Why Quantum-Thermochemical Calculations Must Be Used with Caution to Indicate 'a Promising Lead Antioxidant'

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Introduction. – A recent paper by *Wang et al.*, entitled '*DFT Calculations Indicate that 1,4-Dihydropyridine Is a Promising Lead Antioxidant*' [1] contains a number of serious errors and misinterpretations of the literature. In this paper, we will highlight and correct some of the mistakes in the hope of preventing development work on 'antioxidants' of known or potential toxicity.

Antioxidants. – A chain-breaking and peroxyl-radical-trapping antioxidant (AH) retards the peroxidation of lipids (RH), as outlined by *Eqns. 1–4* [2].

Chain Propagation

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \quad (1)$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \quad (2)$$

Chain Termination

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet} \quad (3)$$

$$ROO^{\bullet} + A^{\bullet} \longrightarrow \text{non-radical products} \quad (4)$$

Effective and biochemically safe antioxidants must exhibit the following properties:

a) No direct reaction with molecular oxygen (*Eqn. 5*).

$$AH + O_2 \longrightarrow A^{\bullet} + HO_2^{\bullet} \quad (5)$$

b) The ionization potential, *IP*, of AH should be high enough to prevent a proton-coupled-electron-transfer with molecular oxygen (*Eqn. 6*).

$$AH + O_2 \longrightarrow [AH^{\cdot+}O_2^{-}] \longrightarrow A^{\bullet} + HO_2^{\bullet} \quad (6)$$

c) The rate constant for H-atom transfer (*Eqn. 3*) must be much higher than the rate constant for *Eqn. 1*, *i.e.* $k_3 >> k_1$.

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d) The radical A$^{\bullet}$ should not, in general, react with O_2 because this will compete with *Eqn. 4* and continue the oxidation chain (*Eqns. 7,a* and *b)*.

$$A^{\bullet} + O_2 \longrightarrow AOO^{\bullet} \xrightarrow{RH} AOOH + R^{\bullet} \quad (7a)$$

$$A^{\bullet} + O_2 \longrightarrow A_{-H} + HOO^{\bullet} \xrightarrow{RH} HOOH + R^{\bullet} \quad (7b)$$

e) The radical A$^{\bullet}$ should not react at an appreciable rate with RH, because this will continue the oxidation chain (*Eqn. 8*).

$$A^{\bullet} + RH \longrightarrow AH + R^{\bullet} \quad (8)$$

f) Both AH and the final products obtained from AH should be non-toxic.

If any of the above conditions are not fulfilled, AH should not be considered an antioxidant, let alone a 'promising' one.

Without dealing with the mechanism of the oxidation of 1,4-dihydropyridine (DHP), it is obvious that the main product will be pyridine. This is born out of the fact that the analogous compound, 1,4-cyclohexadiene (CHD), in the presence of molecular oxygen is quantitatively transformed into benzene in a chain process carried by the hydroperoxyl radical (*Eqns. 9* and *10*) [3].

$$HO_2^{\bullet} + C_6H_8 \longrightarrow H_2O_2 + C_6H_7^{\bullet} \quad (9)$$

$$C_6H_7^{\bullet} + O_2 \longrightarrow C_6H_7OO^{\bullet} \longrightarrow C_6H_6 + HO_2^{\bullet} \quad (10)$$

The toxicity of pyridine is documented as follows: it may cause depression of the central nervous system, irritation of skin and respiratory tract, and large doses may produce gastrointestinal disturbances, kidney and liver damage[1]). The limit for human exposure to air contaminants for pyridine is 5 ppm, to be compared with CCl_4 (10 ppm) or benzene (10 ppm)[1]). Formation of pyridine makes DHP the precursor of a poison and, therefore, DHP should not be considered 'a promising lead antioxdant' without the proviso that only non-toxic DHP derivatives should be explored.

1,4-Dihydropyridine Oxidation. – The purported reactions of DHP in the presence of peroxyl radicals and oxygen is presented in Scheme 1 of [1]. Therein, it is suggested that the peroxyl radical probably does not abstract the N–H H-atom from DHP (*Eqn. 11*) but rather the C_4–H H-atom (*Eqn. 12*), because the computed C_4–H *BDE* was 8.7 kcal/mol lower than the computed N–H *BDE* (see Table 1 of [1]). This suggestion is not in accordance with insights present in the literature.

$$ROO^{\bullet} + C_5H_6NH \longrightarrow ROOH + C_5H_6N^{\bullet} \quad (11)$$

$$ROO^{\bullet} + C_5H_6NH \longrightarrow ROOH + {}^{\bullet}C_5H_5NH \quad (12)$$

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[1]) *National Library of Medicine*, *National Institutes of Health* at http:/www.toxnet.nlm.nih.gov. Before the toxicity of pyridine was fully recognized, its application at around 1940 was suggested as an impregnator of anti-poison gas respirators [4].

For example, at temperatures just above ambient, the rate constants for H abstraction, per active H-atom, by an alkylperoxyl radical are 2×10^4 M^{-1}s^{-1} for diphenylamine (N–H), but only 5.9 M^{-1}s^{-1} for CHD (C–H) [2]. The difference between the N–H [5a] and C–H [5b] *BDE*s for these molecules can be estimated as 10 kcal/mol, with the C–H in CHD being the weaker (76.9 kcal/mol) [5b].

Nevertheless, the N–H abstraction is three orders of magnitude faster than the C–H abstraction. This difference in the dynamic behavior of peroxyls is due to dissimilar intrinsic activation barriers for H-abstraction from NH and CH moieties. *Consequently, computed reaction enthalpies alone must never be used to predict the (relative) kinetics of H-atom abstraction from different heavy atoms.* Therefore, although the C_4–H *BDE* in DHP may be lower than the N–H *BDE*, the rate constant for *Eqn. 11* is most likely to be higher than that for *Eqn. 12*.

Scheme 1 of [1] shows extraordinary intramolecular 1,3-H-atom migrations, which one can only hope are simple misprints. Specifically, the addition of O_2 and the suggested addition of an ROO$^\bullet$ radical to C_2/C_6 of the $^\bullet C_5H_5NH$ radicals is shown with these C-atoms sp^2-hybridized, their original H-atoms having inexplicably migrated to C_4. By analogy with the oxidation of CHD (*Eqn. 10*), oxygen will add to C_2 or C_6 in $^\bullet C_5H_5NH$, and subsequently $HO_2^\bullet$ will be eliminated to yield pyridine (*Eqn. 13*) [3]. Alternatively, when the $C_5H_6N^\bullet$ species is formed, O_2 adds to C_3 or C_5 leading to the same products.

$$^\bullet C_5H_5NH + O_2 \longrightarrow {}^\bullet OOC_5H_5NH \longrightarrow C_5H_5N + HO_2^\bullet \qquad (13)$$

It is claimed that the overall reaction enthalpy for *Eqn. 13*, ΔH_{13}, is 6.67 kcal/mol, which is based on the calculated differences in the N–H *BDE* in $^\bullet C_5H_5NH$ and the O–H *BDE* in $^\bullet OOH$. Thus, *Eqn. 13* is said to be an endothermic reaction. However, the value given for ΔH_{13} cannot possibly be correct. With a N–H *BDE* of 30.12 (as reported in [1]) and using the recommended O–H *BDE* in $HO_2^\bullet$ of 51.6 kcal/mol [6] (and not 23.45 as given in footnotes 3 and 4 of [1]), affords a ΔH_{13} of -21.5 kcal/mol. Hence, *Eqn. 13* is an exothermic reaction. The large discrepancy between the recommended O–H *BDE* in $HO_2^\bullet$ and the much lower value presented in [1] is very likely rooted in the use of the semiempirical AM1 procedure for the geometry optimization, which renders gross deviations in bond lengths and angles[2]).

Based on erroneous thermochemical arguments, it is claimed in [1] that the subsequent reactions of the $^\bullet C_5H_5NH$ radical are '*likely to be H-atom abstraction or addition to peroxide (sic) radicals*' rather than an interaction with O_2, followed by the loss of $^\bullet OOH$ (*Eqn. 13*). In view of the much higher concentration of O_2 than of peroxyl radicals, this is certainly incorrect. *Eqn. 13* must predominate.

The formation of $HO_2^\bullet$ from certain additives, *e.g.*, γ-terpinene [7], can give these additives rather mild antioxidant activities under circumstances where the cross-radical termination reaction (*Eqn. 14*) is faster than termination *via* the bimolecular self-reaction of the alkylperoxyls (*Eqn. 15*), *i.e.*, when $k_{14} >> k_{15}$.

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[2]) The AM1 optimized geometry for $HO_2^\bullet$ contains a r(H–OO) of 1.010, and r(HO–O) of 1.177 Å, while the recommended values are 0.971 and 1.331 Å, resp. The AM1 H–O–O angle is 112.5° (exp. 104.3°). For O_2, AM1 finds a r(O–O) of 1.085, which is not compatible with the experimental value of 1.208 Å. For experimental data see NIST CCCBDB at http://srdata.nist.gov/cccbdb.

$$ROO^{\bullet} + HOO^{\bullet} \longrightarrow ROOH + O_2 \quad (14)$$

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow \text{non-radical products} \quad (15)$$

However, such additives are much less effective than conventional phenolic and aromatic amine antioxidants, in part because the $HO_2^{\bullet}$ radical may propagate the peroxidation chain.

1,4-Dihydropyridine Thermochemistry. – From the experimental work of *Rüchardt et al.* [8], it can be inferred that, in acridane (=9,10-dihydroacridine, the aromatic analog of DHP), the C–H *BDE* is 3–4 kcal/mol lower than that in 9,10-dihydroanthracene (DHA). Since the C–H *BDE*s in CHD and DHA are almost equal, it is reasonable to assume that the C–H *BDE* in DHP is around 72–73 kcal/mol. This C–H *BDE* is several kcal/mol higher than the calculated C–H *BDE* in DHP in [1] (*i.e.*, 70.12 kcal/mol, Table 1, Entry 4). Moreover, it is mentioned in [1] that, based on their computational results, '*We were surprised to find that the C–H* BDE *value for 1,4-dihydropyridine is by 3–5 kcal/mol lower than (the O–H BDE) of* α-**T**....' (α-tocopherol). This assumes that the computational method applied is equally accurate for C–H and O–H bonds, which is not the case. If the authors had performed computations on the C–H *BDE* in CHD, they would have discovered that, irrespective of the computational method, the presence of multiple double bonds in the molecule causes a 2–4 kcal/mol underestimation of its *BDE* [3c]. For example, with CBS-QB3, a high-level chemistry model computation, the C–H *BDE* in CHD is calculated as 74.4 kcal/mol [9], still 2.5 kcal/mol lower than the recommended value [5b].

In [1], the DFT results are also presented for diludin (=diethyl 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate), a derivative of DHP, and a known calcium channel antagonist, and it is stated that '*the ester groups have no positive effect in lowering the* BDE *value of the C_4–H-bond*' (relative to DHP). Despite this assertion, Table 1 of [1] appears to indicate that the C_4–H *BDE* in diludin (Entry 9) is 98 kcal/mol, an increase of 28 kcal/mol over the DHP C_4–H *BDE*! For an isomeric compound (diethyl-1,4-dihydro-pyridine-2,6-dicarboxylate), the C_4–H *BDE* is found to be *ca.* 10 kcal/mol lower than that of DHP. If the C_4–H *BDE* in this compound is indeed 60 kcal/mol, it is unlikely to be an antioxidant because a direct H-abstraction by O_2 (see *Eqn. 5*) is now feasible (reaction enthalpy = 9 kcal/mol). Since the reverse reaction has no activation barrier, it can be estimated that the rate constant for *Eqn. 5* is *ca.* $25\,M^{-1}\,s^{-1}$ at 25° (taking a typical pre-exponential factor of $10^8\,M^{-1}\,s^{-1}$). This implies that, in the presence of atmospheric oxygen (8×10^{-3} M), the shelf life for this particular compound ($t_{50\%}$) is less than 4 s.

It has been shown by theory (and experimentally confirmed) that substituted pyrimidin-5-ols (5-Pys) possess similar reactivities (*Eqn. 3*) to equivalently substituted phenols [10]. Because O–H bonds are involved with both classes of compounds, the computed O–H *BDE*s *do* reflect the relative kinetics of O–H bond cleavage. The *IP*s for 5-Pys are considerably higher than those for comparable phenols, which make the former compounds less vulnerable to air oxidation (*Eqn. 6*). Thus, 5-Pys are excellent peroxyl-radical-trapping agents, but a comprehensive toxicological study is required before they see any use as antioxidants.

Conclusion. – It may be tempting to rely solely on computational methods to discover new 'antioxidants' which have low C–H *BDE*s, and without a doubt many substances could emerge from such an endeavor. However, it is a serious mistake to ignore the basic guidelines formulated in the beginning of this note, because this may lead to suggestions that a compound forming toxic products, or a pro-oxidant compound, or a compound with virtually no shelf life is 'a promising lead antioxidant'.

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