Significance of Branched Bridge-head Substituent in Toxicity of Bicyclic Phosphate Esters

Morifusa ETO, Yoshihisa OZOE,
Toshio FUJITA*¹ and John E. CASIDA*²

Department of Agricultural Chemistry, Kyushu
University, Fukuoka

*¹Department of Agricultural Chemistry,
Kyoto University, Kyoto

*²Division of Entomology and Parasitology,
University of California, Berkeley,
California 94720, U.S.A.

Received May 31, 1976

2,6,7-Trioxa-1-phosphabicyclo[2.2.2]octane 1-oxides

(R) are highly toxic to mammals, yet they do not act as cholinesterase inhibitors.¹⁾ The 4-ethyl homolog is the toxic principle in the smoke produced on burning noncommercial fire-retarded polyurethane foam based on a trimethylolpropane initiated short chain polyol and a phosphate flame retardant.²⁾ Regression analysis of the structure-toxicity relationship for the bicyclic phosphate esters indicates the remarkable influence of the bridge-head substituent R which should be lipophilic and have a definite size approximating C₈H₇ for the highest toxicity.³⁾ The isopropyl compound is the most toxic to mice of the bicyclic phosphate esters previously tested.³⁾ Bowery and coworkers suggest that these bicyclic phosphate esters antagonize the actions of synaptically released GABA and they find that the isopropyl compound is most active in relevant assays.⁴⁾

This paper describes the high toxicity of the tertiary butyl homolog and the importance of steric features of the bridge-head substituent in conferring maximum potency.

4-*tert*-Butyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2] octane 1-oxide was synthesized by the reaction of the corresponding triol and phosphorus oxychloride in the presence of pyridine. mp > 245°C (sublim. 180°C). Anal. Found: C, 46.86; H, 7.20. Calcd. for C₈H₁₆O₄P: C, 46.60; H, 7.28%. NMR (in CDCl₃) δ: 0.95 [9H, singlet, C(CH₃)₃], 4.61 (6H, doublet, CH₂OP). The triol, 2-*tert*-butyl-2-hydroxymethyl-1, 3-propanediol, was prepared from diethyl malonate by condensation with acetone to diethyl isopropylidenemalonate, followed by methylation with methylmagnesium iodide, hydroxymethylation with paraformaldehyde, and finally

reduction of the ester groups with lithium aluminum hydride. A similar method was used to prepare the other new bicyclic phosphate esters. More detailed procedures for the syntheses and chemical properties of other compounds will be described elsewhere. The toxicity was determined by intraperitoneal injection into male Swiss Webster mice (18 to 20 g), with LD₅₀ determinations being made after 24 hr.

When the toxicity (expressed as log 1/LD₅₀) of the bicyclic phosphate is plotted against the size of the R-substituent expressed as the van der Waals volume,⁵⁾ the normal alkyl compounds give a parabola with a maximum toxicity for the propyl group.⁶⁾ However, the branched alkyl derivatives show unexpectedly high toxicity. The isopropyl, isobutyl, *sec*-butyl, and *tert*-butyl compounds are, respectively, 2, 6, 7, and 28 times more toxic than the corresponding normal alkyl compounds, indicating that the van der Waals volume is not sufficient as a steric parameter for branched groups. The *tert*-butyl homolog is the most potent (LD₅₀=0.053 mg/kg), being about 3.4-fold more toxic than the isopropyl compound.

When the toxicity data are plotted against the hydrophobicity parameter $\pi^{6,7)}$ of the R-substituent, all of the straight chain groups including halomethyls give a parabola. The only exception is the methoxymethyl group. However, all of the branched groups are scattered far above the parabola. By introducing appropriate steric parameters into the regression analysis, good correlations are obtained between the observed and calculated toxicity values, provided that the aromatic value of π is applied for the nitro group (Eq. 1~3). The Hancock's steric parameter $E_s^{8,9)}$ corrected for the hyperconjugation effect gives a little better correlation than the Taft's $E_s^{10,11)}$ does (Eq. 1 and 2; Table I). An equally good correlation covering all of the compounds is obtained by replacing these parameters by a very simple term, the number of substituents S at the α - or β -atom of the R-substituent (Eq. 3; Table I; Fig. 1).

$n \quad r$

$$\log 1/\text{LD}_{50} = 2.027\pi - 0.888\pi^2 - 1.203E_s - 1.466 \quad 18 \quad 0.919 \quad (1)$$

$$\log 1/\text{LD}_{50} = 2.060\pi - 0.878\pi^2 - 0.829E_s^c - 1.690 \quad 18 \quad 0.947 \quad (2)$$

$$\log 1/\text{LD}_{50} = 2.051\pi - 0.863\pi^2 + 0.953S - 2.206 \quad 18 \quad 0.945 \quad (3)$$

The application of the aliphatic π value for the nitro group gives a remarkably poor correlation; the correlation coefficients r drop to 0.742, 0.790, and 0.792, respectively, for π - E_s , π - E_s^c , and π - S combinations. The nitro group of the bicyclic phosphate ester appears to have a π -value near the aromatic value (-0.28) rather than the aliphatic value (-0.85). This may be attributable to the lack of an α -hydrogen atom which might give a high hydrophilic character to aliphatic nitro compounds by dissociation.

TABLE I. REGRESSION ANALYSIS OF STRUCTURE-TOXICITY RELATIONSHIP FOR BICYCLIC PHOSPHATE ESTERS $O=P(OCH_2)_2C-R$

	$\pi^a)$	$E_s^{c,b)}$	S	Log 1/LD ₅₀		
				Obs.	Calcd.	
					Eq. 2	Eq. 3
CH ₃	0.5	0.00	0	-1.51 ^{e)}	-0.880	-1.397
C ₂ H ₅	1.0	-0.38	1	0.00 ^{e)}	-0.193	-0.065
<i>n</i> -C ₃ H ₇	1.5	-0.67	1	0.42 ^{e)}	-0.021	-0.119
CH(CH ₃) ₂	1.3	-1.08	2	0.74 ^{e)}	0.399	0.908
<i>n</i> -C ₄ H ₉	2.0	-0.70	1	-0.18 ^{e)}	-0.502	-0.603
CH(CH ₃)C ₂ H ₅	1.8	-1.74	2	0.68	0.615	0.596
CH ₂ CH(CH ₃) ₂	1.8	-1.24	2	0.62	0.201	0.596
C(CH ₃) ₃	1.6	-2.46	3	1.28	1.397	1.726
<i>n</i> -C ₅ H ₁₁	2.5	-0.71	1	-1.57 ^{e)}	-1.439	-1.520
<i>n</i> -C ₆ H ₁₃	3.0	-0.88 ^{d)}	1	-2.70 ^{e)}	-2.682	-2.867
C ₆ H ₅	2.13	-2.09 ^{e)}	2	-0.18 ^{e)}	0.448	0.154
CH ₂ Cl	0.89	-0.55	1	-0.45	-0.096	-0.112
CH ₂ Br	1.10	-0.58	1	-0.16	-0.005	-0.041
CH ₂ OCH ₃	0.02	-0.50	1	-0.98	-1.234	-1.212
COCH ₃	-0.71	-1.80 ^{e)}	2	-1.71 ^{e)}	-2.104	-2.191
CH(OH)CH ₃	-0.36	-1.19 ^{d)}	2	-2.05	-1.559	-1.150
N(CH ₃) ₂	-0.30	-2.31 ^{e)}	2	-0.48 ^{e)}	-0.472	-0.993
NO ₂	-0.28 ^{f)}	-1.50 ^{e)}	2	-0.98 ^{e)}	-1.092	-0.942

^{a)} Calcd. according to Ref. 7) for aliphatic values except NO₂.

^{b)} Obtained from Refs. 8 and 9).

^{c)} Data from Ref. 3).

^{d)} Calcd. according to Ref. 8).

^{e)} Calcd. according to Ref. 9) from E_s which was calculated according to Ref. 11) from an average of van der Waals radii.

^{f)} Aromatic value.

FIG. 1. Correlation of Mouse Toxicity (log 1/LD₅₀) with Hydrophobicity and Steric Factors of the R-Substituent in $O=P(OCH_2)_2C-R$.

The introduction of various electronic parameters does not improve the correlation, *i.e.* an equation combining the Swain's field constant $\mathcal{F}^{12,13}$ and π gives a poor correlation ($r=0.58$).

These regression analyses of the structure-toxicity relationship for the bicyclic phosphate esters indicate the importance of the steric role of R as well as its hydrophobic character for high toxicity. The greater the bulkiness or branching of the bridge-head substituent, provided its hydrophobicity parameter π is near the optimum value of about 1.2, the higher the toxicity of the bicyclic phosphate ester. This bridge-head substituent may interact sterically with a critical synaptic site⁴⁾ which is hydrophobic in nature. Electronic reactions such as phosphorylation and alkylation are probably not involved in the primary action of the bicyclic phosphate esters.

We thank J. L. Engel for skilled technical assistance. This study was supported in part by United States National Institutes of Health Grant 2 P01 ES00049.

REFERENCES

- 1) E. M. Bellet and J. E. Casida, *Science*, **182**, 1135 (1973).
 - 2) J. H. Petajan, K. J. Voorhees, S. C. Packham, R. C. Baldwin, I. N. Einhorn, M. L. Grunnet, B. G. Dinger and M. M. Birky, *ibid.*, **187**, 742 (1975).
 - 3) J. E. Casida, M. Eto, A. D. Moscioni, J. L. Engel, D. S. Milbrath, and J. G. Verkade, *Toxicol. Appl. Pharmacol.*, **36**, 261 (1976).
 - 4) N. G. Bowery, J. F. Collins, R. G. Hill and S. Pearson, Abstract of Papers, British Pharmacological Society Meeting, 1976, p. 19.
 - 5) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).
 - 6) T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
 - 7) A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
 - 8) T. Fujita, C. Takayama and M. Nakajima, *J. Org. Chem.*, **38**, 1623 (1973).
 - 9) C. K. Hancock, E. A. Meyers and B. J. Yager, *J. Am. Chem. Soc.*, **83**, 4211 (1961).
 - 10) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," ed. by M. S. Newman, John Wiley and Sons, Inc., New York, N. Y., 1956, p. 556.
 - 11) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).
 - 12) C. G. Swain and E. C. Lupton, Jr., *J. Am. Chem. Soc.*, **90**, 4328 (1968).
 - 13) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
-