June, 1993

Structure-Toxicity Relationships for α, β-Unsaturated Alcohols in Fish

Ovanes G. Mekenyan, University of Wisconsin - Superior
Gilman D. Veith, United States Environmental Protection Agency
Steven P. Bradbury, United States Environmental Protection Agency
Christine L. Russom, United States Environmental Protection Agency

Available at: https://works.bepress.com/steven_bradbury/65/
Structure-Toxicity Relationships for α, β-Unsaturated Alcohols in Fish

Ovanes G. Mekenyan
Lake Superior Research Institute, University of Wisconsin – Superior, Superior, Wisconsin 54880 USA

Gilman D. Veith*, Steven P. Bradbury and Christine L. Russom
Environmental Research Laboratory – Duluth, 6201 Congdon Blvd., Duluth, Minnesota 55804 USA

Abstract
Previous toxicity testing with fathead minnows (Pimephales promelas) indicated that some unsaturated acetylenic and allylic alcohols can be metabolically activated, via alcohol dehydrogenase, to highly toxic α, β-unsaturated aldehydes and ketones or allene derivatives. Although several in vivo and in vitro toxicological and biochemical endpoints can differentiate these alcohols by toxic mechanism, the use of stereoelectronic molecular descriptors to discriminate these toxicants, and subsequently to predict potency, had not been previously attempted. Exploration of several descriptors indicated that soft electrophilic characteristics of acetylenic or allylic moieties in the suspected metabolites unambiguously discriminated reactive and narcotic toxicants. The acute toxicity of alcohols acting as narcotics was accurately predicted using an existing quantitative structure-activity relationship for nonpolar narcosis. The toxicity of alcohols mediated by metabolic activation was estimated quantitatively using receptor superdelocalizability of specific carbon atoms of the acetylenic or allylic moiety in the putative electrophilic intermediates.

Key words: Propargylic alcohols, toxicity, electrophiles, reactivity, electronic, descriptors, acetylenic alcohols, fish

1 Introduction
Our previous work [1, 2] established that some members in a series of acetylenic and allylic alcohols could be one to three orders of magnitude more acutely toxic in the fathead minnow (Pimephales promelas) than would be predicted from a nonpolar narcosis-based quantitative structure activity relationship (QSAR). More specifically, it was observed that allylic and primary, secondary, and homopropargylic alcohols were 20 to 5000 times more toxic than would be predicted. The signs of intoxication observed with tertiary propargylic alcohols and unconjugated alcohols were consistent with narcosis, and measured 96-h LC50 values were predicted accurately using the narcosis QSAR [3]. It has been hypothesized that the greater than predicted toxicity of allylic and primary and secondary propargylic alcohols is due to metabolic activation to the corresponding electrophilic allenes and α, β-unsaturated aldehydes and ketones, respectively [2]. Support for this hypothesis is consistent with the observation of lordosis, scoliosis, edema, and tetany in fathead minnows exposed to those alcohols suspected of being activated [2], and the finding that these alcohols can be metabolized in vitro using both horse and rainbow trout liver preparations of alcohol dehydrogenase to reactive intermediates [4].

While previous work provides a mechanistic rationale for the observed toxic responses as well as the empirical toxicological and biochemical data to differentiate narcotic from non-narcotic alcohols, identifying specific parameters to discriminate toxic mechanisms and establishing QSARs based on those mechanisms remains incomplete. The lack of a quantitative analysis can be attributed, in part, to the absence of a uniform approach to model in vivo electrophile reactivity. Hermans [5, 6] used the reactivity rate constants with model nucleophiles to predict the toxicity of electrophiles. These empirical rate constants are useful; however, reactive species must first be classified as hard and soft reactants [7], and care is needed not to use a hard model nucleophile to simulate a soft electrophile. The unsaturated aldehyde and ketone metabolites in question, with alkene and alkyne bonds activated by an adjacent carbonyl, are soft electrophiles and have small rate constants (if measurable at all) with a hard nucleophile. They can also have immeasurably fast rate constants with soft nucleophiles such as thiols and amines [6, 8, 9].

2 Materials and Methods
We have used clinical evidence obtained from the fish toxicity tests to classify the toxicants as reactive or non-reactive chemicals with respect to toxicity mechanisms. Behavioral responses and morphological alterations such as hemorrhaging, edema, tetany, and scoliosis were monitored in fathead minnows during 96-h toxicity tests to classify chemicals as either reactive or non-reactive [10].

The exploration of computational methods to discriminate reactive from non-reactive toxicants concordant with the empirical classification was performed using the Optimized QSAR Approach Based on Structural Indexes Set (OASIS) which has been described in detail previously [11-18]. Briefly, the OASIS system is a highly integrated modeling package which provides an extensive description of molecular structure...

* Mention of specific systems or approaches does not constitute endorsement by EPA.
and a powerful set of statistical tools to explore for potential structure-activity relationships. The OASIS system computes substituent constants, physicochemical properties, and topological and stereoelectronic indexes to quantify chemical structure.

Finally, the electronic structure of molecules is described for their ground state within the MO LCAO approximation. Various semiempirical MO methods (AM1, MNDO (C), CNDO/2, MINDO/3, PM/3) are used to assess electronic structure implementing SCF-calculations optimized by quantum chemical or other force field methods [13].

We reasoned that the soft electrophiles described in this work will react with biological molecules through reactions controlled by molecular orbital interactions rather than by atomic charges as in charge controlled reactions of hard electrophiles. The electronic indexes which describe the local reactivity of soft electrophiles include acceptor superdelocalizability indexes (\(\Sigma_{\mu}^{\text{N}}\)), frontier (“positive hole”) on LUMO orbitals (\(\Sigma_{\mu}^{\text{LUMO}}\)), and atomic polarizability, \(\mu\) [19, 20]. As a global measure of molecular soft electrophilic behavior, the sum of the acceptor superdelocalizability indexes (\(\Sigma_{\mu}^{\text{N}}\)), the frontier charges, \(\Sigma_{\mu}^{\text{LUMO}}\) for some particular molecular fragment, and the energies of the frontier molecular orbitals, \(E_{\text{LUMO}}, E_{\text{HOMO}}\) were evaluated. The more reactive molecules as soft electrophiles are characterized with lower-lying LUMO orbitals as well as with higher values of \(\Sigma_{\mu}^{\text{N}}\), \(\Sigma_{\mu}^{\text{LUMO}}\)

3 Results and Discussion

The toxicity of the unsaturated alcohols expressed as \(\log(1/\text{LC}_{50})\) in the fathead minnow is presented in Table 1 along with some of the molecular descriptors found to be significant for structure-toxicity relationships of the series under investigation. The structures of each chemical and their corresponding carbonyl were modelled separately. We also generated a set of 83 conformers of the toxicants to verify that the chosen conformations of the chemicals did not affect the results.

Our previous work has shown that these chemicals can be grouped into conventional classes of tertiary propargylic which do not undergo metabolic activation, and the proelectrophiles such as secondary and primary propargylic, homopropargylic, and those allylic alcohols which can be activated. A structure-toxicity relationship for each subclass using only the log P of the

Table 1. Measured and estimated toxicity, 96-h \(\log(1/\text{LC}_{50})\), of acetylenic and allylic alcohols, and important electronic and hydrophobic parameters.

<table>
<thead>
<tr>
<th>NO.</th>
<th>CHEMICALS</th>
<th>Log(1/\text{LC}_{50}) (mol/l)</th>
<th>Stereoelectronic Variables</th>
<th>log (P^a)</th>
<th>(\log(1/\text{LC}_{50})) (mol/l)</th>
<th>QSAR (3)</th>
<th>QSAR (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Tertiary propargylic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2-Methyl-3-butyn-2-ol</td>
<td>1.41</td>
<td>0.2845</td>
<td>0.0512</td>
<td>0.33</td>
<td>2.07</td>
<td>2.16</td>
</tr>
<tr>
<td>2</td>
<td>3-Methyl-1-pentyn-3-ol</td>
<td>1.91</td>
<td>0.2847</td>
<td>0.0561</td>
<td>0.86</td>
<td>2.37</td>
<td>2.45</td>
</tr>
<tr>
<td>3</td>
<td>1-Ethynyl-1-cyclohexanol</td>
<td>2.67</td>
<td>0.2844</td>
<td>0.0638</td>
<td>1.66</td>
<td>2.76</td>
<td>2.87</td>
</tr>
<tr>
<td>4</td>
<td>2-Phenyl-1-cyclohexanol</td>
<td>3.11</td>
<td>0.2843</td>
<td>0.0042</td>
<td>1.68</td>
<td>2.76</td>
<td>2.70</td>
</tr>
<tr>
<td>5</td>
<td>3,6-Dimethyl-1-heptyn-3-ol</td>
<td>3.46</td>
<td>0.2847</td>
<td>0.0559</td>
<td>2.32</td>
<td>3.14</td>
<td>3.18</td>
</tr>
<tr>
<td>6</td>
<td>1,1-Diphenyl-2-propyn-1-ol</td>
<td>4.07</td>
<td>0.2834</td>
<td>0.0060</td>
<td>2.71</td>
<td>3.20</td>
<td>3.22</td>
</tr>
<tr>
<td>7</td>
<td>3,4-Dimethyl-1-pentyn-3-ol</td>
<td>2.73</td>
<td>0.2846</td>
<td>0.0660</td>
<td>1.26</td>
<td>2.57</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>B. Secondary propargylic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3-Butyn-2-ol</td>
<td>3.78</td>
<td>0.3051</td>
<td>0.7343</td>
<td>-0.06</td>
<td>4.07</td>
<td>4.11</td>
</tr>
<tr>
<td>9</td>
<td>1-Heptyn-3-ol</td>
<td>4.80</td>
<td>0.3049</td>
<td>0.7318</td>
<td>1.52</td>
<td>4.88</td>
<td>4.90</td>
</tr>
<tr>
<td>10</td>
<td>1-Octyn-3-ol</td>
<td>5.48</td>
<td>0.3049</td>
<td>0.7318</td>
<td>2.05</td>
<td>5.16</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>C. Primary propargylic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2-Propyn-1-ol</td>
<td>4.59</td>
<td>0.3071</td>
<td>0.7154</td>
<td>-0.37</td>
<td>4.12</td>
<td>3.90</td>
</tr>
<tr>
<td>12</td>
<td>2-Butyn-1-ol</td>
<td>3.84</td>
<td>0.3030</td>
<td>0.6590</td>
<td>0.16</td>
<td>3.96</td>
<td>3.99</td>
</tr>
<tr>
<td>13</td>
<td>2-Butyn-1,4-diol</td>
<td>3.21</td>
<td>0.3050</td>
<td>0.6455</td>
<td>-1.83</td>
<td>3.13</td>
<td>2.94</td>
</tr>
<tr>
<td>14</td>
<td>2-Decyn-1-ol</td>
<td>5.16</td>
<td>0.3038</td>
<td>0.6644</td>
<td>3.33</td>
<td>5.72</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>D. Homopropargylic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>3-Butyn-1-ol</td>
<td>3.29</td>
<td>0.2963</td>
<td>0.4619</td>
<td>-0.50</td>
<td>2.90</td>
<td>3.04</td>
</tr>
<tr>
<td>16</td>
<td>4-Pentyn-2-ol</td>
<td>3.38</td>
<td>0.2956</td>
<td>0.4306</td>
<td>-0.08</td>
<td>3.04</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>E. Alkenes-ols</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1,5-Hexadien-3-ol</td>
<td>3.41</td>
<td>0.2963</td>
<td>0.4858</td>
<td>0.51</td>
<td>3.46</td>
<td>3.65</td>
</tr>
<tr>
<td>18</td>
<td>1-Hexen-3-ol</td>
<td>3.52</td>
<td>0.2964</td>
<td>0.5094</td>
<td>1.12</td>
<td>3.76</td>
<td>4.00</td>
</tr>
<tr>
<td>19</td>
<td>cis-3-hexen-1-ol</td>
<td>2.42</td>
<td>0.2870</td>
<td>0.0172</td>
<td>1.34</td>
<td>2.87</td>
<td>2.57</td>
</tr>
<tr>
<td>20</td>
<td>trans-3-hexen-1-ol</td>
<td>2.57</td>
<td>0.2871</td>
<td>0.0185</td>
<td>1.34</td>
<td>2.88</td>
<td>2.57</td>
</tr>
</tbody>
</table>

\(^a\) as carbonyls metabolites, \(^b\) as alcohol from CLOGP version 3.53
chemical can be developed [2]. However, any simple classification based on substructure moieties is unreliable because predicting reactivity is unreliable, especially for the chemical classes in which some members are proelectrophiles.

If these unsaturated alcohols are activated to soft electrophiles and the toxicity is due to the reactivity of the metabolites, then it can be assumed that molecular descriptors which quantify orbital-controlled processes will correlate to toxicity. Oxidation of the alcohol to carbonyl yields metabolites with both activated $\pi$ bonds as well as the carbonyl moiety as reactive centers. Thus, some of these chemicals may react as aldehydes as well as Michael addition at the $\pi$ bond [8]. The most significant positions for a “soft” nucleophilic attack are the unsaturated $\beta$-carbon (atom 1) one atom removed from the carbonyl and the carbonyl carbon itself (atom 3).

Table 1 shows that the acceptor superdelocalizability of the unsaturated carbon (atom 1) is less than approximately 0.285 for all tertiary propargylic alcohols and is greater than 0.295 for reactive propargylic metabolites.

Interestingly, the two allylic alcohols (3-hexen-1-ol isomers) are metabolized to aldehydes with acceptor superdelocalizability lower than the other proelectrophiles but still larger than 0.285. These results suggest that the classification of non-reactive and reactive unsaturated alcohols and their metabolites can be accomplished using superdelocalizability.

![Figure 1. Variation of log (1/LC50) with acceptor superdelocalizability, $S^*_a$. (● are unmetabolized alcohols, □ are activated to reactive metabolites).](image)

Figure 1 presents the variation in toxicity of the electrophilic metabolites with the acceptor superdelocalizability on the unsaturated carbon. It is seen that the non-reactive (solid circles) and reactive chemicals (open squares) are unambiguously discriminated by the superdelocalizability index. The data show that the toxicity of the non-reactive chemicals varies by almost three orders of magnitude, as do the corresponding values of log P.

Veith et al. [2] classified tertiary alcohols and the two allylic alcohols (Nos. 19 and 20, Table 1) as non-reactive, narcotic chemicals, the toxicity for which were accurately predicted by the QSAR for narcosis [3]. Although Bradbury and Christiansen [4] found that the allylic alcohols were activated in vitro, the reactivity of the metabolites was seemingly insufficient to cause measurable excess toxicity in vivo. Since the 3-hexene-1-ol isomers can be metabolized to aldehydes but not to $\alpha,\beta$-unsaturated carbonyls, the in vitro enzyme inhibition [4] could be due to the carbonyl reactivity alone. This would also be consistent with other observations that, as the hydrophobicity of aldehydes increases, the toxic effects in vivo more closely resemble those of narcotics than those of electrophiles [6].

Table 1 provides electronic parameters for quantitative analysis. The QSAR for the non-reactive alcohols ($S^*_a \leq 0.285$) is:

$$\log (1/LC_{50}) = 1.10 (\pm 0.17) + 1.08 (\pm 0.10) \log P$$

where $n$ is the cardinality of the correlation sample, $r$ is the correlation coefficient, $s^2$ is the variance, $F$ is Fisher’s criterion, and $W$ is the normalized parameter weight. Equation (1) is consistent with the narcosis QSAR and the experimental evidence presented previously [2, 3].

For the reactive primary and secondary propargylic alcohols (with $S^*_a > 0.308$), the toxicity increased with log P as shown in Eq. 2:

$$\log (1/LC_{50}) = 4.12 (\pm 0.19) + 0.41 (\pm 0.11) \log P$$

The statistics show that hydrophobicity alone is inadequate in accounting for the variation in toxicity of the metabolites. However, QSARs with only electronic descriptors were worse, and the $r^2 = 0.76$ for log P was great enough to preclude a second variable for this small set of chemicals.

Because of the relative orthogonality of the hydrophobic (log P) and electronic ($S^*_a$ and $f^*_\text{UMO}$) factors [R ($S^*_a$/log P) = 0.36, R ($f^*_\text{UMO}$/log P) = 0.34], two QSARs were developed using both hydrophobic and electronic parameters as shown in Eqs. 3 and 4.

$$\log (1/LC_{50}) = -28.6 (\pm 3.4) + 0.53 (\pm 0.09) \log P + 107.1 (\pm 11.4)S^*_a$$

$$n = 20 \quad \alpha = 0.02 \quad r^2 = 0.85 \quad s^2 = 0.19 \quad F = 48.9 \quad W_{\log P} = 0.63 \quad W_{S^*_a} = 0.96$$

$$\log (1/LC_{50}) = 1.84 (\pm 0.20) + 0.50 (\pm 0.09) \log P + 3.13 (\pm 0.35f^*_\text{UMO})$$

$$n = 20 \quad \alpha = 0.03 \quad r^2 = 0.84 \quad s^2 = 0.19 \quad F = 44.5 \quad W_{\log P} = 0.60 \quad W_{f^*_\text{UMO}} = 0.97$$

The normalized weights of the electronic descriptors are greater than that of log P for the entire data set of narcotics and proelectrophiles. The weight of $S^*_a$ is 0.96 in Eq. (3) while that for log P is 0.63. Similarly, the weight of $f^*_\text{UMO}$ is 0.94 com-
pared to 0.60 for log P in Eq. (4). The toxicity values estimated from Eqs. (3) and (4) are presented in Table 1.

Equations (3) and (4) are regression models that obviously mix at least two modes of toxic action involving narcosis and soft electrophilicity. Log P dominates for the narcotic alcohols, while both log P of the alcohols and either $S^N_1$ or $f_{\text{LUMO}}^{1}$ of the metabolites control the toxicity of the proelectrophiles. Equations (3) and (4) also support the possibility that two reactive centers in the metabolites may be involved in the interactions that lead to toxicity. Both the $\beta$-unsaturated and carbonyl carbons appear as significant factors in the QSARs; however, $S^N_1$ is statistically more significant than $f_{\text{LUMO}}^{1}$ for the unsaturated carbon and $f_{\text{LUMO}}^{1}$ is more significant than $S^N_1$ for the carbonyl carbon. Of course, these electronic descriptors co-vary in conjugated systems [$R (S^N_1/f_{\text{LUMO}}^{1}) = 0.98$] so that the reaction sites cannot be discriminated without explicitly identifying reaction products. Finally, the toxicity of the allene intermediates formed in the transformation of homopropargylic alcohols was explained, which supports the hypothesis of Alston et al. regarding the mechanism of activation [21].

The electronic connection between the carbon-carbon $\pi$ bond and the carbonyl explains why the reactive toxicity of the proelectrophiles reflects the electronic properties of the conjugated carbonyl and not just the inherent reactivity of the carbon-carbon or triple bonds. Terminal olefins and acetylenes can inhibit cytochrome P-450 metabolism and have been used as insecticide synergists [22]. The enzyme destruction is a “suicide” inhibition involving the activation of $\pi$-bonds by the iron-oxygen complex. The narcosis produced by the tertiary propargylic alcohols was explained, which supports the hypothesis of Alston et al. regarding the mechanism of activation [21].

The covariance between the hydrophobic and electronic factors for these chemicals was $R = 0.36$ for $S^N_1$/log P and $R = 0.34$

for $f_{\text{LUMO}}^{1}$/log P. The orthogonality means that any soft electrophile will be an outlier in the “baseline” toxicity plot involving only log P and toxicity [3]. In contrast, Eq. (3) represents a response plane in a three-dimensional “toxicity space” as illustrated in Fig. 2. The data in Table 1 are distributed in Fig. 2 where log P has been used as a global molecular descriptor of hydrophobicity and $S^N_1$ has been used as the electronic descriptor for electrophilic micro-environments in the molecules. Extension of the global nature of superdelocalizability indexes so that soft electrophilicity of other classes of chemicals can be included in more comprehensive QSARs is needed. Refinements of this approach will be presented in subsequent papers.

Acknowledgements

The authors express their applications to Ronald H. Oveson and Roger W. LePage for their invaluable contribution to the graphical presentations for this work.

4 References


Received on June 22nd 1992; accepted on January 15th 1993.