercetin; **II**, dihydroquercetin; **III**, luteolin; **IV**, catechin; **V**, fisetin; **VI**, naringenin; **VII**, kaempferol; **VIII**, rutin (rut = rutoside); **IX**, cyanidine chloride; **X**, caffeic acid; **XI**, nordihydroguaiaretic acid; **XII**, chlorogenic acid; **XIII**, propyl gallate; **XIV**, vitamin E; **XV**, BHT (Scheme 1). It is now possible to compare theoretical indexes with experimental results to determine which parameter is best. Moreover, Roginsky *et al.* (10) drew two conclusions from their experiments. First was that phenolic hydroxyls of ring B of flavonoids were their primary active sites. Second was that most flavonoids were less active than regular phenolic antioxidants, such as vitamin E and BHT. These results not only are helpful for selecting proper theoretical parameters but also are useful for reevaluating flavonoids. Hence, using existing experimental data, semiempirical quantum chemistry method AM1 was employed to calculate different indexes of antioxidants (Scheme 1) for the selection of proper parameters to predict antioxidant activity. Moreover, by comparing the results calculated by different semiempirical methods, including AM1, Modified Neglect of Diatomic Overlap (MNDO), and Parametric Method 3 (PM3), the best method was also determined.

METHODS

As the rutoside in rutin (**VIII**) and side chain $C_{16}H_{33}$ in vitamin E (**XIV**) had little effect on antioxidant activity, CH_3 was substituted for them in the calculations. The calculation methods were as follows. First, molecular mechanic method MMX (11) in program PCMODEL was used to optimize the molecular structures, and then AM1 (12), MNDO (13), and PM3 (14) in program MOPAC7 were employed to calculate indexes measuring bond strength. Heats of formation of mother molecules (HOFm) and the states of removing phenolic H

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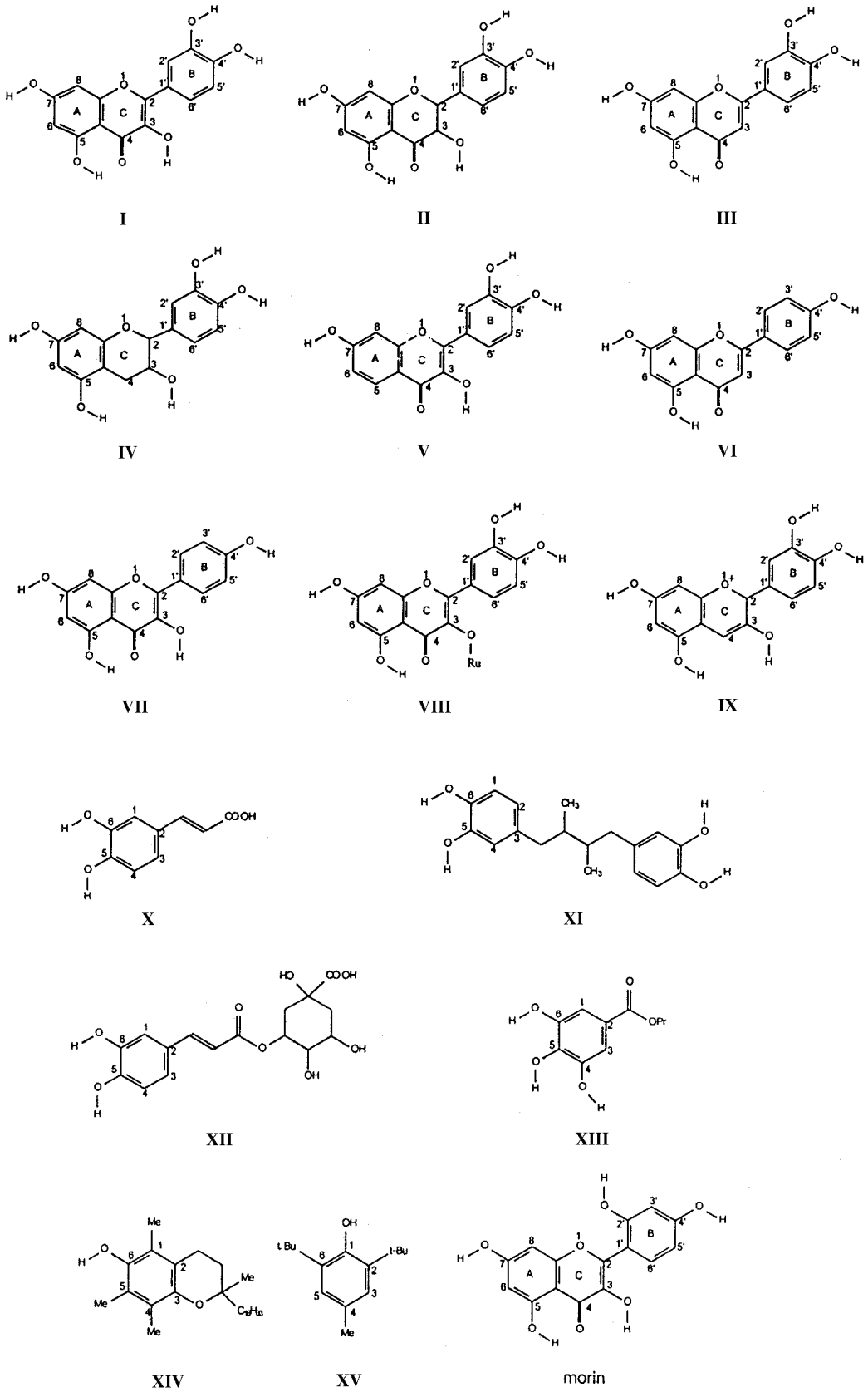


SCHEME 1

(HOFr) were obtained to calculate ΔHOF: ΔHOF = HOFr – HOFm. For comparison, mother molecules and free radicals were all calculated using RHF (restricted Hartree Fock). ΔHOF values obtained by the RHF method were almost equal to those obtained from the UHF (unrestricted Hartree Fock) after recalculation by one SCF Restricted Open Shell (ROHF) (5). Overall the RHF method for calculating ΔHOF values was most self-consistent.

RESULTS AND DISCUSSION

Results calculated by the AM1 method and relative rate constants of 15 antioxidants determined by Roginsky *et al.* (10) are listed in Table 1. Phenoxyl free radicals of antioxidants containing catechol moiety (**I**, **II**, **III**, **IV**, **V**, **VIII**, **IX**, **X**, **XI**, **XII**, **XIII**) may be in two conformations, forming intramolecular hydrogen bonds or not (Scheme 2). Obviously the former is more stable in energy, so their ΔHOF values were calculated using this conformation. In order to compare different methods, such as AM1, MNDO, and PM3, we also calculated ΔHOF of the antioxidants by MNDO and PM3; the results are listed in Table 2.

Bond order and bond length. Theoretically, the bond order and bond length of O–H can measure its strength to a certain extent. For smaller bond orders, the bond is weaker, the hydrogen can be removed more easily, and the phenolic hydroxyl is more active. Bond length also measures bond strength. Larger bond length corresponds to weaker bond, and therefore to smaller bond order. Consequently the values of bond order and bond length of the antioxidants are opposite (Table 1). Accordingly, phenolic hydroxyls in ring A may be active sites of flavonoids, because 5-OH has the smallest bond order and largest bond length. Obviously this is not consistent with the experimental results that ring B is the active moiety (10). Furthermore, recent studies (15) on quercetin and morin indicated that although they were only different in phenolic hydroxyl substitution site in ring B (Scheme 1), quercetin was much more active than morin in scavenging free radicals. This provided further evidence to prove hydroxyls in ring B are the primary active sites. Bond orders and bond lengths of flavonoids cannot explain this phenomenon. Comparing bond orders and bond lengths of different molecules with their relative rate constants of scavenging free radicals, we cannot find any relationships between them. For instance, although compound **IX** is the most active one and compound **VI** is the least active one, bond order of the former (0.9055) is larger than that of the latter (0.8942). In brief, bond order and bond length cannot be used to compare antioxidant activity regardless of the lo-

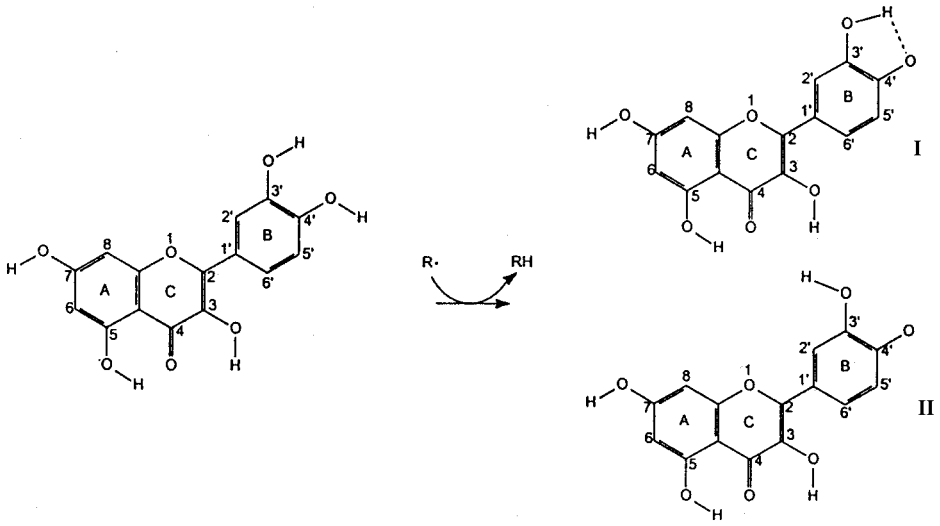
TABLE 1
Antioxidant Theoretical Parameters Calculated by Austin Model 1 and Relative Rate Constants of Scavenging Free Radicals

		Bond order	Bond length (0.1 nm)	ΔHOF (kJ/mol)	k_3/k_1 [a]
I	3′-OH	0.9234	0.9713	133.28	110
	4′-OH	0.9253	0.9696	133.03	
	3-OH	0.9148	0.9761	129.68	
	5-OH	0.8970	0.9743	195.24	
	7-OH	0.9251	0.9723	195.69	
II	3′-OH	0.9231	0.9701	127.07	23
	4′-OH	0.9254	0.9679	131.99	
	3-OH	0.9257	0.9695	277.46	
	5-OH	0.8989	0.9718	178.50	
	7-OH	0.9265	0.9698	184.79	
III	3′-OH	0.9224	0.9704	137.50	31
	4′-OH	0.9248	0.9684	136.88	
	5-OH	0.8898	0.9747	195.51	
	7-OH	0.9261	0.9701	185.40	
IV	3′-OH	0.9237	0.9695	130.09	52
	4′-OH	0.9263	0.9677	134.92	
	3-OH	0.9312	0.9678	248.42	
	5-OH	0.9233	0.9680	155.06	
	7-OH	0.9293	0.9691	160.34	
V	3′-OH	0.9237	0.9701	137.48	105
	4′-OH	0.9252	0.9680	135.03	
	3-OH	0.9166	0.9738	132.47	
	7-OH	0.9275	0.9694	176.13	
VI	4′-OH	0.9303	0.9664	155.90	0.5
	5-OH	0.8942	0.9731	190.28	
	7-OH	0.9267	0.9691	185.11	
VII	4′-OH	0.9299	0.9679	160.78	50
	3-OH	0.9167	0.9729	136.74	
	5-OH	0.8915	0.9746	192.71	
	7-OH	0.9264	0.9692	190.01	
VIII	3′-OH	0.9240	0.9700	137.83	31
	4′-OH	0.9254	0.9681	139.81	
	5-OH	0.8958	0.9731	195.08	
	7-OH	0.9253	0.9705	193.97	
IX	3′-OH	0.9194	0.9724	153.34	310
	4′-OH	0.9228	0.9697	147.75	
	3-OH	0.9055	0.9786	59.54	
	5-OH	0.9174	0.9713	131.17	
	7-OH	0.9094	0.9759	158.12	
X	5-OH	0.9213	0.9708	138.01	23
	6-OH	0.9250	0.9679	140.55	
XI	5-OH	0.9269	0.9675	132.87	250
	6-OH	0.9242	0.9699	132.41	
XII	5-OH	0.9220	0.9703	134.22	150
	6-OH	0.9255	0.9681	136.77	
XIII	4-OH	0.9242	0.9684	145.73	160
	5-OH	0.9168	0.9705	131.19	
	6-OH	0.9215	0.9703	146.08	
XIV	6-OH	0.9291	0.9670	127.74	290
XV	1-OH	0.9174	0.9640	129.18	240

[a] k_3 is the rate constant of antioxidants scavenging free radicals, and k_1 is the rate constant of free radicals reacting with methyl linoleate and oxygen. See Reference 10 for the detailed description.

TABLE 2
ΔHOF Values (kJ/mol) of Antioxidants Calculated by Modified Neglect of Diatomic Overlap (MNDO) and Parametric Method 3 (PM3)

	I	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**X**	**XI**	**XII**	**XIII**	**XIV**	**XV**
MNDO	123.20	135.69	133.74	115.89	155.87	114.32	135.26	137.86	130.71	129.04	134.12	125.04	100.17
PM3	112.33	124.73	113.73	112.74	147.28	116.05	125.46	123.31	117.75	127.29	114.38	123.59	136.41



SCHEME 2

cation of the hydroxyl group on the molecule. So these two parameters are not good for characterizing antioxidant activity.

ΔHOF value. ΔHOF value also measures bond strength. The smaller the ΔHOF, the more stable the phenoxyl radical and the weaker the O–H bond in the phenol, so the more active is the antioxidant (5). Table 1 shows that ΔHOF values of phenolic hydroxyls in ring B are much lower than in ring A, and while ring C is a chromone (**I**, **III**, and **V**, etc.) the ΔHOF value of the phenolic hydroxyl in ring C is similar to that of ring B. These data are in good agreement with the previous conclusion (10) that ring B is the active site and the 3-OH group has a more favorable effect than the 5-OH group.

Furthermore, there is good correlation between relative rate constants of scavenging free radicals and the lowest ΔHOF value of each molecule. For instance, the ΔHOF value of compound **IX** is 59.54 kJ/mol, much lower than that of compound **VI**, 155.90 kJ/mol. ΔHOF values of most flavonoids are higher than those of regular phenolic antioxidants, such as vitamin E and BHT, which is also consistent with the conclusion (10). Moreover, for most of the antioxidants evaluated, except compounds **II** and **IX**, the correlation between log k_3/k_1 and ΔHOF showed good linearity (Fig. 1, $r = 0.9491$). Considering that the structures of the 15 compounds evaluated possess many differences and only one parameter ΔHOF is used, the correlation between experimental antioxidant activity and theory is fairly good. Moreover, it can be seen from Figures 2 and 3 that ΔHOF values calculated by MNDO and PM3 will not be better than those calculated by AM1 in correlating with relative rate constants of scavenging free radicals.

But why is ring B of flavonoids the active site and ring A is not? Preliminary investigations show that three factors result in this phenomenon. First, catecholic phenoxyl free radicals of ring B can form intramolecular hydrogen bonds (Scheme 2, **I**), which will increase the stability of the radical. Second, catecholic phenoxyl free radicals of ring B can transform to an *ortho* benzoquinonoid free radical through resonance, which will make the unpaired electron well-distributed on atoms and reduce the internal energy. Noting that ring A has no catecholic phenols, it is easy to understand why ring B is more active than ring A. Third, electron-attracting property of the chromone of flavonoids on ring A makes ring A less active. Detailed discussion on the phenomenon and investigation on why flavonoids are less active than other regular phenolic antioxidants will be published elsewhere.

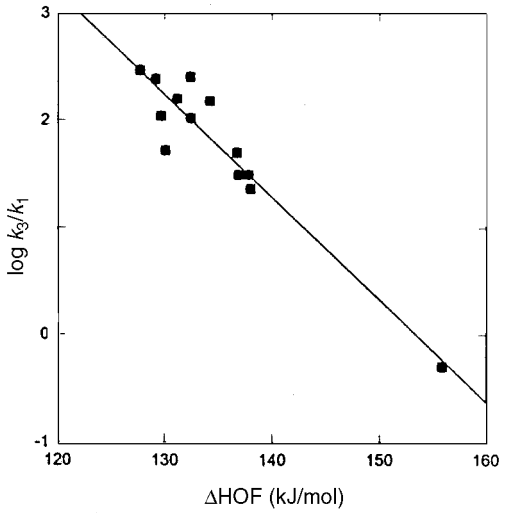


FIG. 1. Correlation between log k_3/k_1 and ΔHOF of phenolic free radicals calculated by Austin Model 1; k_3 is the rate constant of antioxidants scavenging free radicals, and k_1 is the rate constant of free radicals reacting with methyl linoleate and oxygen. See Reference 10 for the detailed description. The linear equation between log k_3/k_1 and ΔHOF is: log $k_3/k_1 = 14.6491 - 0.0955$ ΔHOF, $r = 0.9491$.

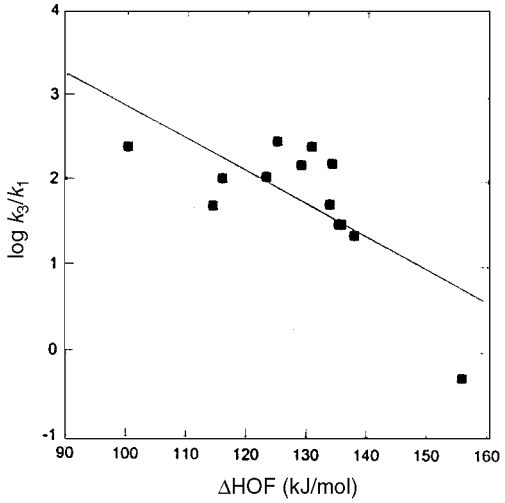


FIG. 2. Correlation between log k_3/k_1 and ΔHOF calculated by Modified Neglect of Diatomic Overlap; k_3 is the rate constant of antioxidants scavenging free radicals, and k_1 is the rate constant of free radicals reacting with methyl linoleate and oxygen. See Reference 10 for the detailed description. The linear equation between log k_3/k_1 and ΔHOF is: log k_3/k_1 = 6.6797 − 0.0381 ΔHOF, r = 0.7098.

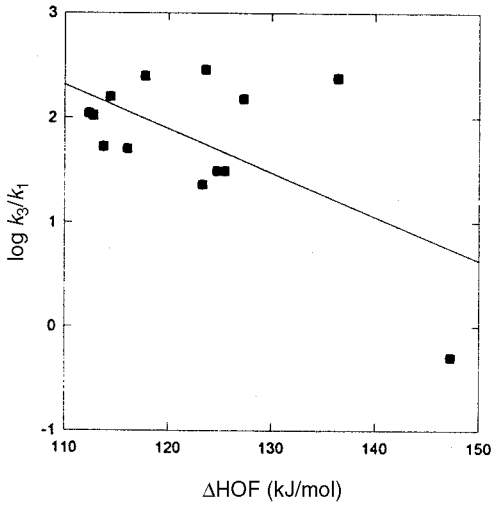


FIG. 3. Correlation between log k_3/k_1 and ΔHOF calculated by Parametric Method 3; k_3 is the rate constant of antioxidants scavenging free radicals, and k_1 is the rate constant of free radicals reacting with methyl linoleate and oxygen. See Reference 10 for the detailed description. The linear equation between log k_3/k_1 and ΔHOF is: log k_3/k_1 = 6.9461 − 0.0421 ΔHOF, r = 0.5911.

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