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Structure-Toxicity Relationships for Unsaturated Alcohols to *Tetrahymena pyriformis:* C_5 and C_6 Analogs and Primary Propargylic Alcohols

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Previous investigations have used 1-octanol/water partition coefficient (log K_{ow}) dependent quantitative structure-activity relationships (QSAR) as a basis of predicting toxicity of nonreactive, nonionic narcotics including saturated monoalcohols to the guppy (*Poecilia reticulata*, Könemann 1981), the fathead minnow (*Pimephales promelas*, Veith et al. 1983) and the ciliate (*Tetrahymena pyriformis*, Schultz et al. 1990c). However, other investigations (Lipnick 1985; Veith et al. 1989; Dawson et al. 1990) have shown selected unsaturated alcohols, especially primary propargylic alcohols, to exhibit excess toxicity to fish and to frog embryo and larvae. Excess toxicity (T_e) was defined by the ratio of predicted toxicity to observed toxicity (Lipnick et al. 1987) with predicted toxicity values being calculated by the baseline nonpolar narcosis QSAR.

The purposes of this investigation were to: (1) determine the biological response in the *Tetrahymena* population growth impairment assay of exposure to selected unsaturated monoalcohols; (2) compare the observed and predicted toxicity, and (3) develop a log K_{ow} dependent QSAR for 2-alkyn-1-ols.

MATERIALS AND METHODS

The chemicals selected for testing include a series of C_5 and C_6 straight-chain alken-1-ols and alkyn-1-ols, as well as, selected primary propargylic alcohols. Each chemical was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, USA or MTM Research Chemicals Lancaster Synthesis Inc., Windham, New Hampshire, USA, and each had a purity of 95 percent or better.

The population growth impairment testing was done in the *Tetrahymena pyriformis* batch system (Schultz et al. 1990b). This is a two-day assay using population density measured spectrophotometrically at 540 nm as the endpoint. Each chemical was tested in a range-finder, followed by testing in duplicate for three more replicates. Each replicate was a six-to-ten step concentration

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series using freshly prepared stock solutions. Only replicates with control values in late log-growth-phase (absorbance from 0.6 to 0.9) were used in the analyses. The 50 percent growth inhibitory concentration, IGC_{50}, was determined for each alcohol using Probit Analysis of Statistical Analysis System (SAS) software (SAS Institute Inc. 1989), with Y as the absorbance normalized as percentage of control and X as the toxicant concentration in parts per million. This 50 percent effect concentration was adjusted from pipetted volume to actual concentration by multiplying by density and recalculated for mM concentration.

For the straight-chain C_5 and C_6 unsaturated alcohols, the experimental toxicity measured as the density adjusted IGC_{50} in mM was compared with that predicted from the log K_{ow} dependent equation developed from data for a robust set of basic monoalcohols. This baseline, nonpolar narcosis, QSAR was generated using log IGC_{50}^{-1} as the measurement of relative toxicity (Y) and the hydrophobicity constant log K_{ow} was used as the single molecular descriptor (X). The log K_{ow} value of each saturated alcohol was obtained from MEDCHEM CLOGP version 3.53 either as a calculated value or, when available, a measured one (Leo and Weininger 1988). The equation was generated from General Linear Model for simple regression analysis from SAS. Similarly, a QSAR was developed for the 2-alkyn-1-ols. Log K_{ow} values for the unsaturated alcohols were calculated using MEDCHEM CLOGP version 3.53 and correcting for the polar interaction between the carbon-carbon triple bond and hydroxy moiety (Hansch et al. 1967).

In addition, the log K_{ow} value for selected unsaturated alcohols was experimentally determined by the shake-flask, equilibrium method and gas chromatography. Briefly, 8 mls of an 1-octanol and water phases in a suitable ratio were placed in a 10 ml flask and shaken vigorously for 1 hr. The head space was checked for evaporated substance, none was found, and the formed emulsion was eliminated by centrifugation. The aqueous phase was used for alcohol determination. A CHROM 5 gas chromatograph in conjunction with a FID detector and a 120 cm X 3 mm (ID) column packed with 80/100 mesh Chromosorb 101 and heated to 200°C were used. The chromatographic data were quantitated from calibration curves. Each determination was repeated 8 - 12 times with mean values and standard errors being reported.

RESULTS AND DISCUSSION

Earlier work with straight-chain saturated monoalcohols (Schultz et al. 1990a) was expanded to include branched derivatives. Toxicity, density and hydrophobicity data for these chemicals are reported in Table 1. Regression analysis of log K_{ow} versus the log of the density-adjusted IGC_{50}^{-1} values (mM^{-1}) for the alcohols listed in Table 1 resulted in the equation,

$$\log IGC_{50}^{-1} = 0.80 (\log K_{ow}) - 2.04;$$
$$n = 34,\ r^2 = 0.982,\ s = 0.171,\ f = 1744.53$$

Table 1. Hydrophobicity, density and toxicity of saturated monoalcohols

Alcohol	CAS number[a]	log K_{ow}[b]	Density	IGC_{50} (mM)	log IGC_{50}^{-1}
1. methanol	67-56-1	-0.77[c]	0.791	585.400	-2.67
2. ethanol	64-17-5	-0.31[c]	0.785	259.670	-2.31
3. 1-propanol	71-23-8	0.25[c]	0.804	69.360	-1.75
4. 1-butanol	71-36-3	0.88[c]	0.810	33.270	-1.43
5. 1-pentanol	71-41-0	1.56[c]	0.811	13.220	-1.03
6. 1-hexanol	111-27-3	2.03[c]	0.814	2.940	-0.38
7. cyclohexanol	108-93-0	1.23	0.963	6.057	-0.77
8. 1-heptanol	111-70-6	2.57[c]	0.822	0.964	0.10
9. 1-octanol	111-87-5	3.15[c]	0.827	0.316	0.58
10. 1-nonanol	143-08-8	3.69	0.827	0.169	0.85
11. 1-decanol	112-30-1	4.23	0.829	0.056	1.33
12. 1-undecanol	112-42-5	4.77	0.830	0.010	2.08
13. 1-dodecanol	112-53-8	5.13[c]	0.820	0.008	2.18
14. 1-tridecanol	112-70-9	5.67	0.822	0.005	2.39
15. 2-propanol	67-63-0	0.05[c]	0.785	97.060	-1.88
16. (±)-2-butanol	15892-23-6	0.6[f]	0.803	43.114	-1.54
17. 2-pentanol	6023-29-7	1.21	0.812	17.780	-1.16
18. 2-octanol	4128-31-8	2.77	0.819	1.218	0.00
19. 2-nonanol	628-99-9	3.25	0.827	0.291	0.62
20. 3-pentanol	584-02-1	1.21[c]	0.815	21.500	-1.24
21. 3-octanol	20296-29-1	2.77	0.819	1.137	0.03
22. (±)-4-decanol	2051-31-2	3.86	0.826	0.171	0.85
23. 2-methyl-1-propanol	78-83-1	0.65	0.803	29.356	-1.37
24. 2-methyl-2-propanol	75-65-0	0.75	0.786	77.556	-1.79
25. 2-methyl-1-butanol	137-32-6	1.42	0.815	11.000	-0.95
26. 3-methyl-1-butanol	123-51-3	1.42	0.809	13.430	-1.04
27. 3-methyl-2-butanol	598-75-4	1.28	0.818	12.110	-1.00
28. (tert)-pentanol	75-85-4	1.21	0.805	18.490	-1.17
29. neo-pentanol	75-84-3	1.57	0.818	9.070	-0.87
30. 3, 3-dimethyl-1-butanol	624-95-3	1.62	0.844	6.464	-0.74
31. 4-methyl-1-pentanol	626-89-1	1.25	0.821	5.302	-0.64
32. 2-propyl-1-pentanol	58175-57-8	2.81	0.830	0.885	0.13
33. 2-ethyl-1-hexanol	104-76-7	2.87	0.833	0.817	0.17
34. 3-ethyl-2, 2-dimethyl-3-pentanol	66793-96-2	1.88	0.852	1.732	-0.17

[a] Chemical Abstract Service registry number.
[b] 1-octanol/water partition coefficient calculated by MEDCHEM CLOGP version 3.53.
[c] Experimentally measured values.

$Pr > f = 0.0001$ [1].

This model was considered baseline toxicity and was used for comparisons.

A summary of the hydrophobicities, density and relative toxicities for the twelve straight-chain C_5 and C_6 unsaturated alcohols examined in this investigation are listed in Table 2. A comparison of the observed static 2-day *Tetrahymena* population growth impairment revealed the alkyn-1-ols to be more toxic than alken-1-ols. In addition, 2-position unsaturated derivatives were more toxic than other isomers. An examination of T_e values showed the 2-alkyn-1-ols and, to a lesser extent, the 3-alkyn-1-ols exhibit excess toxicity.

Moreover, an in-depth analysis of primary propargylic alcohols was undertaken. This included toxicity testing of nine straight-chain 2-alkyn-1-ols, one phenyl-substituted derivative, and two diols. A summary of the hydrophobicity, density and toxicity data for these primary propargylic alcohols are presented in Table 3. The log K_{ow} values were distributed over more than five orders of magnitude. The log IGC_{50}^{-1} values varied for almost four orders of magnitude.

An examination of T_e values revealed for those compounds having a single hydroxy group, that excess toxicity is inversely related to molecular size. Moreover, the two diols exhibited a more extensive excess toxicity. Even when log K_{ow} values were adjusted to account for two carbon-carbon triple bond and hydroxy moiety interactions, Eq. [1]-based excess toxicity for each diol was greater than forty. These trends in excess toxicity are consistent with those reported by Veith et al. (1989).

Regression analysis of log K_{ow} versus the log of the density-adjusted IGC_{50}^{-1} for the 2-alkyn-1-ols resulted in the equation,

$\log IGC_{50}^{-1} = 0.64 (\log K_{ow}) - 0.61;$
$n = 10,\ r^2 = 0.974,\ s = 0.166,\ f = 332.14$
$Pr > f = 0.0001$ [2].

As expected from the high coefficient of determination, an examination of residual values showed no statistical outliers. Comparison of observed toxicity with that predicted by Eq. [2] showed the toxicity of the two diols to be outside the upper 95 percent confidence limit as they were two orders of magnitude more toxic than predicted. Even when calculated log K_{ow} values involved corrections to account for two carbon-carbon triple bond and hydroxy moiety interactions, the Eq. [2]-based T_e values for each diol was still greater than four.

The primary propargylic alcohols are considered proelectrophiles. They are thought to be metabolically activated to the appropriate alpha-unsaturated

Table 2. Hydrophobicity, density and toxicity of selected C_5 and C_6 unsaturated monoalcohols

Alcohol	CAS number[a]	K_{ow}[b] exp.[c]	se[d]	log K_{ow}[b] calc.[e]	exp.[c]	Density	Observed IGC_{50} (mM)	log IGC_{50}^{-1}	Predicted[f] IGC_{50} (mM)	T_e[g]
1. (cis) 2-penten-1-ol	1576-95-0	-.---	-.---	0.81	-.--	0.853	13.321	-1.13	24.774	1.86
2. 4-penten-1-ol	821-09-0	-.---	-.---	0.81	-.--	0.840	17.905	-1.25	24.774	1.38
3. 2-pentyn-1-ol	6261-22-9	6.065	0.805	0.69	0.78	0.910	3.740	-0.57	30.974	8.28
4. 3-pentyn-1-ol	10229-10-4	3.364	0.278	0.06	0.53	0.912	22.995	-1.36	123.595	5.37
5. 4-pentyn-1-ol	5390-04-5	3.415	0.301	0.06	0.53	0.907	31.916	-1.50	123.595	3.87
6. (trans) 2-hexen-1-ol	928-95-0	57.81	7.013	1.34	1.76	0.849	2.964	-0.47	9.311	3.14
7. (cis) 3-hexen-1-ol	928-96-1	-.---	-.---	1.34	-.--	0.848	6.459	-0.81	9.311	1.44
8. 4-hexen-1-ol	928-92-7	40.51	2.582	1.34	1.61	0.851	5.675	-0.75	9.311	1.64
9. 5-hexen-1-ol	821-41-0	31.30	2.721	1.34	1.50	0.842	7.002	-0.85	9.311	1.33
10. 2-hexyn-1-ol	764-60-3	13.78	2.673	1.22	1.14	0.893	2.421	-0.38	11.641	4.81
11. 3-hexyn-1-ol	1002-28-4	9.459	1.129	0.47	0.98	0.900	10.599	-1.03	46.452	4.38
12. 5-hexyn-1-ol	928-90-5	6.136	1.269	0.47	0.79	0.895	17.927	-1.25	46.452	2.59

[a] Chemical Abstract Service registry number.

[b] 1-octanol/water partition coefficient.

[c] Experimentally determined by equilibrium method and gas chromatography.

[d] Standard error.

[e] Calculated by MEDCHEM CLOGP version 3.53 and corrected for the polar interaction of the carbon-carbon triple bond and the hydroxy moiety.

[f] Predicted from calculated log K_{ow} values and the QSAR, log $IGC_{50}^{-1} = 0.80$ (log K_{ow}) -2.04.

[g] Excess toxicity parameter defined as IGC_{50} (pred)/IGC_{50} (obs).

Table 3. Hydrophobicity, density and toxicity of primary propargylic alcohols

Alcohol	CAS number[a]	K_{ow}[b] exp.[c]	se[d]	log K_{ow}[b] calc.[e]	exp.[c]	density	Observed IGC_{50} (mM)	log IGC_{50}^{-1}	Predicted[g] IGC_{50} (mM)	T_e[h]
1. 2-propyn-1-ol	107-19-7	0.464	0.057	-0.37	-0.33	0.963	11.853	-1.08	219.149	18.49
2. 2-butyn-1-ol	764-01-2	1.098	0.190	0.16	0.04	0.938	10.861	-1.04	82.353	7.58
3. 2-pentyn-1-ol	6261-22-9	6.065	0.805	0.69	0.78	0.910	3.740	-0.57	30.947	8.27
4. 2-hexyn-1-ol	764-60-3	13.78	2.673	1.22	1.14	0.893	2.421	-0.38	11.629	4.80
5. 2-heptyn-1-ol	1002-36-4	61.67	6.377	1.75	1.79	0.882	1.547	-0.19	4.370	2.82
6. 2-octyn-1-ol	20739-58-6	-.---	-.---	2.27	-.--	0.880	0.642	0.19	1.673	2.61
7. 2-nonyn-1-ol	5921-73-3	-.---	-.---	2.80	-.--	0.873	0.220	0.66	0.629	2.86
8. 2-decyn-1-ol	4117-14-0	2600.00	590.0	3.33	3.40	0.870	0.103	0.99	0.236	2.29
9. 2-tridecyn-1-ol	51887-25-3	-.---	-.---	4.92	-.--	1.000	0.005	2.30	0.013	2.51
10. 3-phenyl-2-propyn-1-ol	1504-58-1	-.---	-.---	1.59	-.--	1.078	0.842	0.07	5.872	6.97
11. 2-butyn-1,4-diol	110-65-6	-.---	-.---	-1.83[f]	-.--	1.000	15.603	-1.19	3248.33	208.19
12. 3-hexyne-2,5-diol	3031-66-1	-.---	-.---	-0.77[f]	-.--	1.009	2.887	-0.46	458.712	158.89

[a] Chemical Abstract Service registry number.
[b] 1-octanol/water partition coefficient.
[c] Experimentally determined by equilibrium method and gas chromatography.
[d] Standard error.
[e] Calculated by MEDCHEM CLOGP versions 3.53 and corrected for polar interaction of the carbon-carbon triple bond and the hydroxy moiety.
[f] Corrected for only one polar interaction.
[g] Predicted from calculated log K_{ow} values and the QSAR, log IGC_{50}^{-1} = 0.80 (log K_{ow}) - 2.04.
[h] Excess toxicity parameter defined as IGC_{50} (pred)/IGC_{50} (obs).

aldehyde by the activity of alcohol dehydrogenase (Lipnick 1985; Veith et al. 1989). The aldehyde, in turn, acts as a Michael-type acceptor for nucleophilic moieties in biotic macromolecules (Lipnick et al. 1985). Roberts (1989) presented a rationale for the aquatic toxicity of electrophiles and proelectrophiles. In his explanation, the toxic action of electrophiles and proelectrophiles was due to involvement in "alkylation" reactions with biotic nucleophiles. He assumed these nucleophiles to reside in a lipid environment, i.e., biological membranes, and the rate-limiting step in toxicity to be the alkylation reaction. It followed that QSARs for electrophiles and proelectrophiles were expected to require both a term quantitating the rate of alkylation and a term quantitating transport of the chemical to the lipid phase (Roberts, 1989). Lipid phase transfer is proportional to log K_{OW} and hence is incorporated into Eq. [2]. However, no alkylation term was required for strong predictability with Eq. [2]. This reflects the fact that proelectrophiles used in the development of Eq. [2] were all 2-alkyn-1-ols. Therefore, the rate of alkylation had the potential of being constant and dropping out of the equation. Since both alkyn-diols tested had the potential for two electrophilic moieties per molecule, the alkylation rate constant for these chemicals was not expected to be the same as for the 2-alkyn-1-ols.

In summary, the relative toxicity of selected unsaturated alcohols were evaluated in the static *Tetrahymena pyriformis* population growth assay. The alkyn-1-ols were found to be more toxic than alken-1-ols. Moreover, 2-position unsaturated derivatives were more toxic than other isomers. A linear log K_{OW} dependent relationship was generated (slope = 0.64; intercept = -0.61; r^2 = 0.974) for selected 2-alkyn-1-ols. However, the diols were observed to be more toxic than predicted.

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