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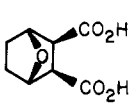
Endothal and Cantharidin Analogues: Relation of Structure to Herbicidal Activity and Mammalian Toxicity

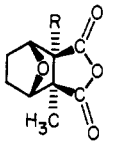
Masafumi Matsuzawa,[1] Michael J. Graziano,[2] and John E. Casida*

Analogues of the herbicide endothal (*exo,exo*-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) with various ring substituents and including several geometrical isomers were synthesized via Diels–Alder reactions. They were assayed as inhibitors of root elongation in barnyard grass and wild mustard and for acute toxicity to mice on intraperitoneal administration. Studies with 43 analogues demonstrate the importance for herbicidal activity of the spatial arrangement of the bridged oxygen and the exo,exo positions of the two carboxylic acid groups. The exo,exo positions facilitate intramolecular hydrogen bonding and formation of stable metal ion complexes. The herbicidal activity of endothal is reduced on adding ring substituents due to steric hindrance around the bridged oxygen. Mammalian toxicity generally follows the same pattern except that 2,3-dimethyl substitution to form cantharidin increases activity. Somewhat similar structure–activity relationships for herbicidal activity and toxicity to mice suggest the possibility of a related oxabicycloheptane target site in plants and mammals.

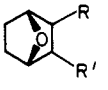
Endothal is an important herbicide, desiccant, and defoliant that is also toxic to mammals (Tischler et al., 1951; Simsiman et al., 1976; Keckemet, 1980). It is similar in



endothal (1)



cantharidin (2, R = CH_3)
palasonin (R = H)



2,3-disubstituted
7-oxabicyclo[2.2.1]heptane

structure to the natural products cantharidin (2,3-dimethylendothal anhydride), an extremely toxic vesicant and counterirritant produced by blister beetles (Sollman, 1949; Cavill and Clark, 1971), and palasonin (2-methylendothal anhydride), an anthelmintic isolated from the seeds of the tree *Butea frondosa* (Raj and Kurup, 1967; Bochis and Fisher, 1968). The common structural features of these highly bioactive compounds are the 7-oxabicyclo[2.2.1]-heptane system with *exo,exo*-2,3-dicarboxylic acid or -2,3-dicarboxylic anhydride substituents. Several related dicarboxylic acids, some with the oxabicycloheptane system, also have herbicidal activity (Koch, 1970).

The relative toxicities of **1**, **2**, and related compounds to plants and mammals may be dependent on the oxabicycloheptane system and specific ring substituents. This study therefore examines the structure–activity relationships of various endothal analogues for inhibition of root growth in barnyard grass and wild mustard and for acute toxicity to mice.

MATERIALS AND METHODS

Syntheses. *General Procedures and Intermediates.* The endothal and cantharidin analogues were usually prepared by Diels–Alder reactions of appropriate dienes and dienophiles followed by reduction and hydrolysis (Figure 1). No attempt was made to resolve optical isomers formed from certain reactions. In general, the furans in dry ether (10–20 volumes) were stirred at room temperature for 24 h with equimolar maleic anhydride or other dienophile alone or in the presence of a catalytic amount of boron trifluoride etherate (for 3-furancarboxylic acid

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.
[1]Present address: K-I Chemical Research Institute Co., Ltd., Fukude-cho, Iwata-gun, Shizuoka, 437-12, Japan.
[2]Present address: Department of Pathology and Experimental Toxicology, Warner-Lambert Pharmaceutical Research Division, Ann Arbor, MI 48105.

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Table I. Effect of Ring Substituents on the Herbicidal Activity and Mouse Toxicity of *exo,exo*-7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acids and Anhydrides and Geometrical Isomers

no.	derivative	mp, °C (lit.[a])	root growth IC_{50},[b] ppm		mouse LD_{50},[c] mg/kg
			barnyard grass	wild mustard	
		Unsubstituted			
1	endothal	141–143 (122–123)	0.8	2.1	14 ± 2
3	endothal anhydride	108–110 (116–117)	0.8	4.3	4.0 ± 1.4
		1- and 1,4-Substituents			
4	1-methyl	177–178 (158)	135	66	>400
5	1-ethyl	167–168	1250	935	>400
6	1,4-dimethyl	115–116	5000	1300	>400
		2- and 2,3-Substituents			
7	2-bromo	168–170	8.3	17	72 ± 15
8	2,3-dimethyl disodium salt	>290	9.5	3.3	1.8 ± 0.3
2	2,3-dimethyl anhydride (cantharidin)	210–211 (210–211)	6.2	6.2	1.0 ± 0.4
9	2,3-trimethylene anhydride	130–131	12	4.0	5.0
		endo-5-Substituents			
10	methyl	186–187	1.8	18	35 ± 7
11	butyl	147–148	22	20	
12	hydroxymethyl	135–137	22	50	135 ± 12
13	methoxymethyl	114–116	15	92	
14	carboxy	163–165	11	15	50 ± 8
15	phenyl	138–140	49	395	182 ± 32
		5,6-Substituents			
16	*endo,endo*-5,6-dimethyl	159–160	100	175	>400
17	*exo*-5,6-epoxy	199–201 (198–205)	90	79	>400
18	*exo,exo*-5,6-dihydroxy	202–204 (199–200)	1600	820	>400
19	*exo,exo*-5,6-methylenedioxy	229–230	1500	800	>400
20	*endo,exo*-5,6-dibromo	162–164 (168)	2900	3000	>400
21	*endo,exo*-5,6-dichloro	160–162 (163)	2300	2200	>400
22	*exo,exo*-5,6-dibromo	>270	6400	3500	>400
23	5,6-phenylene anhydride	214–215	1200	1100	165 ± 16
24	2,3-dimethyl-5,6-phenylene anhydride	175–178 (180–181)	1400	790	>400
		Geometrical Isomers of Endothal, 1,4-Dimethylendothal, and Cantharidin			
25	*endo,exo*-endothal	173–174 (179–180)	>2000	>2000	>400
26	*endo,endo*-endothal	160–161 (169–170)	305	245	>400
27	*endo,endo*-1,4-dimethylendothal	221–221.5 (202–203)	127	290	>400
28	*endo*-2,3-dimethyl anhydride	193–194 (193–194)	140	153	>400

[a] References for synthesis and melting points of known compounds are as follows: Alder and Backendorf (1938a), **1**, **3**, **4**, **6**, **25–27**; Dauben et al. (1980, 1985), **2**, **28**; Wang (1980) and Yur'ev and Zefirov (1961), **17**; Daniels and Fisher (1963), **18**; Jolivet (1960), **20**, **21**; McCormick and Shinmyozu (1982), **24**; Kwart and Burchuk (1952), **25**. Compound **9** was provided by W. G. Dauben. [b] Mean of two experiments differing by 1.7-fold on an average basis. [c] Confidence intervals (95%).

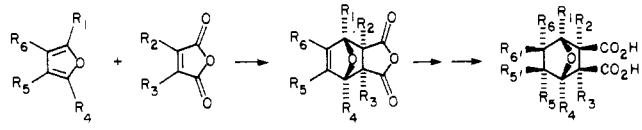


Figure 1. Synthesis of *exo,exo*-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid derivatives.

and cyclopentene-1,3-dione). Filtration gave crystals of the exo adducts that were reduced over 10% palladium on carbon (12% by weight) in dry dimethoxyethane under 1 atm of hydrogen. After filtration to remove the catalyst and evaporation of the filtrate in vacuo, the residues were refluxed in water overnight. The *exo,exo*-7-oxabicyclo-[2.2.1]heptane-2,3-dicarboxylic acids crystallized at 5 °C.

The oxabicycloheptane examples synthesized are listed in Tables I and II. References to known compounds are provided in the footnotes of these tables. New analogues were synthesized by minor variations in the reported procedures, i.e. Furdick and Drabek (1965) for **22**, McCormick and Shinmyozu (1982) for **23**, Tamura et al. (1977) for **33**, Eggelte et al. (1978) for **34**, Kotsuki et al. (1979) for **37**, and Rice et al. (1953) for **43**. The furan starting materials were commercially available or were synthesized by known methods, i.e. 3-methylfuran (Kutney et al., 1971), 3,4-dimethylfuran (Rawson et al., 1979), and 3-phenyl- and 3-*n*-butylfurans (Liotta et al., 1983). Typical syntheses are given below for four compounds.

endo-5-Methylendothal (***10***). To a solution of 3-methylfuran (3.8 g, 46.3 mmol) in anhydrous ether (40 mL) was added powdered maleic anhydride (4.5 g, 46 mmol). The flask was stoppered and the mixture stirred for 30 min until a clear solution was obtained. After being allowed to stand overnight at room temperature, the flask was unstoppered and stirring resumed until crystals appeared. The white suspension was refrigerated (24 h) and the solid isolated by filtration in 81% yield. The isolated adduct (6.5 g, 36 mmol) in anhydrous dimethoxyethane (50 mL) was stirred over 10% palladium on carbon (0.8 g) under 1 atm of hydrogen for 12 h. The solid recovered from filtration and evaporation was refluxed in water (10 mL) overnight. Compound **10** crystallized at 5 °C; 4.8 g (66%).

exo,exo-5,6-(Methylenedioxy)endothal (***19***). A suspension of **18** (Daniels and Fischer, 1963) (1.0 g, 4.6 mmol) in aqueous formaldehyde (2 mL, 37%) and concentrated hydrochloric acid (1.25 g) was heated until a clear solution was obtained (70 °C, 2 h). Crystals of **19** appeared on standing (2 h). They were filtered after cooling to 0 °C and washed with cold water; 1.0 g (95%).

5,6-Dehydroendothal (***30***). A suspension of *exo,exo*-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid anhydride (Diels and Alder, 1929) (2.4 g, 14.4 mmol) in aqueous hydrogen peroxide (3 mL, 30%) was stirred at 0 °C overnight. The clear solution obtained was evaporated under a stream

Table II. Effect of Other Substituents on the Herbicidal Activity and Mouse Toxicity of exo,exo-2,3-Disubstituted 7-Oxabicyclo[2.2.1]heptanes and Related Compounds

no.	derivative	mp, °C (lit.[a])	root growth IC_{50},[b] ppm		mouse LD_{50},[b] mg/kg
			barnyard grass	wild mustard	
		Dehydroendothal Isomers			
29	2,3-dehydro	163–165 (163)	>2000	>2000	>400
30	5,6-dehydro	141–142	1.6	75	55 ± 15
		Monocarboxy Derivatives			
31	*exo*-2-COOH	75–75.5 (76–77)	800	84	350 ± 38
32	*endo*-2-COOH	77–78 (76–77)	740	326	>400
		exo,exo-2,3-Cyclic Substituents			
33	2,3-C(O)SC(O)	78–80	1.4	5.6	0.31 ± 0.17
34	2,3-C(O)OCH_2	97–98	106	550	194 ± 49
35	2,3-C(O)CH_2C(O)	207–210	114	135	>400
		Replacements for 7-Oxa Substituent of 2,3-Dicarboxylic Acids			
36	*endo,endo*-7-CH_2	161–162 (160–161)	1750	190	>400
37	*exo,exo*-7-S	130–132	>200	>200	>400
		Endothal Esters, Amide, and Imides			
38	2-CO_2H, 3-CO_2Et	103–104 (108)	36	650	110 ± 11
39	2-CO_2Et, 3-CO_2Et	n_D^{20} 1.468 (47)	8.0	47	50 ± 17
40	2-CO_2H, 3-CONH(Ph-Cl-4)	188–190 (190)	21	105	16 ± 5
41	2,3-C(O)NHC(O)	184–186 (185)	159	315	>400
42	2,3-C(O)NEtC(O)	162–165 (165–166)	150	330	>400
43	2,3-C(O)N(Ph-Cl-4)C(O)	185–186	112	230	100 ± 27

[a] References for synthesis and melting points are as follows: Alder and Backendorf (1938a,b), **29**, **36**; Nelson and Allen (1972), **31**, **32**; Jolivet (1960), **38**, **39**; Joshi et al. (1983), **40**; Grogan and Rice (1963), **41**, **42**. The 5,6-dehydro-2,3-dicarboxylic anhydride precursor for compound **37** was supplied by H. Kotsuki. [b] See Table I.

of nitrogen to half of the original volume to give **30** [1.4 g (53%)], which was isolated by filtration. The expected product (**17**) was not formed.

Endothal (***33***). Anhydride **3** (2.0 g, 11.9 mmol) and sodium sulfide nonahydrate (4.0 g, 16.7 mmol) were thoroughly mixed with occasional grinding in a mortar at room temperature for 1 h. The pasty greenish brown mixture was slowly added to ice-cooled 20% hydrochloric acid (25 mL), which was extracted with chloroform. The soluble products were chromatographed on a silica gel column with *n*-hexane–ether (1:1) and then recrystallized from *n*-hexane–ether; 0.48 g (22%). Application of this procedure to **2** did not yield cantharidin thioanhydride.

NMR Structural Assignments. The structure of each compound was confirmed by its ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra recorded at 300 and 75 MHz, respectively, with a Bruker WM-300 wide-bore spectrometer. Data for key compounds as solutions in dimethyl-d_6 sulfoxide (Me_2SO-d_6) are given in Table III. Geometrical assignments are based on vicinal ^1H–^1H coupling constants ($^3J_{HH}$) compared with those established in the bicyclo[2.2.1]heptane system, e.g. $^3J_{1,2\text{-endo}}$ = 0–2 Hz and $^3J_{1,2\text{-exo}}$ = 3–4 Hz (Jackman and Sternhill, 1969; Nelson and Allen, 1972). These assignments are supported by the γ-steric shielding effect (Marchand, 1982) on the γ-carbon chemical shift introduced by an endo substituent relative to that by an exo substituent. For example, C-5 in **25** and C-5 (C-6) in **26** are shielded by about 3 ppm relative to the *exo,exo*-dicarboxylate, endothal. Similarly, the carbon atoms γ to an endo substituent (C-3 in **10–15**, C_2 and C_3 in **16**, C_2 in **20**) are relatively shielded compared to **1**. The exo geometry of the 5,6-methylenedioxy group in **19** is established by the strong nuclear Overhauser effect (NOE) due to endo,endo dipolar interaction observed between H-2,3 and H-5,6 protons in an NOE difference spectrum.

Physicochemical Parameters. Hydrophobicity was estimated as $R_m = \log (1/R_f - 1)$ (Boyce and Milborrow, 1965) by reversed-phase thin-layer chromatography on Analtech F chromatoplates (0.25 mm) developed with water–methanol (9:1). Ionization constants (pK_{a_1} and pK_{a_2}) were measured by potentiometric titration and calculated by Noyes method (Albert and Serjeant, 1962). Stability constants of the 1:1 cupric ion complexes were determined by potentiometric titration and calculated by Bjerrum's method (Albert and Serjeant, 1962). Additional stability constants of 1:1 endothal complexes with various metal ions are given by Li et al. (1983). van der Waals volumes (V_{ws}) are taken from Moriguchi et al. (1976) and Moriguchi and Kanada (1977).

Bioassays. *Inhibition of Root Growth.* Herbicidal activity was assayed by the seed-pack growth pouch method (Russo, 1980). The herbicide solution (15 mL) was neutralized with 4% sodium bicarbonate followed by addition of 0.2 M pH 7.4 phosphate buffer (0.1 mL) and introduction into the pouch. The growth pouches were sterilized at 120–130 °C for 20 min and rapidly cooled (5 °C). The pouches were placed in the upright position, and seeds (15–18 for each pouch) of wild mustard (*Brassica kaber*) and barnyard grass (*Echinochloa crusgalli*) were planted in the trough formed by the paper wick. The growth pouches containing the seeds were kept in the dark for 3 days at 23 °C at which time the root lengths were measured (average 5.4 and 4.9 cm for wild mustard and barnyard grass, respectively). The parts per million (ppm) values of test compound required for 50% inhibition of root elongation (IC_{50}) were estimated from the average values of two experiments with a 4-fold dilution series.

Mouse Toxicity Assays. LD_{50} values were determined 72 h after intraperitoneal administration of the compounds to male albino Swiss–Webster mice (18–22 g) with saline or methoxytriglycol as the carrier vehicle. Four to six mice were used at each concentration in determining the reported LD_{50} values.

RESULTS

Structure–Herbicidal Activity. *Effect of Ring Substituents on exo,exo-7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acids and Anhydrides and Geometric Isomers* (*1–28*) (*Table I*). The herbicidal activity of endothal anhydride (**3**) is essentially the same as that of endothal

Table III. ^{13}C and ^{1}H NMR Spectral Characteristics of Key Compounds (δ)[a]

		^{13}C NMR								
no.	derivative	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	Me (other)
					Endothal Isomers					
1	exo,exo	77.8	51.6	51.6	77.8	28.7	28.7	172.5	172.5	
25	endo,exo	76.9	51.3	50.5	79.8	25.2	29.0	172.2	173.6	
26	endo,endo	78.0	48.4	48.4	78.0	25.7	25.7	171.9	171.9	
					Endo-5-Substituents					
10	methyl	78.7	52.0	45.2	81.7	36.8	34.6	172.6	172.3	15.1
12	hydroxymethyl	79.3	52.8	46.7	81.1	44.0	33.1	173.1	173.3	62.0 (hydroxymethyl)
14	carboxy	79.9	52.4	47.0	80.4	48.4	32.7	173.2	173.2	174.1 (carboxy)
15	phenyl	79.4	51.9	45.8	81.7	46.4	32.4	172.4	172.4	126.5, 128.0, 128.6, 136.7 (phenyl)
					5,6-Substituents					
16	*endo,endo*-dimethyl	82.8	45.1	45.1	82.8	35.5	35.5	172.8	172.8	10.0
17	*exo*-epoxy	76.0	48.6	48.6	76.0	48.6	48.6	171.7	171.7	
19	*exo,exo*-methylenedioxy	81.4	45.7	45.7	81.4	80.6	80.6	171.7	171.7	96.4 (methylenedioxy)
20	*endo,exo*-dibromo	82.1	45.9	49.1	86.2	54.7	54.0	170.5	171.3	

		^{1}H NMR										
no.	derivative	H_1	H_2	H_3	H_4	H_5	$H_{5'}$	H_6	$H_{6'}$	H_7	H_8	Me (other)
						Endothal Isomers						
1	exo,exo	4.78 (d)	3.04 (s)		4.78 (d)	0.87		1.68 (m)		10.5 (br)	10.5 (br)	
25	endo,exo	4.83 (d)	3.00 (d)	3.43 (t)	4.77 (dt)	1.56		1.78 (m)		8.90 (br)	8.90 (br)	
26	endo,endo	4.66 (m)	3.21 (m)		4.66 (m)	1.64		2.10 (m)		NA	NA	
						Endo-5-Substituents						
10	methyl	4.71 (d)	3.16 (AB q)		4.58 (d)	2.17 (m)		2.00 (dt)	1.03 (dd)	8.0 (br)	8.0 (br)	1.09 (d)
12	hydroxymethyl	4.70 (d)	3.10 (AB q)		4.67 (d)	2.26 (m)		1.80 (dt)	1.10 (dd)	NA	NA	3.70 (dd), 3.47 (t) (hydroxymethyl)
14	carboxy	4.88 (d)	3.05 (AB q)		4.77 (d)	3.00 (m)		1.92 (dt)	1.78 (dd)	NA	NA	4.25 (br) (carboxy)
15	phenyl	4.80 (d)	2.85 (AB q)		4.78 (d)	2.49 (m)		2.04 (dt)	1.82 (dd)	NA	NA	7.21–7.36 (m) (phenyl)
						5,6-Substituents						
16	*endo,endo*-dimethyl	4.60 (m)	3.22 (s)	3.22 (s)	4.60 (m)	2.23 (m)		2.23 (m)		NA	NA	0.92 (d)
17	*exo*-epoxy	4.58 (s)	2.91 (s)	2.91 (s)	4.50 (s)	3.45 (s)			3.45 (s)	10.11 (br)	10.11 (br)	
19	*exo,exo*-methylenedioxy	4.56 (s)	2.85 (s)	2.85 (s)	4.56 (s)	4.28 (s)			4.28 (s)	10.0 (br)	10.0 (br)	4.94 (s), 4.73 (s) (methylenedioxy)
20	*endo,exo*-dibromo		3.38 (AB q)		4.73 (s)	4.41 (d)		4.43 (t)		3.3 (br)	9.9 (br)	

[a] Spectra measured in Me_2SO-d_6 with tetramethylsilane as the internal standard. Abbreviations are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; q, quartet; m, multiplet; br, broad; NA, not applicable.

(1). Alkyl substitution at the 1- and 1,4-positions greatly reduces the herbicidal activity in the sequence none (1) > 1-methyl (4) > 1-ethyl (5) > 1,4-dimethyl (6). However, in contrast to the unsubstituted compounds (1, 3), the 1-substituted and 1,4-disubstituted analogues are more active against wild mustard than barnyard grass. Substitution at the 2- and 2,3-positions (2, 7–9) generally reduces herbicidal activity but not nearly to the same extent as 1-substitution and 1,4-disubstitutions. Endo-5-substitution (10–15) and 5,6-disubstitution (16–24) also weakens the activity, although in the latter case to a much greater degree. The endo,endo isomers (26, 28) of endothal and cantharidin are of very low activity, and the trans isomer (25) of endothal is not active. In contrast, *endo,endo*-1,4-dimethylendothal (27) is more active than its exo,exo isomer (6).

Effect of Other Substituents on exo,exo-2,3-Disubstituted 7-Oxabicyclo[2.2.1]heptanes and Related Compounds (29–43) (Table II). The 2,3-dehydro analogue (29) is inactive, but the 5,6-dehydro compound (30) shows strong activity, particularly for barnyard grass. *exo*-Monocarboxylic acid 31 and endo isomer 32 are weakly active for wild mustard. Endothal thioanhydride (33) shows high activity similar to endothal anhydride (3), but the lactone (34) and 2,3-dione (35) exhibit only moderate activities. Modification of the bridged oxygen to the methylene (36) and sulfide (37) derivatives also weakens the potencies. Diethyl ester 39 is more potent than ethyl ester 38, amide 40, and imides 41–43, and in each case barnyard grass is more sensitive than wild mustard.

Structure–Mouse Toxicity (Tables I and II). Endothal anhydride (3) is more toxic than the corresponding dicarboxylic acid (1). 2,3-Dimethyl or 2,3-trimethylene substitution (2, 8, 9) increases the toxicity relative to endothal, whereas substitution at the 1- (4, 5), 1,4- (6), and 5,6- (16–24) positions essentially eliminates all activity. Substitution at the 2- (7) or endo-5-positions (10, 12, 14, 15) also reduces the toxicity, but to a lesser extent. The geometric isomer series (25–28) is not toxic. The 5,6-dehydro analogue (30) is moderately toxic whereas the 2,3-dehydro analogue (29), the monocarboxylic acids (31, 32), the 2,3-lactone (34), and the 2,3-dione (35), 7-methylene (36), and 7-sulfide (37) derivatives are all of little or no activity. Surprisingly, the new compound endothal thioanhydride (33) is the most toxic of the analogues assayed. Esterification (38, 39) and imide formation (41–43) reduce the toxicity, but amidation (40) does not change the activity relative to endothal.

Quantitative Structure–Activity Relationships (QSAR) (Table IV). Structure–activity relationships of 22 or 23 *exo,exo*-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acids and related compounds were analyzed by the multiple-regression technique using physicochemical substituent parameters for the V_{ws} of ring substituents and R_m values for hydrophobicity. $\sum V_w(\alpha)$ and $\sum V_w(\beta)$ are, respectively, the summations of the V_{ws} for exo and endo substituents, counting the groups at R_1 and R_4 as exo substituents.

The potency for inhibiting root growth of barnyard grass shows a good correlation with $\sum V_w(\alpha)$ and $\sum V_w(\beta)$ (eq 1; Figure 2). In eq 1, n is the number of data points used

$$\begin{aligned} &pIC_{50}(\text{barnyard grass}) = \\ &\underset{(\pm 0.012)}{-0.101\sum V_w(\alpha)} \underset{(\pm 0.007)}{- 0.012\sum V_w(\beta)} \underset{(\pm 0.33)}{+ 2.16} \qquad (1) \\ &n = 22,\ s = 0.572,\ r = 0.801,\ F_{2,19} = 38.2 \end{aligned}$$

in the correlation, s is the standard deviation, r is the correlation coefficient, F_{V_1,V_2} is the F value of the correlation where $V_1 = m$ and $V_2 = n - m - 1$, m is the number of independent variables, and figures in parentheses are the 95% confidence intervals of the corresponding coefficients. R_m values as hydrophobicity gave no significant correlation or improved correlation with barnyard grass.

Wild mustard gives a moderate correlation by adding the R_m term to $\sum V_w(\alpha)$ and $\sum V_w(\beta)$ (eq 2). Mouse tox-

$$\begin{aligned} &pIC_{50}(\text{wild mustard}) = \\ &\underset{(\pm 0.013)}{-0.075\sum V_w(\alpha)} \underset{(\pm 0.007)}{- 0.015\sum V_w(\beta)} \underset{(\pm 0.247)}{+ 0.479R_m} \underset{(\pm 0.366)}{+ 1.586} \qquad (2) \\ &n = 23,\ s = 0.577,\ r = 0.667,\ F_{3,18} = 12.0 \end{aligned}$$

Table IV. Herbicidal Activity and Physicochemical Parameters for *exo,exo*-7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acids

	pIC_{50}			$\sum V_w$[a]	
no.	barnyard grass	wild mustard	R_m	α	β
1	2.37	1.95	−0.14	5.2	5.2
4	0.17	0.48	0.37	20.6	5.2
5	−0.77	−0.64	0.12	36	5.2
6	−1.37	−0.78	0.63	36	5.2
7	1.50	1.19	0.37	5.2	21.5
8	1.43	1.89	1.12	5.2	36
9[b]	1.24	1.72	1.12	5.2	31
10	2.04	1.05	0.14	5.2	20.6
11	1.04	1.08	0.69	5.2	50
12	0.93	0.64	−0.69	5.2	46
13	1.19	0.40	−0.32	5.2	61.4
14	1.32	1.18	−0.58	5.2	35
15	0.73	−0.18	0.12	5.2	74.6
16	0.33	−0.09	0.27	5.2	36
17	0.35[c]	0.40	−0.69	3.5	5.2
18	−0.86	−0.58	−1.12	18.8	5.2
19	−0.81	−0.54	−0.69	21.8	5.2
20	−0.93	−0.94	0.41	21.5	21.5
21	−0.95	−0.94	0.25	18	18
22	−1.27	−1.01	0.29	38	5.2
23[b]	−0.74	−0.71	0.43	24.5	24.5
24[b]	−0.76	−0.51	1.00	24.5	55.3
30	2.06	0.39	−0.48	3.9	3.9

[a] Summation of V_{ws}: α = R_1, R_4, and exo substituents ($R_{5'}$, $R_{6'}$); β = endo substituents (R_2, R_3, R_5, R_6). See Tables I and II for structures. [b] Anhydride. [c] Excluded from quantitative structure–activity analysis.

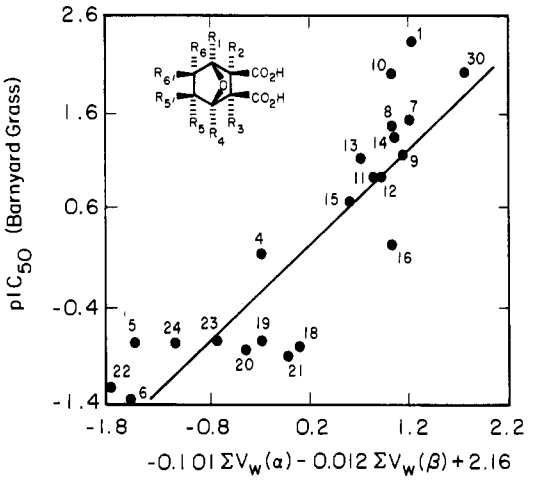


Figure 2. Relationship between van der Waals volumes of ring substituents of *exo,exo*-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acids and anhydrides and potency for inhibiting root growth of barnyard grass. $\sum V_w(\alpha)$ and $\sum V_w(\beta)$ are the sums of van der Waals volumes of R_1, R_4, $R_{5'}$, and $R_{6'}$ and R_2, R_3, R_5, and R_6, respectively. pIC_{50} is the negative logarithm of the millimolar concentration for 50% inhibition. Least-squares correlation coefficient r = 0.801 for the compounds in Table IV excluding 17.

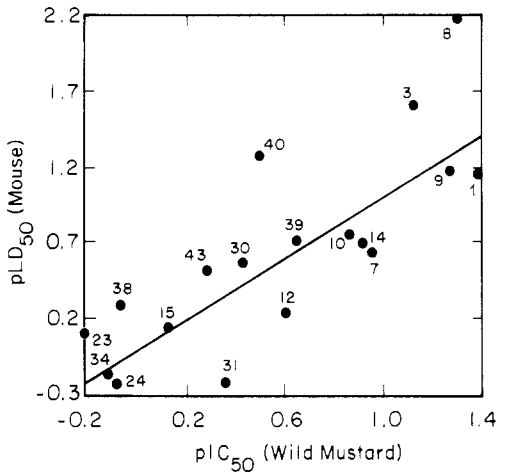


Figure 3. Relationship between potency of 7-oxabicyclo-[2.2.1]heptane-2,3-dicarboxylic acids and related compounds for inhibiting root growth of wild mustard and intraperitoneal toxicity to mice. pIC_{50} as in Figure 2. pLD_{50} is the negative logarithm of the millimole/kilogram dose for 50% mortality. $r = 0.634$ ($n = 18$) based on compounds with actual values for both IC_{50} and LD_{50} in Tables I and II except **2** and **33**.

Table V. Ionization and Stability Constants of 1:1 Cupric Ion Complexes of Endothal Analogues[a]

struct	pK_{a_1}	pK_{a_2}	log $K_{Cu^{2+}}$
1	4.02 ± 0.02	5.86 ± 0.02	5.81 ± 0.02
25	2.90 ± 0.04	4.31 ± 0.06	2.93 ± 0.20
26	3.04 ± 0.02	5.84 ± 0.03	4.17 ± 0.06
31	3.94 ± 0.04		2.96 ± 0.06

[a] For related studies see Li et al. (1983).

icity is not satisfactorily correlated directly with these substituent parameters. On the other hand, a moderate correlation is evident for 18 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acids and related compounds between inhibition of root growth of wild mustard and toxicity to mice (Figure 3).

Ionization and Stability Constants of 1:1 Cupric Ion Complexes of Endothal Analogues (Table V). Ionization constants and stability constants for the cupric complexes were determined to evaluate factors involved in stabilizing the endothal–metal complex. Endothal (**1**) gives the highest pK_a and log $K_{Cu^{2+}}$ values. The endo,endo isomer **26** gives a high pK_{a_2} value, but pK_{a_1} and log $K_{Cu^{2+}}$ are low relative to endothal. Stabilization of the hydrogen bond and metal complex is achieved by rigid *cis*-dicarboxylic acid groups on the same side as the bridged oxygen atom.

Hydrolytic Stability (Figure 4). Endothal anhydride (**3**) in aqueous Me_2SO-d_6 is hydrolyzed with a half-life of ~0.2 h at pD 4.5 and ~7 h in D_2O at 40 °C. Endothal thioanhydride (**33**) is much more stable with a half-life of ~40 h at pD 4.5. The 2,3-dimethyl (**2**) and imide (**41**) analogues have greatly enhanced stability.

DISCUSSION

Endothal is a more potent herbicide than any of its analogues containing additional substituents on the 7-oxabicycloheptane ring; i.e., it appears to have the optimal spatial characteristics for fit at the site of action. Substituents in the C-1 and C-4 or exo positions at C-5 and C-6 reduce herbicidal activity more drastically than do substituents in the C-2 and C-3 or endo positions at C-5 and C-6. QSAR analysis establishes a correlation between the potencies for inhibiting root growth of barnyard grass and the variables $\sum V_w(\alpha)$ and $\sum V_w(\beta)$, and a moderate correlation is also achieved for wild mustard when R_m

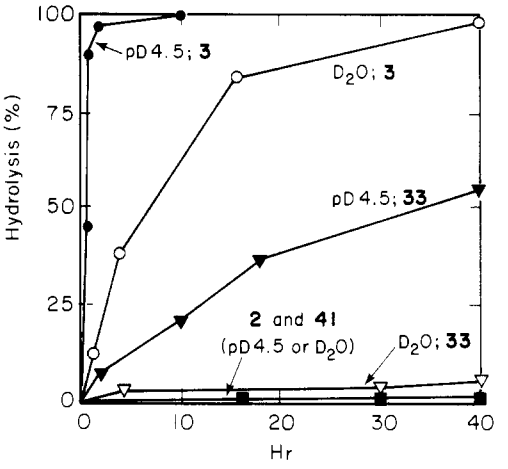
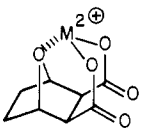


Figure 4. Relative hydrolysis rates of cantharidin (**2**), endothal anhydride (**3**), endothal thioanhydride (**33**), and endothal imide (**41**) as 0.25 M solutions in D_2O–Me_2SO-d_6 (1:4) or pD 4.5 0.04 M sodium phosphate in D_2O–Me_2SO-d_6 (1:4) at 40 °C. Hydrolysis was monitored by NMR. Signals for H_1 and H_2 in the hydrolyzed compounds appear 0.2 and 0.5 ppm upfield from the corresponding signals in the original compounds (**3**, **33**) due to the shielding effect of the carboxylic acid groups.

values are included in the analysis. Furthermore, geometric isomers **25** and **26** of endothal and **28** of cantharidin show little toxicity to plants and mice, indicating the significance of possible interactions involving the carboxylate groups and the bridged oxygen. These interactions are also evident by the formation of 1:1 metal ion complexes with endothal and its analogues, possibly of the type shown below.



Interaction with a metal (this study; Li et al., 1983) may be related to the herbicidal activity (Rakitin and Imamaliev, 1959) and perhaps the mammalian toxicity of endothal and cantharidin analogues (Allison and Williamson, 1960). Stability constants for cupric ion complexes with endothal and its geometric isomers and related compounds indicate a direct relationship between the number of oxygens available for chelation and their configuration; i.e., the metal ion is stabilized with three oxygen centers. Thus, the herbicidal activities for the endothal isomers (**1**, **25**, **26**) follow the same order as their metal ion complex stability constants.

Absorption and translocation of the endothal analogues are probably related in part to their hydrophobicity and hydrolysis rates. Thus, R_m improves the correlation obtained for inhibition of wild mustard root growth. The compounds vary greatly in hydrolytic stability. Although it seems likely that the toxic form is the dicarboxylate, it is possible that some dehydro analogues may react by Michael addition.

This investigation shows that three positions in endothal and its analogues appear to be involved in their target site interaction: i.e., the bridged oxygen atom and two suitably positioned carboxyl groups. Consideration of stability constants suggests, but does not in itself establish, that a metal ion may be involved at the target site. It is clear that the biological activity of these compounds is greatly reduced by steric hindrance with substituents at C-1, C-4, or the exo positions of C-5 and C-6. Somewhat similar structure–activity relationships for herbicidal activity and

toxicity to mice suggest the possibility of a related oxabicycloheptane target site in plants and mammals.

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Pyrolysis of Triallate

Douglas E. Johnson* and James C. DeLane

The thermal chemistry of triallate [*S*-(2,3,3-trichloro-2-propenyl) diisopropylthiocarbamate, **1**] was examined to determine whether the thermal chemistry of thiocarbamate *S*-ester herbicides can be predicted from thermal reactions of simpler carbamates. Flash vacuum pyrolysis of **1** at 475–575 °C yields a complex mixture of products. Products were identified by gas chromatography–mass spectrometry analysis of the pyrolysate. 1,1,2-Trichloro-1-butene (**7**) was the major product. Minor products included 1,1,2,5,6,6-hexachloro-1,5-hexadiene (**21**), 1,1-dichloro-1,2-butadiene (**5**), and 4,5-dihydro-3-isopropyl-4-methylthioazolidin-2(3*H*)-one (**18**). The major reaction mechanism involves formation and reactions of 1,1,2-trichloroallyl and diisopropylthiocarbamoyl radicals. Thermal reactions of structurally analogous carbamates can be used to predict the thermal chemistry of other thiocarbamate *S*-ester herbicides.

Previous studies of herbicide thermal chemistry have focused on the bulk stability of the herbicide and largely ignored the thermal decomposition products (Saito et al., 1981; Stojanovic et al., 1972). Herbicide thermal reaction products and reaction mechanisms could be predicted if the thermal chemistry of suitable analogues was well established. The thermal chemistry of thiocarbamate *S*-ester

Department of Chemistry, North Dakota State University, Fargo, North Dakota 58105.

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