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Structure-Toxicity Relationships for Selected Lactones to *Tetrahymena pyriformis*

T. W. Schultz,[1,2] A. D. Deweese[2]

[1] College of Veterinary Medicine, The University of Tennessee, 2407 River Drive, Knoxville, TN 37996-4500, USA
[2] Graduate Program in Ecology and Evolutionary Biology, The University of Tennessee, 2407 River Drive, Knoxville, TN 37996-4500, USA

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Lactones are oxygen-containing heterocyclic compounds that have a carbonyl substituent alpha to the in-ring oxygen. Based on their ring size (four-, five-, six-, or seven-membered), lactones are defined as beta (β), gamma (γ), delta (δ), or epsilon (ε), respectively. These molecular structures have an impact on the mode of toxic action. Based on their structure, lactones may elicit a biological response in one of two ways: reversible narcosis or electrophilic reactivity. Whereas, six and seven membered lactones are thought to act as nonpolar narcotics, the small stressed-ringed lactones may react covalently with macromolecular nucleophiles (Hermens 1990). While none were tested in the present study, it is worth noting that larger ringed lactones, eight-membered rings and greater, also should be stressed-ringed.

Toxicity testing with the freshwater ciliate *Tetrahymena pyriformis* allows for the examination of a large number of organisms that possess characteristics of both single eucaryotic cells and whole organisms (Schultz 1996). Because of these traits, *Tetrahymena* has been used to generate toxicity data for the development of structure-toxicity relationships (Schultz et al. 1997).

The purpose of this study was to examine the aquatic toxicity of selected lactones. The specific aims were: (1) to determine the toxic potency for each lactone in the *T. pyriformis* population growth impairment assay; (2) to compare the observed toxicity to that predicted by the baseline toxicity model for neutral organic chemicals, and (3) to examine toxic potency QSARs developed with selected hydrophobic and quantum chemical descriptors.

METHODS AND MATERIALS

Eleven lactones were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) or Lancaster Synthesis Inc. (Windham, NH, USA). While β-propiolic lactone had a purity of only 90%, the remaining lactones had purity of 95% or better. None were repurified prior to use. Stock solutions of each lactone were prepared in dimethyl sulfoxide.

Tetrahymena pyriformis population growth impairment testing was executed following the protocol described by Schultz (1996). This static 40-hr assay used

Correspondence to: T. W. Schultz

population density measured spectrophotometrically at 540 nm as its endpoint. Test conditions allow for 8-9 cell cycles in control cultures.

Each lactone was tested in a range finder prior to testing in duplicate for three additional replicates. Two controls, one with no test material but inoculated with *T. pyriformis,* and the other, a blank, which had neither toxicant nor ciliates were used to provide a measure of the acceptability of the test and a basis for interpreting treatment data. Each definitive test replicate consisted of six to eight different concentrations with duplicate flasks of each concentration. Only replicates with control-absorbency values > 0.6 but < 0.75 were used in the analyses. The effect levels are based on unmeasured lactone concentrations. The 50% growth inhibitory concentrations, IGC_{50}, were determined by Probit Analysis of Statistical Analysis System (SAS) software (SAS Institute Inc. 1989).

Excess toxicity (T_e) evaluations were conducted for each chemical by calculating the ratio of predicted toxicity to observed toxicity (Lipnick et al. 1987). Experimental toxicity was measured as the IGC_{50} in mM. Predicted non-polar narcosis toxicity was determined from the relationship,

$$\log (IGC_{50}^{-1}) = 0.74 (\log K_{ow}) - 1.86;$$

$$n = 148, r^2 = 0.958, s = 0.21, F = 3341, Pr > F = 0.0001 \qquad \text{Eq. [1]}.$$

developed by Schultz et al. (1997) from data for neutral organic chemicals.

Logarithms of the 1-octanol/water partition coefficients (log K_{ow}) values for γ–butyrolactone and γ-decanolactone were secured as measured values from CLOGP for Windows (BIOBYTE Corp., Claremont, CA) software. Log K_{ow} values for β-, α-, and ε-lactones were obtained as estimated values from CLOGP for Windows. The log K_{ow} values for remaining γ-lactones were estimated by adding 0.56 log units for each methylene group (a value determined for the measured values for γ-butyrolactone and γ-decanolactone).

The energy of the frontier orbitals, lowest unoccupied molecular orbital (E_{LUMO}) and highest occupied molecular orbital (E_{HOMO}), were determined. Initially, each lactone was built and its energy minimized with NEMESIS for PC molecular modelling software. Subsequently, each molecule was geometrically optimized and molecular orbital quantum chemical calculations performed using the AM1 Hamiltonian in the MOPAC6 program.

Quantitative structure-toxicity relationships were examined using the log of the inverse of the IGC_{50} (log (IGC_{50}^{-1})) in mM as the dependent variable, and log K_{ow} and frontier orbital descriptors as the independent variables. Data were modeled using least-squares regression (general linear model procedure of SAS). Model adequacy was quantified with the coefficient of determination (r^2 value). The root of the mean square for error (s value), the Fisher statistic (F value), and the probability greater than the F value (Pr > F) were also noted.

RESULTS AND DISCUSSION

Acute toxicity has been compiled into two categories: baseline toxicity or bioreactive toxicity (Schultz et al. 1997). Baseline toxicity is a minimal toxic effect. It is characterized by the nonpolar narcosis mode of action (McKim et al. 1987) and is deemed to be the retardation of general cytoplasmic activity (Veith and Broderius 1990). This mode of action has been examined several times, including the recent review of van Wezel and Opperhuizen (1995).

In contrast to nonpolar narcosis, bioreactive chemicals exhibit excess toxicity (T_e). T_e is defined as potency greater than two times the predicted value based on nonpolar narcotic QSAR (Lipnick et al. 1987). This T_e is especially evident for low log K_{ow} bioreactive toxicants.

Bioreactive toxicity is a more complex process than narcosis (Schultz et al. 1997). It can be further subdivided into covalent and non-covalent mechanisms. While non-covalent mechanisms react reversibly, covalent reactions act irreversibly. Non-covalent mechanisms are often referred to as narcoses (Russom et al. 1997). Covalent mechanisms are primarily electrophilic in character (Hermens 1990). Quantum chemistry provides an accurate and detailed description of electronic effects (Karelson et al. 1996).

The presence of two oxygen atoms in a lactone produces a relatively hydrophilic compound. This propensity to reside in the aqueous phase limits the toxic potency of lactones. A computation of the Chemical Abstract Service registry numbers, toxicity, hydrophobicity, frontier orbitals energy values and excess toxicity are given in Table 1. While hydrophobicity varies over 3 orders of magnitude, toxicity varies over only 2 orders of magnitude. The orbital energies of the lactones exhibited limited differences.

It is generally agreed that different sized cyclic molecules have different stability. Three and four membered cyclic molecules are considered particularly unstable (Jacobs 1997). This instability is referred to as a ring strain. Ring strain is the result of atomic orbitals not being able to overlap at their optimum angles (Jacobs 1997). Because the orbitals cannot overlap at their preferred angle, bonds are weaker. The net result is that ring-strained molecules are more reactive and, as demonstrated in Table 1, more toxic.

The effect of ring size on toxicity is best quantified by T_e values that are also presented in Table 1. Excess toxicity is associated with the small ring-stressed lactones. The β-lactones exhibit higher T_e values than the γ-lactones. While the δ-lactones exhibit T_e values near 2, the ε-lactones exhibit T_e values near unity. Both δ-lactones and ε-lactones model well as nonpolar narcotics.

While the number of β-, δ-, and ε-lactones precludes development of any subclass-based QSARs, the number and diversity of γ-lactones is sufficient for QSAR evaluation. The following hydrophobic-dependent model was generated for the γ-lactones,

$$\log (IGC_{50}^{-1}) = 0.64 (\log K_{ow}) - 1.36;$$
$$n = 7, r^2 = 0.947, s = 0.22, F = 89, Pr > F = 0.0001 \quad \text{Eq. [2].}$$

As expected, log K_{ow} did not model the toxic potency of all eleven lactones simultaneously ($r^2 = 0.395$).

Table 1. Toxicity and Molecular Descriptor Values for Selected Lactones.

Lactone	CAS Number[a]	log $1/IGC_{50}$	Log K_{ow}[b]	E_{LUMO}[c]	E_{HOMO}[d]	T_e[e]
β-propiolic	57-57-8	-0.13	-1.36	0.913	-11.374	228
β-butyric	36536-46-6	-0.76	-0.84	0.989	-11.292	60.4
γ-butyric	96-48-0	-1.72	-0.64[m]	1.099	-11.239	4.71
α-methyl-γ-butyric	1679-47-6	-1.19	-0.28	1.158	-11.060	3.16
γ-valeric	108-29-2	-1.66	-0.08	1.160	-11.175	2.11
γ-hexanoic	695-06-7	-1.24	0.48	1.162	-11.168	1.95
γ-octanoic	104-50-7	-0.38	1.60	1.161	-11.158	1.95
γ-nonanoic	104-61-0	0.01	2.16	1.161	-11.151	1.78
γ-decanoic	706-14-9	0.49	2.72[m]	1.162	-11.141	2.01
δ-decanoic	705-86-2	-0.08	2.47	1.181	-10.813	0.83
ε-hexanoic	502-44-3	-1.26	0.31	1.226	-10.847	0.93

[a]Chemica Abstract Services registry number
[b]1-octanol/water partition coefficient
[c]energy of the lowest unoccupied molecular orbital
[d]energy of the highest occupied molecular orbital
[e]excess toxicity as compared to baseline nonpolar narcosis
m = measured value

One approach to modeling acute toxicity is the response-surface (Veith and Mekenyan 1993). This approach models toxic potency as a plane determined by hydrophobicity and reactivity.

Toxic reactivity is often quantitated by quantum chemical descriptors especially molecular orbital energies. While the $E_{HOMO} - E_{LUMO}$ gap (i.e., the difference between the frontier energies) is an index of molecular stability, it is not a good predictor of lactone toxicity either separately or in conjunction with log K_{ow} ($r^2 = 0.016$ and 0.397, respectively).

The E_{LUMO} value is related to electron affinity and characterized the susceptibility of a chemicals towards nucleophilic attack. While E_{LUMO} values do not model all eleven lactones simultaneously ($r^2 = 0.002$), when the two orthogonal variables log K_{ow} and E_{LUMO} ($r^2 = 0.403$) are combined, the result is a quality model,

$$\log (IGC_{50}^{-1}) = 0.63 (\log K_{ow}) - 7.29 (E_{LUMO}) + 7.11;$$
$$n = 11, r^2 = 0.884, s = 0.28, F = 30, Pr > F = 0.0001 \quad \text{Eq. [3].}$$

The rationale for E_{LUMO} when combined with log K_{ow} being a quality descriptor of acute toxicity of lactones may reside in the fact that the ring-stressed β-lactones, especially β-propiolactone, are susceptible to nucleophilic attack. However, alkyl substitutions (e.g., β-butyrolactone) sterically hinder such interaction, and this impairment is less than that imposed with the γ-lactones.

In conclusion, the results of this investigation indicate that ring size has an impact of lactone toxicity. Small stressed-ringed lactones are reactive and exhibit toxic potencies in excess of baseline narcosis. Non-stressed-ringed lactones act as simple narcotics. A response plane of hydrophobicity and a gross electrophilic reactivity model lactone toxicity.

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