Comparative QSAR and radical toxicology: the aromatic CH_2OH moiety

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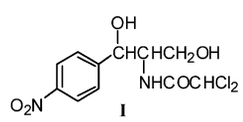
Evidence is summarized to show *via* quantitative structure–activity relationships (QSAR) that the aromatic CH_2OH group when conjugated with electron-releasing substituents displays toxic effects that suggest a radical reaction mechanism. The evidence can be used to explain the inhibitory action of lucanthone toward schistosomiasis. That is, lucanthone *per se* is not active, but its metabolite in which a methyl group has been converted to CH_2OH is. This calls to mind the activity of chloramphenicol, which was a very effective anti-bacterial agent, but also caused serious blood dyscrasias in some patients. Presumably this could have been due to the toxic action of radicals.

Introduction

As we continue to build our chem-bioinformatics system now based on 15900 quantitative structure–activity relationships (QSAR) from chemical–chemical and chemical–biological interactions, we find new opportunities for doing comparative QSAR at the interface of chemistry and biology. Recently, we have become especially interested in radical reactions[1–4] of which there appear to be two types: good and bad.[5] What defines the borderline between the two classes is unclear. Anyone who reads the newspapers constantly sees reports on the importance of radical scavengers. Of course, when such a substance destroys a bad radical, it becomes a new radical. One wonders if it is possible to consume too much of the food containing natural scavengers.[6] In this report, we are concerned about means of establishing evidence for the ability of certain aromatic functional groups to form radicals that can then be compared with their toxic actions in biological systems.

Results

For a long time we have been concerned with understanding the toxic action of chloramphenicol, **I**.[4,7,8] Chloramphenicol was a very effective anti-bacterial agent that was first isolated from *Streptomyces venezuelae* from a soil sample collected in Venezuela.[9] However, because of serious blood dyscrasias and other toxic reactions its use has been discontinued except in unusual circumstances.[9] Early research established that replacement of the benzylic H with D resulted in an isotope effect in its action on *E. coli* by lowering the activity.[8] It has been shown that the nitro group could be replaced by other substituents that were effective against microorganisms. Chloramphenicol contains two asymmetric carbons. Only the D-isomer is effective; that is, there is something about the geometry of the transition state that is critical in the toxic action. We have been able to obtain eqn. (1) from previously published data that suggest a radical mechanism. Eqn. (1) is



I

Inhibition of *E. coli* by 4-X-chloramphenicols[7]

$$\log k = 3.04\ (\pm 0.77)\ E_R + 0.18\ (\pm 0.12) \log P + 0.61\ (\pm 0.21) \quad (1)$$

$$n = 9, \quad r^2 = 0.947, \quad s = 0.110, \quad q^2 = 0.872$$

(X = NO_2, COMe, SMe, I, CN, Br, OMe, Cl, $CHMe_2$, SO_2Me, C_6H_5 and 3-NO_2)

only for a limited set of data for which we have E_R values. The most generally useful parameter for correlating radical reactions is σ^+,[1] but in this instance σ^+ cannot replace E_R in QSAR 1.

E_R is based on radical abstraction of $^{\bullet}H$ from X–C_6H_4-$CH(CH_3)_2$.[10a] A summary of E_R values has been reported.[11] The correlation with E_R plus the isotope effect strongly point to a radical mechanism being involved in the toxicity with *E. coli.* However, the fact that σ^+ cannot replace E_R in eqn. (1) raises questions. One needs further evidence for additional support.

The data in eqn. (1) can be considered from another point of view. Eqn. (2) was formulated using $\sigma^{\bullet}$, a radical parameter

$$\log k = 0.26\ (\pm 0.13) \log P + 2.04\ (\pm 0.45)\ \sigma^{\bullet} + 0.48\ (\pm 0.19) \quad (2)$$

$$n = 7, \quad r^2 = 0.981, \quad s = 0.079, \quad q^2 = 0.927$$

derived by Creary *et al.*[10b] Creary's[10b] parameter is available only for 7 data points, however, it reinforces the idea of a radical reaction. There is rather good agreement between E_R and Creary's $\sigma^{\bullet}$. For 14 substituents where both parameters are available for *para* substituents $r^2 = 0.926$. One data point 4-NMe_2 was poorly related and omitted. A most interesting fact is that there is no correlation between σ^+ and E_R or $\sigma^{\bullet}$. Obviously different mechanisms are involved.

There are two ways in which radical action of benzyl alcohols could be mediated. $H^{\bullet}$ abstraction could occur with a CH bond or an OH bond. Calculated homolytic bond dissociation energies (BDE) show that the former is more favorable (BDE = 89.7 kcal mol^{-1} for the former while for the latter it is 107.2 kcal mol^{-1}).[4] Of course, the reaction need not go homolytically. The H could be removed as H^-. Still, in either

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Table 1 Oxidation of benzyl alcohols by chemical agents

No.	QSAR Parameters	n	r^2	Oxidizing agent	Ref.	r^2 for σ^+ *vs.* σ or σ^-
1	$-2.02\ (\pm0.27)\ \sigma$	7	0.987	HNO_3	12	0.904
2	$-1.77\ (\pm0.02)\ \sigma$	8	1.00	Quinolinium dichromate[a]	13	0.925
3	$-1.64\ (\pm0.03)\ \sigma$	10	1.00	Permanganate	14	0.939
4	$-1.62\ (\pm0.64)\ \sigma - 1.45\ (\pm0.55)\ B1_2$	8	0.969	*N*-Bromoacetamide	15	0.826
5	$-2.11\ (\pm0.08)\ \sigma^+$	9	0.998	Chloramine-T[b]	16	0.930
6	$-2.10\ (\pm0.22)\ \sigma^+$	10	0.984	Bromine	17	0.975
7	$-1.51\ (\pm0.40)\ \sigma^+$	9	0.919	*N*-Bromosuccinimide	18	0.925
8	$-2.15\ (\pm0.20)\ \sigma^+$	4	0.999	Trimethylester of coenzyme PQQ	19	0.983
9	$-2.65\ (\pm0.19)\ \sigma + 0.68\ (\pm0.16)\ B1_2$	22	0.980	Ethyl chlorocarbamate[c]	20	0.512
10	$-2.09\ (\pm0.04)\ \sigma^+$	9	1.000	*N*-Chlorobenzenesulfonamide	21	0.930
11	$-0.75\ (\pm0.24)\ \sigma^+$	6	0.948	BrO_3^-	22	0.962
12	$-1.57\ (\pm0.32)\ \sigma^-$	9	0.952	Pyridinium dichromate[d]	23	0.836

[a] $[C_9H_7NH^+]_2Cr_2O_7^{2-}$. [b] 4-Me-$C_6H_4SO_2N(Cl)H$. [c] $C_2H_5N(Cl)COO^-$. [d] $[C_5H_5NH^+]_2Cr_2O_7^{2-}$.

case one would expect through-resonance between substituent and reaction center to be important. That is, either σ^- or σ^+ should be important. We have found in many radical reactions that σ^+ is by far the most important parameter.[1] While our review did not find any examples for radical reactions of benzyl alcohols,[1] one example for a similar situation, X–$C_6H_4CH_2OMe$ was uncovered where the abstraction agent was $Cl_3C^{\bullet}$ and the correlating term was $-0.36\sigma^+$. A large amount of work has been done on the chemical oxidation of benzyl alcohols and it is summarized in Table 1.

Table 1 lists all the examples for the oxidation of benzyl alcohols that are at present in our chem-bioinformatics system. There is one common feature among the twelve data sets: all have negative ρ values indicating that electron-releasing substituents promote oxidation. While 6 examples are best correlated by σ^+, 5 are best correlated by σ and 1 by σ^-. The reasons for this are not clear. Collinearity between σ, σ^+ and σ^- is high in most cases. The most convincing example is number 9 that is based on the largest number of data points and has the lowest degree of collinearity. Considering the wide variety of oxidizing reagents, and hence mechanisms, the results are interesting. A number of these reagents have been used in a variety of radical reactions with a variety of substrates other than benzyl alcohols that are correlated by σ^+.

Of 418 QSAR for presumably radical reactions in chemical and biological systems that were reviewed[1] only ten were best correlated by E_R. Two of these involved toxicity to biological systems of mixed cells.

Growth inhibition of moulds (*aspergillus*, *penicillum*, *cladosporium* and *mucor*) by X–$C_6H_4CH_2OH$.
Data from Carter *et al.*[24] employed to derive eqn. (3)

$$\log 1/C = 1.41\ (\pm0.85)\ E_R + 0.67\ (\pm0.08) \log P + 0.78\ (\pm0.21) \quad (3)$$

$n = 18$, $r^2 = 0.962$, $s = 0.160$ $q^2 = 0.940$ outlier: 2-NO_2

(X = 4-Cl, 2,4-diCl, 3,4-diCl, 2,4,5-triCl, 3,4,5-triCl, 2-Br, 4-Br, 4-I, 4-Me, 2,4-diMe, 4-Cl-3,5-diMe, 2-NO_2, 4-CN, 2-OH, 3-OH and 4-OH)

Growth inhibition of Gram negative bacteria (*Proteus vulgaris*, *E. coli* and *Pseudomonas*) by X–$C_6H_4CH_2OH$.
Data from Carter *et al.*[24] employed to derive eqn. (4)

$$\log 1/C = 2.36\ (\pm0.47)\ E_R + 0.67\ (\pm0.06)\ C\log P + 0.66\ (\pm0.15) \quad (4)$$

$n = 15$, $r^2 = 0.980$, $s = 0.092$, $q^2 = 0.965$, outlier: 4-Cl

[X substituents are the same as for eqn. (3)]

In each of the above examples we find a positive ρ as in QSAR 1 for the chloramphenicols. *T. pyriformis* shows σ^+ correlated toxicity.

T. pyriformis vs. X–$C_6H_4CH_2OH$

$$\log 1/C = 0.93\ (\pm0.28)\ \sigma^+ + 1.03\ (\pm0.13)\ C\log P + 1.17\ (\pm0.26) \quad (5)$$

$n = 19$, $r^2 = 0.947$, $s = 0.132$, $q^2 = 0.928$ outlier: 4-CMe_3

(X = 2-Me, 3-Me, 4-Me, 4-C_2H_5, 4-$CHMe_2$, 4-CMe_3, 2-C_6H_5, 4-C_6H_5, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2-I and 3-I)

In the study of phenol toxicity to fast growing cells and rat embryos we have found a correlation[2] between toxicity and σ^+ that encouraged us to investigate other functional groups for their toxicity. The results that we found with benzyl alcohols (QSAR 6) were a surprise.

Adding electronic terms to eqn. (6) does not improve it. The slope and intercept of eqn. (6) are typical of 'nonspecific'

I_{50} of 4-X–$C_6H_4CH_2OH$ to L1210 leukemia cells[4]

$$\log 1/C = 0.51\ (\pm0.15)\ C\log P + 2.09\ (\pm0.26) \quad (6)$$

$n = 11$, $r^2 = 0.870$, $s = 0.158$, $q^2 = 0.804$

(X = CN, C_6H_5, CMe_3, Cl, SMe, NO_2, NH_2, COOMe, Me, Br, OC_4H_9 and OMe)

toxicity. This is intriguing in light of the phenol toxicity to the same system. With the phenols it was found that those with electron-attracting substituents did not show activity correlated by σ^+. Only electron-releasing substituents produced activity correlated with σ^+. Phenols with electron-attracting substituents yielded QSAR 7.

I_{50} of X–C_6H_4OH with electron-attracting substituents to L1210 leukemia cells[2]

$$\log 1/C = 0.62\ (\pm0.16) \log P + 2.35\ (\pm0.31) \quad (7)$$

$n = 15$, $r^2 = 0.845$, $s = 0.232$, $q^2 = 0.800$

Eqn. (7) is very similar to eqn. (6). The results with eqn. (7) led us to postulate that a weak oxidant in the leukemia cells (possibly superoxide) was involved in activating the phenols. Phenols with electron-releasing substituents correlate with σ^+ [eqn. (8)]. If this is indeed true, we conclude that it is not strong enough to activate the benzyl alcohols.

I_{50} of X–C_6H_4OH with electron-releasing substituents to L1210 leukemia cells[2]

$$\log 1/C = -1.35\ (\pm 0.15)\ \sigma^+ + 0.18\ (\pm 0.04) \log P + 3.31\ (\pm 0.11) \quad (8)$$

$$n = 51, \quad r^2 = 0.985, \quad s = 0.227, \quad q^2 = 0.882$$

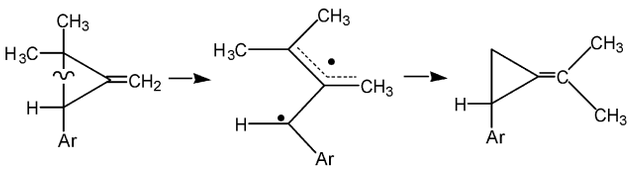
Using homolytic bond dissociation energy yields a somewhat better equation with $r^2 = 0.920$.

Discussion

The above examples show a certain degree of consistency. Eqns. (1)–(4) help us understand how the drug chloramphenicol exerts its toxic effect toward bacteria. The fact that only the D-isomer is active indicates the important nature of the transition state.

Although we have much to learn about biological reaction mechanisms, it is becoming increasingly clear that the possibilities for doing so are real.

The present query is: what is correlation with σ^+ or E_R telling us about reaction mechanisms? It should be noted that the radical parameter $\sigma^\bullet$ formulated by Creary *et al.* (Scheme 1)[10b] from the rearrangement of methylenecyclopropanes is highly collinear with E_R. However, we have used E_R since there are more values for the common substituents.

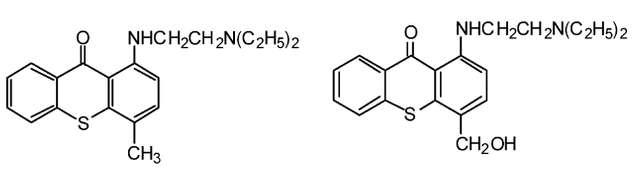


Scheme 1

At present by far the largest number of radical reactions are best correlated by σ^+.[1] These are largely reactions with highly active chemical systems. Turning to biological systems we have an excellent example of phenols inhibiting leukemia cells correlated by σ^+ or BDE. However, phenols with large *ortho* substituents inhibiting the same cells are best correlated by E_R (unpublished results). This would suggest, as with the chloramphenicols, that the geometry of the transition state is important in determining the reaction mechanism. This might also be involved in QSAR 3 and 4.

Eqn. (5) shows a good correlation with σ^+, but has a positive ρ value. In a different organism [eqn. (6)] we find an equation without an electronic term.

We have spent considerable time analyzing the oxidation of benzyl alcohols in part because of our interest in chloramphenicol's toxicity to *E. coli* and, rarely, to humans. However, we have a broader concern about the toxicity of the aromatic CH_2OH function. Of special interest is lucanthone, a drug that has found use in the treatment of schistosomiasis. It was a surprise to find that lucanthone was not the active species. Oxidation occurs *in vivo* to produce a CH_2OH group essential for activity.[25] This could have been expected from our results since the NH-moiety, conjugated with the CH_3, has a strong negative σ^+ value. One might have expected the metabolism to simply result in loss of drug, but the results with QSAR 3–5 suggest that activation of the CH_2OH moiety could occur to yield special chloramphenicol-like toxicity. The result with lucanthone can be associated with toluenes. It is also of interest that toluene caused birth defects in pregnant women who inhaled it for recreation.[27] An enormous amount of work has been devoted to the study of hydrogen abstraction from toluenes by a variety of radicals. We found[1] 57 examples best correlated by σ^+, 21 by σ, 3 by σ^- and one biological reaction correlated by σ^+ [eqn. (9)]. There is wide variation in ρ values depending in part on reaction conditions, but more on the activity of the radical and the ease of removal of the $H^\bullet$ from CH_3. The weaker the radical, other factors being equal, the larger ρ is; that is, substituent assistance is important. The stronger the radical, the smaller ρ is, and substitutent assistance is not so important.



Hydroxylation of CH_3 in X–$C_6H_4CH_3$ by microsomal P450 data for equation for mutation from ref. 26

$$\log k_{cat}/K_m = -0.71\ (\pm 0.62)\ \sigma^+ + 1.37\ (\pm 0.41)\ \mathrm{MR} + 4.39\ (\pm 0.29) \quad (9)$$

$$n = 7, \quad r^2 = 0.956, \quad s = 0.157, \quad q^2 = 0.907 \quad \text{outlier: 4-NO}_2$$

Our overall result shows that the toxicity of the aromatic CH_2OH function can be associated with radical toxicity. There is also evidence that the aromatic CH_3 can be converted to CH_2OH *in vivo*. The result with lucanthone shows that this could be favorable in drug development. However, chloramphenicol toxicity to patients does indicate a down side. The problem is that CH_2OH toxicity in people is very slow to become evident. Hence, one should be cautious in designing drugs with aromatic CH_2OH or CH_3 conjugated to substituents with strong electron-releasing properties.

While the results from our present review are interesting, we still do not have a clear understanding of what kind of cells have the ability to respond negatively to the CH_2OH and CH_3 functions. For example, benzyl alcohols do not show σ^+ reactivity with L1210 cells, but do with *T. pyriformis*. Phenols show σ^+ toxicity with leukemia cells, but not with *T. pyriformis*. We have much to learn about the behavior of various types of cells with various functional groups.

Acknowledgement

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