Fungitoxicity of Dioxanes, Dioxolanes, and Methylenedioxybenzenes

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Contents

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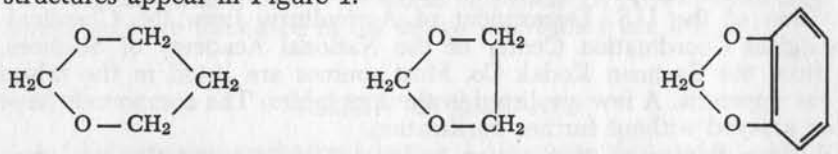
INTRODUCTION

The relation of structure to biological activity of synthetic compounds continues to be a baffling area of biological research. Additions to knowledge in this field improve the understanding of the chemistry of living organisms, however, and help to design better drugs, fungicides, hormones, and other biologically active materials.

We offer here a study of the relation of structure to the fungicidal activity of three related heterocyclic nuclei, each containing two oxygen atoms separated by a carbon atom.

The compounds are called 1,3-dioxanes if the ring is 6-membered, 1,3-dioxolanes if the ring is 5-membered, and 1,3-methylenedioxybenzenes if the ring is 5-membered and has a benzene ring condensed to it. Their structures appear in Figure 1.



1,3-Dioxane 1,3-Dioxolane 1,3-Methylenedioxybenzene

FIGURE 1.

Aside from their intrinsic interest, we are concerned with these oxygen compounds also because they bear a resemblance to known fungitoxic heterocyclic compounds with 2-nitrogen atoms separated by one carbon atom. The best known one of these is an imidazoline derivative known as glyodin, an analogue of dioxolane. This is a commercial fungicide described by Wellman and McCallan (1946).

There are also mixed heterocycles of oxygen and nitrogen separated by one carbon atom. And, if you please, you can substitute sulfur for oxygen, its neighbor in the periodic table.

In due course, these will be interrelated in the manuscript. We shall discuss the results in the order dioxane, dioxolane, and methylenedioxybenzene.

Heterocyclic nitrogen compounds have surely "had their day in court" as fungicides. Important practical fungicides like captan, glyodin, and

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copper oxinate contain nitrogen heterocycles. We have published a paper dealing with the fungitoxicity of numerous heterocyclic nitrogen compounds (Horsfall and Rich, 1951).

Heterocyclic oxygen compounds are notable by their scarcity in fungicidal literature, however. At one time furfural (Flor, 1927) was proposed. Owens (1959) discusses the fungitoxic and disease controlling properties of the 5-nitrofurans. The next year Avery Rich (1960) reported the fungitoxic and disease controlling properties of a 5-ketofuran. It is interesting to note in passing that both structures depend on a strong electronegative group at position five. 2-Furylbenzothiazole is reported by Miron and Ripeanu (1961) to be effective on spores of *Tilletia* sp. We find this compound reasonably toxic to our spores. Rich and Horsfall (1961) showed that N-(2-furanyl) or N-(2-furfuryl) carbamates were not toxic to their test fungi.

Griseofulvin, a fungitoxic antibiotic, contains a 5-membered oxygen heterocycle, and protoanemonin, a furan derivative is fungitoxic according to Holden *et al.* (1947).

Dehydroacetic acid (Wolf, 1950) seems to be the most fungitoxic of the 6-membered oxygen heterocycles. γ-Pyrone is a weak fungicide in our trials.

MATERIALS AND METHODS

Most of the compounds were obtained from the Entomology Research Division of the U.S. Department of Agriculture, from the Chemical-Biological Coordination Center of the National Academy of Sciences, or from the Eastman Kodak Co. Most sources are listed in the tables in the appendix. A few are listed in the text tables. The compounds have been assayed without further purification.

We use the spore germination technique of assaying the test compounds as described by Horsfall and Rich (1951). We use two test organisms, *Stemphylium sarcinaeforme* (Cav.) Wilt. and *Monilinia fructicola* (Wint.) Honey. These names are abbreviated to St. and Mo., respectively, in the data tables. We frequently find that the difference between these two organisms is instructive in understanding the biological effects of compounds. All compounds are dissolved in a suitable solvent, usually at 10, 1.0, 0.1, and 0.01 grams per liter, and 0.2 ml. is pipetted with a graduated syringe into duplicate depression or culture slides to give 1300, 130, 13, and 1.3 $\mu g/cm^2$. The solutions are allowed to dry so that a uniform deposit is produced.

The depression is filled with 0.4 ml. of spore suspension in which the number of spores is adjusted to 50 per low power field of the microscope. The spores are incubated overnight at 25° C. and are examined next morning. The mean percentage not germinated is recorded. The one or two per cent of natural mortality is disregarded. If natural mortality is abnormally high, the test is discarded and rerun. Essentially all of the compounds were tested in duplicate on different days. Doubtful ones were tested three or four times.

In comparing compounds, we shall use a "toxicity index" which is the sum of the mean percentage kill for the four doses for the several tests. This is a relative number with a possible maximum of 400. This index has been used in an earlier paper (Rich and Horsfall, 1961).

Since we do not test for death of spores, we speak of fungitoxic effect, not fungicidal effect.

Because the standard technique does not adequately assess the fungitoxicity of compounds that are volatile or that form volatile derivatives, all of the aldehydes and many of the dioxanes were assayed without preliminary drying. For this test we dissolve the compound in water or acetone and add 2 μliters to the spore suspension in each depressed slide. The minute amount of acetone is not toxic.

Being as interested in practical fungicides as in the theory of fungicidal action, we were disappointed to find but few highly toxic compounds in the list of analogues tested. Highly toxic compounds like nabam would have an index of 400. On the other hand the results are interesting, and so we present them herewith as a contribution to the literature on the relation of structure to biological activity.

EXPERIMENTAL DATA

Because the emphasis is to relate the activity of the compound to its structure, the compounds are arranged by structure in the tables in the main body of the paper. In the text they may be mentioned by name or accession number. To provide for complete cross referencing, they are named; the coded source is given; and they are arranged by accession number in the major categories in Tables A, B, and C of the Appendix. The sources themselves are shown in Appendix D. A few miscellaneous compounds are identified in the tables where they are listed.

TOXICITY OF DIOXANES

A 1,3-dioxane is synthesized from a 1,3-propane diol and an aldehyde. The structure of the 1,3-dioxanes is shown in Figure 1 and the experimental data are arranged horizontally in Table 1 in accordance with the substituent in the 2-position. They are arranged vertically in groups in accordance with substituents elsewhere on the ring.

Differential Action on the Test Organisms

In studying fungicidal action, we have usually found additional information by examining the differential responses of our two test organisms, *Stemphylium* and *Monilinia* (Rich and Horsfall, 1961).

Data on 99 dioxanes are presented in Table 1. Only one (No. 3512) is appreciably more toxic to *Stemphylium* than to *Monilinia*. Of the compounds with a toxicity index greater than 150, 20 are toxic to *Monilinia*, but only 6 are toxic to *Stemphylium*. Thus, *Monilinia* is more sensitive to the dioxanes, as it is to many classes of compounds. This differential sensitivity will be useful in discussing mechanisms of action later.

Effect of Substituents in the 2-Position

All of the test compounds of necessity are substituted in the 2-position because the aldehyde carbon from which they are made becomes the number 2-carbon of the dioxane. From Table 1, it is clear that some

Table 1. Toxicity index of 1,3-dioxanes

The 2-substitution	Other substitutions and group number														
	1			2			3			4			5		
	No other subs.			4-Methyl			2,4-Dimethyl			4,6-Dimethyl			2,4,6-Trimethyl		
	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.
Me and Et	.....	.....	.....	.....	.....	.....	3869	0	6	.....	.....	.....	3870	12	23
Propyl	.....	.....	.....	.....	.....	.....	3855	100	100	4062	155	167	3866	0	83
Propenyl	.....	.....	.....	4171	50	157	.....	.....	.....	4214	0	27	.....	.....	.....
Hexyl	3512	201	114	.....	.....	.....	3556	0	0	3524	50	101	3874	50	141
Nonyl	3968	0	71	3520	2	83	.....	.....	.....	3949	0	23	.....	.....	.....
Phenyl	.....	.....	.....	3860	100	100	.....	.....	.....	3973	100	100	4165	0	32
o-cl-Phenyl	3932	75	198	3958	53	153	.....	.....	.....	3951	100	200	.....	.....	.....
p-cl-Phenyl	3941	197	207	3852	55	200	.....	.....	.....	3891	100	200	.....	.....	.....
p-Me-o-Phenyl	3942	100	143	3595	100	106	.....	.....	.....	3535	50	137	.....	.....	.....
Benzyl	.....	.....	.....	.....	.....	.....	4053	67	100	.....	.....	.....	4043	5	100
Styryl	3872	200	200	3938	200	200	.....	.....	.....	3952	167	200	.....	.....	.....
Furyl	3851	67	83	3853	101	101	.....	.....	.....	3862	100	100	.....	.....	.....
In a 6-ring	4063	32	76	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
In a 5-ring	.....	.....	.....	3963	62	57	.....	.....	.....	3931	5	50	.....	.....	.....

Table 1. Toxicity index of 1,3-dioxanes (continued)

The 2-substitution	Other substitutions and group number														
	6			7			8			9			10		
	4,4,6-Trimethyl			5,5-Dimethyl			2,5,5-Trimethyl			5-Ethyl-5-Butyl			2-Me-5-et-5-bu		
	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.
Me and Et	.....	.....	.....	.....	.....	.....	3935	1	5	.....	.....	.....	4223	0	50
Propyl	4036	0	21	4058	5	0	3943	29	29	4005	0	100	3955	0	130
Propenyl	3974	50	69	4056	73	92	.....	.....	.....	4210	8	184	.....	.....	.....
Hexyl	3564	0	100	.....	.....	.....	.....	.....	.....	3959	0	0	4222	0	0
Nonyl	3805	0	0	4054	0	5	.....	.....	.....	.....	.....	.....	.....	.....	.....
Phenyl	.....	.....	.....	3946	65	154	4047	0	100	4042	36	33	4187	3	3
o-cl-Phenyl	3964	3	197	3882	0	223	.....	.....	.....	.....	.....	.....	.....	.....	.....
p-cl-Phenyl	3957	25	193	3936	9	111	.....	.....	.....	.....	.....	.....	.....	.....	.....
p-Me-o-Phenyl	3930	0	98	4006	0	61	.....	.....	.....	.....	.....	.....	.....	.....	.....
Benzyl	.....	.....	.....	.....	.....	.....	4064	100	101	.....	.....	.....	4061	6	0
Styryl	3937	68	46	.....	.....	.....	.....	.....	.....	4215	20	3	.....	.....	.....
In a 6-ring	3947	50	105	4068	53	64	.....	.....	.....	4059	6	214	.....	.....	.....
In a 5-ring	.....	.....	.....	.....	.....	.....	.....	.....	.....	3956	0	161	.....	.....	.....

Table 1. Toxicity index of 1,3-dioxanes (continued)

The 2-substitution	Other substitutions and group number															
	11			12			13			14			15			
	5-Ethyl-4-propyl			2-Me-5-et-4-prop			5-Met-5-nitro			5-Ethyl-5-nitro			2,5-Dimet-5-nitro			
	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	
Me and Et	.....	.....	.....	3960	2	81	.....	.....	.....	3926	30	100	3884	50	102	
Propyl	4045	4	79	3537	24	92	4002	100	140	4016	127	172	4046	81	100	
Propenyl	4206	36	100	.....	.....	.....	3971	101	164	3969	100	161	.....	.....	.....	
Hexyl	3529	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
Nonyl	3652	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
Phenyl	3945	18	92	4186	0	45	3934	0	0	3933	0	110	.....	.....	.....	
o-cl-Phenyl	3954	0	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
p-cl-Phenyl	3949	0	23	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
p-Me-o-Phenyl	3536	0	53	.....	.....	.....	3939	0	0	.....	.....	.....	4229	0	0	
Benzyl	.....	.....	.....	4069	34	31	.....	.....	.....	.....	.....	.....	.....	.....	.....	
Styryl	3965	2	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
In a 6-ring	3560	34	176	.....	.....	.....	3928	0	0	.....	.....	.....	.....	.....	.....	
In a 5-ring	3967	0	100	.....	.....	.....	4050	65	147	3966	102	128	.....	.....	.....	

substituents in the 2-position add more activity than others to the dioxane molecule. Although the aliphatic series is not complete, we may infer from groups 1, 2, 3, and 4 in the table that the peak of activity is reached between 3 and 6 carbons in the aliphatic tail. The 9-carbon (nonyl) tail seems definitely too long. The best of the 2-substituents are *o*-chlorophenyl, *p*-chlorophenyl, and styryl.

The promoting effect of the 2-styryl group may be compared with that of the 2-propenyl group in the table. These differ only in that the former has a benzene ring and the latter a methyl group.

The promoting effect of the 2-styryl group on the dioxanes reminds us of the promoting effect of the styryl group on the fungitoxicity of pyridine as reported by Horsfall and Rich (1951). Pyridine itself is essentially nonfungitoxic to our organisms, but if it is substituted with the lipophylic styryl group in the 2-position (i.e. adjacent to the nitrogen atom), it acquires a toxicity index of 254 for *Stemphylium* and 300 for *Monilinia*. Likewise, a styryl group can activate the methylenedioxyphenyl nucleus, as we shall see later.

The 2-(*p*-methoxyphenyl) substituent seems clearly less effective than the other 2-phenyl substituents presumably because it is less lipophilic.

The performance of these compounds suggests right away that the 2-substituents are acting as lipid solubilizers—that is, that they are "shaped charges" as Rich and Horsfall (1952) have dubbed them, and aid in driving the dioxane through the semipermeable membrane into the interior of the cell. A chlorophenyl group is a well known "shaped charge" for fungicides and it performs well here for dioxanes.

Effect of Other Ring Substitutions

The dioxane ring may be substituted in the 4, 5, or 6 position. We have compounds for many of the possibilities, but not for all. The most dramatic effect occurs in the 5,5-dialkyl substitutions as shown in groups 7, 8, 9, and 10 in the table. To manipulate the molecule with a 5,5-dialkyl substitution quenches the toxicity to *Stemphylium*, but it has little effect on *Monilinia*.

The differential effect on *Stemphylium* confronts us with two basic possibilities: (1) that 1,3-dioxane forms an addition product at position 5- with some *Stemphylium* metabolite but not with a *Monilinia* metabolite, and (2) that permeation into *Stemphylium* is differentially reduced. Suppose we consider these possibilities in turn.

The first possibility seems doubtful, somehow. We tested it by using Feigl's (1946) reagent (1,2-naphthoquinone-5-sulfonic acid) for addition compounds on carbon atoms with two hydrogen atoms. We tested dioxanes with numerous ring substitutions including the 5,5-dialkyl substitutions. There was indication of addition products, but no correlation with toxicity.

The permeation possibility seems more feasible. The substitutions at position 5- are alkyl and fat soluble. Hence, they could conceivably affect permeation under the shaped charge hypothesis. Successfully permeating fungicides generally are balanced between polar and nonpolar groups. If the molecule is too polar, adding fat soluble groups will redress the balance and improve permeation. This appears to be the case for the phenyl derivatives at position 2- as discussed in the last

section. If, however, the molecule is already balanced, the adding of a fat soluble group may well reduce permeability.

We have extensive evidence from other studies (Horsfall, 1951; Rich and Horsfall, 1961) that permeation into *Stemphylium* is more easily reduced by excessive aliphatic substitution than is that of *Monilinia*, and, hence, we cannot ignore this possibility to account for the depressing effect of the 5,5-disubstitution.

One might squeeze a bit of evidence for this from groups 13, 14, and 15 where $-NO_2$ is the second substituent on the number 5-carbon. Horsfall (1956) suggests that a nitro group may enhance permeation. Here it seems to enhance potency to *Stemphylium* for the 2-propyl (compare No. 4002 and 4016 with No. 4058) and the 2-propenyl analogues (compare No. 3971 and 3969 with No. 4056). It does not, however, do the same for the 2-phenyl (No. 3934 and 3933 vs. 3946) or the 2-(*p*-methoxyphenyl) (No. 3939 vs. 4006) analogues. This curious result should not be unexpected. As we suggested in the section on the 2-substitution, the 2-propyl group is probably not adequate for proper permeation, but the 2-phenyl substituent probably is. If the nitro group adds permeation properties, then, it should be effective on the 2-propyl, not on the 2-phenyl analogues.

There are other alkyl substituents on the ring that are worth consideration under the permeation hypothesis. The 4,4,6-trimethyl substitutions on either side of position 5- appear in group 6 of the table. Not one of nine 4,4,6-compounds shows toxicity to *Stemphylium*. *Monilinia* toxicity seems hardly affected. Here, too, excess hydrocarbons inhibit activity to *Stemphylium*, presumably by inhibiting permeation.

Following this line of reasoning, one discovers that he can overload the lipophilicity of a dioxane molecule even to *Monilinia*. Compare on *Monilinia*, for example, the 5,5-dimethyl compounds in group 7 in each of the four phenyl series with their corresponding 5-ethyl-4-propyl homologues in group 11. In all cases the four extra carbons on the ring seem to reduce activity even for *Monilinia*.

Our general conclusion, then, for the ring substitutions tested, is that the hydrocarbons can improve permeation and activity for molecules that permeate poorly, and reduce permeation and activity for molecules that permeate well.

Effect of Dioxane Intermediates

Since a 1,3-dioxane is synthesized from an aldehyde and a 1,3-propanediol, we must recognize the possibility that the dioxane may hydrolyze back to these intermediates. If we are to understand the toxicity of the dioxane molecule, we must first assess the toxicity of the intermediates and the possibility that they may be formed by hydrolysis in the fungicide-fungus system.

Effect of 1,3-propane diols:—The toxicity of the available 1,3-propane diols is shown in Table 2. It is clear that they cannot account for the toxicity of a dioxane whether or not hydrolysis occurs. The nitro derivatives (Nos. 320 and 321) are no more toxic than the others, and thus cannot account for the special case of the nitrodioxanes discussed above.

Effect of aldehydes:—The aldehydes are different from the 1,3-propane diols. The fungitoxicity of aldehydes has been known for nearly 75

Table 2. Fungitoxicity of 1,3-propane diols

No.	Source	Name of propane diol	Dioxane derivative	Toxicity Index St.	Toxicity Index Mo.
320	Com'l Solvents	2-Nitro-2-ethyl-1,3-propane diol	5-Nitro-5-ethyl	45	54
321	Com'l Solvents	2-Nitro-2-methyl-1,3-propane diol	5-Nitro-5-methyl	0	0
1058	CBCC	2-Methyl-2,4-pentane diol	4,4,6-Trimethyl	0	0
1064	CBCC	2-Methyl-1,3-pentane diol	4-Ethyl-5-methyl	100	50
4251	CBCC	1,3-Butane diol	4-Methyl	0	0
5529	EK	1,3-Propane diol	No substitution on ring	20	86
5578	EK	2,2-Diethyl-1,3-propane diol	5,5-Diethyl	0	0
5580	EK	2,2,4-Trimethyl-1,3-pentane diol	5,5-Dimethyl-4-isopropyl	0	0
5581	EK	2,2-Dimethyl-1,3-propane diol	5,5-Dimethyl	0	0
5582	EK	2-Ethyl-1,3-hexane diol	5-Ethyl-4-propyl	0	0

years beginning with formaldehyde. Brian *et al.* (1946) reported the toxicity of cinnamic aldehyde, the parent aldehyde for our styryl derivatives of dioxane.

In Tables 3 and 4 we give the data on six aryl and three alkyl aldehydes alongside the data on their dioxane derivatives. The data show that aldehydes that have a benzene ring in the molecule (Nos. 157, 5524, 5525, 5527, 4039, and 5528) are more toxic than those that do not (Nos. 771, 774, and 5526).

One could ask if any dioxane is more toxic than its parent aldehyde. The general rule seems to be that the dioxanes are less toxic than the parent aldehyde. This is a puzzling phenomenon. It could be due to permeation difficulties. In any case, we can safely say that conversion of an aldehyde to a dioxane does not make it more fungitoxic.

Effect of rate of hydrolysis:—The data just given in the section on toxicity of the aldehydes do not add much weight to the hypothesis that dioxane toxicity is due to hydrolysis to an aldehyde. Still, by no means do they disprove it. The discrepancy between the toxicity of the aldehyde and the dioxane may simply be due to slow hydrolysis of the dioxane. If so, this can be subjected to trial by examining the rate of hydrolysis of a few dioxanes.

Dioxanes do not hydrolyze significantly at the normal physiological pH of 6 or 7. Therefore, we had to resort to low pH levels to study rate of hydrolysis. We used the recommended solution of 0.087M acetic acid in ethyl alcohol. The pH was about 3.15.

Aldehyde was measured rather than diol because Feigl (1946) lists a good reagent (*o*-anisidine) for aldehydes. The rate of hydrolysis of 1,3-dioxane to aldehyde was measured spectrophotometrically by tracing the formation of the Schiff's bases formed in the reaction of aldehydes with *o*-anisidine. The reagent (0.05 ml. saturated *o*-anisidine in glacial acetic acid) was added to the dioxane (10 ml. ethanolic solution). The amount of each Schiff's base formed was computed from absorbancy at 385 mμ for cinnamaldehyde and 2-styryl-1,3-dioxanes, at 372.5 mμ for *o*-chlorobenzaldehyde and 2-(*o*-chlorophenyl)-1,3-dioxanes, and at 365 mμ for *p*-chlorobenzaldehyde and 2-(*p*-chlorophenyl)-1,3-dioxanes.

Data on the rate of aldehyde formation in the *o*-chlorophenyl, *p*-chlorophenyl, and styryl derivatives of 1,3-dioxane are matched with the toxicity data in Table 3. The first data that we obtained led us to think that toxicity of dioxanes is due to formation of aldehydes. Thus, examination of the data in Table 3 shows clearly that dioxanes that hydrolyze readily are fungitoxic. A striking case is that of the 4,6-dimethyl derivatives in each of the three groups. It looks as if 4,6-dimethyl substitution encourages hydrolysis to aldehyde. Other fungitoxic derivatives that liberate considerable amounts of aldehyde are: No. 3932 in the *o*-chlorobenzaldehyde series, and Nos. 3872, 3938, and 3952 in the cinnamic aldehyde series.

As we accumulated hydrolysis data on other derivatives, however, this explanation weakened. Considering the easily hydrolyzable 4,6-dimethyl derivatives, we note in Table 3 that they are no more fungitoxic than several dioxanes in the same series that do not hydrolyze readily.

Thus, some analogues are toxic to both organisms and liberate no aldehydes at all in the test. Examples are numbers 3941, 3852, and 3936 in the *p*-chlorophenyl series and No. 3882 in the *o*-chlorophenyl

Table 3. Fungitoxicity and rate of hydrolysis of aldehydes and 1,3-dioxane derivatives

Cpd. No.	Ring substituents	Toxicity Index St.	Toxicity Index Mo.	Rate of aldehyde formation ppm/min.
5524	*o*-Chlorobenzaldehyde (Derivs. called *o*-cl-phenyl)	200	200	.....
3932	None	75	198	1.25
3958	4-Met	53	153	0.67
3951	4,6-Dimet	100	200	3.50
3964	4,4,6-Trimet	3	197	0.33
3882	5,5-Dimet	0	223	0.00
3954	5-Et-4-prop	0	3	0.00
3961	5,5-Dimet-4-*iso*prop	0	0	0.00
5525	*p*-Chlorobenzaldehyde (Derivs. called *p*-cl-phenyl)	164	179	.....
3941	None	197	207	0.00
3852	4-Met	55	200	0.00
3891	4,6-Dimet	100	200	1.92
3957	4,4,6-Trimet	25	193	0.67
3936	5,5-Dimet	9	111	0.00
3949	5-Et-4-prop	0	23	0.50
3929	5,5-Dimet-4-*iso*prop	0	30	0.00
4039	Cinnamic aldehyde (Derivs. called styryl)	247	207	.....
3872	None	200	200	46.0
3938	4-Met	200	200	27.5
3952	4,6-Dimet	167	200	42.0
3937	4,4,6-Trimet	68	46	28.0
4215	5-Et-5-bu	20	3	6.5
3965	5-Et-4-prop	2	0	12.0

Table 4. Fungitoxicity of aldehydes and 1,3-dioxane derivatives

No.	Ring substituents	Toxicity Index St.	Mo.
157	Benzaldehyde (Derivs. called phenyl)	150	150
3860	4-Met	100	100
3973	4,6-Dimet	100	100
3946	5,5-Dimet	65	154
4042	5-Ethyl-5-bu	36	33
3945	5-Ethyl-5-prop	18	92
3934	5-Ethyl-5-nitro	0	0
5528	Phenylacetaldehyde (Derivs. called benzyl)	200	150
4048	2-Met	100	100
4053	2,4-Dimet	67	100
4043	2,4,6-Trimet	5	100
4064	2,5,5-Trimet	100	101
4061	2-Met-5-et-5-bu	6	0
4069	2-Met-5-et-4-prop	34	31
5526	Heptaldehyde (Derivs. called hexyl)	100	100
3512	None	201	114
3524	4,6-Dimet	50	101
3564	4,4,6-Trimet	0	100
3959	5-Et-5-bu	0	0
3529	5-Et-4-prop	0	0
3556	2,4-Dimet	0	0
3874	2,4,6-Trimet	50	141
4222	2-Met-5-et-5-bu	0	0
5527	*o*-Methoxybenzaldehyde (Derivs. called *o*-me-*o*-phenyl)	150	200
3905	4,6-Dimet	100	100
3911	4-Hydroxy	102	159
771	Propionaldehyde (Derivs. called ethyl)	0	100
3869	2,4-Dimet	0	6
3870	2,4,6-Trimet	12	23
3960	2-Met-5-et-4-prop	2	81
3935	2,5,5-Trimet	0	5

Table 4. Fungitoxicity of aldehydes and 1,3-dioxane derivatives (continued)

No.	Ring Substituents	Toxicity Index St.	Mo.
4223	2-Met-5-et-5-bu	0	50
3588	2,5,5-Trimet-4-*iso*prop	0	0
3926	2-Met-5-et-5-nitro	65	100
3884	2,5-Dimet-5-nitro	25	102
774	n-Butyraldehyde (Derivs. called propyl)	100	119
3855	2,4-Dimet	100	100
4062	4,6-Dimet	155	167
3866	2,4,6-Trimet	0	83
4036	4,4,6-Trimet	0	21
4058	5,5-Dimet	5	0
3943	2,5,5-Trimet	23	23
4005	5-Et-5-bu	0	100
3955	2-Met-5-et-5-bu	0	130
4045	5-Et-4-prop	4	77
4002	5-Met-5-nitro	100	140
4016	5-Et-5-nitro	127	172
4046	2,5-Dimet-5-nitro	81	100
3537	2-Met-5-et-4-prop	24	92

series. On the other hand, the styryl derivative No. 3937 liberates as much aldehyde as No. 3938, but it is far less toxic.

Another interesting comparison is between *p*-chlorobenzaldehyde and cinnamic aldehyde. These two aldehydes have about the same molecular weight but the latter is more fungitoxic to both organisms: consider, then, that the dioxanes based on cinnamic aldehyde liberate from 10 to 20 times as much aldehyde as those of the *p*-chlorophenyl analogues. Despite more aldehyde and more toxicity of the aldehyde, the dioxanes based on cinnamic aldehyde are not significantly more toxic than those based on *p*-chlorobenzaldehyde.

We can adduce a small amount of other data on the aldehyde hypothesis. If a dioxane be doubly substituted in the 2-position, it will not hydrolyze to an aldehyde because the hydrogen is missing. Are such dioxanes toxic? Such compounds appear in groups 3, 5, 8, 10, 12, and 15 in Table 1. When the compounds are paired with their monosubstituted aldehyde analogues in the adjacent groups, they are found to be no more and no less toxic. In fact, two of them approach the maximum toxicity to *Monilinia* displayed by any of the dioxanes (Nos. 3874 in group 5 and 3955 in group 10). The toxicity of the 2-substituted compounds at least cannot be ascribed to hydrolysis to the aldehydes.

Thus, we can see from all the data that we need not postulate that dioxanes act by liberating aldehydes. Some unstable dioxanes may very well form toxic aldehydes, but we need not conclude that aldehyde formation is necessary for fungitoxic activity in dioxanes. Thus, we can proceed to analyze other possibilities.

Effect of Nitrogen and Sulfur Analogues

We turn now to a comparison of the 1,3-dioxanes with pyrimidines, oxazines, and thiazines, where the heteroatoms also occupy the 1- and 3-positions. We list in Table 5 the most toxic compounds we have of these four kinds. It is clear that of the compounds tested the 2-heptadecyltetrahydropyrimidine (No. 1375) is the most fungitoxic. This is the compound first published by Rader *et al.* (1952) obviously as a follow up to glyodin, which will be discussed in the section on dioxolanes.

It is interesting that it takes a 17 carbon tail in the 2-position to fully activate the pyrimidine compound, whereas, a 9 carbon tail in the 2-position (No. 3968) of the dioxane is too long.

It seems too bad that we do not have for comparison an oxazine (see No. 1689) substituted in the 2-position. Presumably, it would be more toxic than the one we have.

Discussion of Structure of Dioxanes

As we suspected, the oxygen-containing heterocyclic nucleus, 1,3-dioxane, is fungitoxic. True, it is not highly fungitoxic, but it is fungitoxic. It seems to require a fat-solubilizing group, however, to move it into the cell. The best of the fat-solubilizing groups available to us are the 2-(*p*-chlorophenyl), 2-(*o*-chlorophenyl), and the 2-styryl substituents.

The 5-position seems to have some significance to activity. If this position is doubly substituted, the potency to *Stemphylium* evaporates,

Table 5. Comparative fungitoxicity of 1,3-dioxanes and certain nitrogen and sulfur analogues

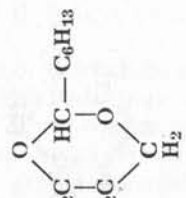
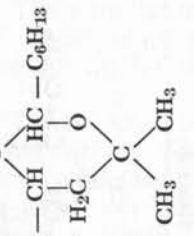
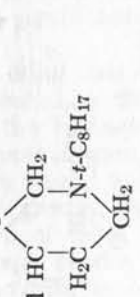
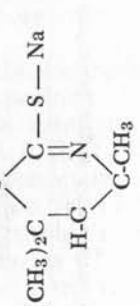
Number	Source	Name	Structure	Toxicity Index St.	Toxicity Index Mo.
3512	USDA	2-Hexyl-1,3-dioxane		201	114
3564	USDA	2-Hexyl-4,4,6-trimethyl-1,3-dioxane		0	100

Table 5. Comparative fungitoxicity of 1,3-dioxanes and certain nitrogen and sulfur analogues (continued)

Number	Source	Name	Structure	Toxicity Index St.	Mo.
1375	Shell	2-Heptadecyl-4,4,6-trimethyltetrahydropyrimidine		395	400
1689	R&H	3-*t*-Octyl-5-chlorohexahydro-1,3-oxazine		110	155
4793	Koppers	Na salt of 4,6,6-trimethyl-1,3-thiazine-2-thiol		0	100

but the effect on *Monilinia* is much less drastic. At first we felt that the data suggested the formation of addition products with *Stemphylium* metabolites. Upon further reflection, however, this conclusion seems too facile. For one thing *Monilinia* data do not agree with this. It is difficult to postulate an addition product with metabolites of one fungus and not another.

Generally, the 5,5-disubstitution seems to overload the lipophilic properties of the molecule and reduce permeation rather than reduce reactability. This explanation is reinforced by the effect of a nitro group as one of the two substituents at position 5-. The nitro group presumably promotes permeation and may, therefore, cancel some of the weak permeation induced by the alkyl groups occupying the other valence on the number 5-carbon.

At first we thought, too, (see abstract of Lukens and Horsfall, 1965) that decomposition to aldehyde may account for the potency of the dioxanes, but data on other compounds has weakened this position. Although some dioxanes may degrade to aldehydes, toxicity of the dioxanes as a group is poorly correlated with aldehyde formation or with the aldehydes that would be formed.

TOXICITY OF DIOXOLANES

The fungitoxicity data on dioxolanes appear in Appendix B and in Tables 6 and 7 in the text where they are arranged, as before, vertically in accordance with the substituents at position 2- and horizontally in accordance with the substituents elsewhere on the ring.

The dioxolane nucleus as shown in Figure 1 is a 5-membered ring instead of a 6-membered ring as in dioxane. The aldehyde component is the same but the diol is a 1,2-diol, not a 1,3-diol. Just as in the dioxanes, we must consider the possibility of hydrolysis of dioxolane to the aldehyde and the diol.

Differential Action on the Test Organisms

We are interested to know whether the 5-membered ring is more or less toxic than the 6-membered ring. The evidence is in Tables 6 and 7, especially in the direct comparison in Table 7.

There are 54 dioxolanes in Table 6 and 99 dioxanes in Table 1. For *Stemphylium* 27 per cent of the dioxolanes reach a toxicity index of 150 or more but only 6 per cent of the dioxanes. Similarly for *Monilinia*, 30 per cent of the dioxolanes reach an index of 150 or more, but only 14 per cent of the dioxanes. Thus, the dioxolanes do seem to be more fungitoxic.

This may be only a statistical conclusion, however. All it says is that one has a better chance of finding a toxic analogue in a 5-membered ring than in a 6-membered ring. One may make specific comparisons, however, in Table 7. Here the difference seems less pronounced. The *p*-chlorophenyl derivatives are among the most toxic of the dioxanes. They are among the most toxic of the dioxolanes as well. The nonyl derivatives are weak in the dioxanes. They are weak in the dioxolanes.

Table 6.* Toxicity index of 1,3-dioxolanes

The 2-substitution	Other substitutions and group number															
	1			2			3			4			5			6		
	No other subs.			2-Methyl			4-Methyl			2,4-Dimethyl			4,5-Dimethyl			2,4,5-Trimethyl		
	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.
Propyl	.....	.....	.....	.....	.....	.....	4004	153	91	.....	.....	.....	4034	75	17	3601	0	0
Propenyl	.....	.....	.....	.....	.....	.....	4180	129	16	.....	.....	.....	4168	0	79	.....	.....	.....
Hexyl	3799	130	100	3873	100	200	3545	103	151	.....	.....	.....	3610	109	100	.....	.....	.....
Nonyl	3532	119	200	.....	.....	.....	3562	0	15	.....	.....	.....	3565	0	4	.....	.....	.....
Phenyl	.....	.....	.....	4228	0	25	.....	.....	.....	4218	5	0	3863	100	104	4213	0	4
o-Cl-phenyl	3886	100	200	.....	.....	.....	3859	100	114	.....	.....	.....	3856	50	145	.....	.....	.....
p-Cl-phenyl	3858	200	200	.....	.....	.....	3880	215	209	.....	.....	.....	3876	200	247	.....	.....	.....
p-Me-o-Phenyl†	3970	100	100	.....	.....	.....	3857	100	100	.....	.....	.....	3585	100	100	.....	.....	.....
Benzyl	.....	.....	.....	4055	100	100	.....	.....	.....	4052	100	100	.....	.....	.....	4065	100	103
Styryl	4221	187	200	.....	.....	.....	4188	150	175	.....	.....	.....	3883	172	200	.....	.....	.....
Furyl	.....	.....	.....	.....	.....	.....	3854	100	100	.....	.....	.....	3868	103	100	.....	.....	.....
In a 6-ring	3586	103	78	.....	.....	.....	3801	193	104	.....	.....	.....	3800	194	101	.....	.....	.....
In a substituted 6-ring	4044	0	43	.....	.....	.....	4057	38	27	.....	.....	.....	4041	0	0	.....	.....	.....

* Fifteen numbers as follows appear in Appendix B but not here. These are 745, 1933, 2835, 3350, 3716, 3906, 3907, 3927, 3944, 3948, 3953, 4060, 4196, 4198, and 4219.
† See also the 3,4-methylenedioxyphenyl derivatives Nos. 3904 and 3908 in Table 9.

Table 7. Comparative toxicity indexes for dioxanes and dioxolanes

The 2-substitution	No other substitution						4-Methyl						4,6-Dimethyl			4,5-Dimethyl		
	Dioxane			Dioxolane			Dioxane			Dioxolane			Dioxane			Dioxolane		
	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.	No.	St.	Mo.
Propyl	.....	.....	.....	.....	.....	.....	.....	.....	.....	4004	153	91	4062	155	167	4034	75	17
Propenyl	.....	.....	.....	.....	.....	.....	4171	50	157	4180	129	16	4214	0	27	4168	0	79
Hexyl	3512	201	114	3799	130	100	.....	.....	.....	3545	105	151	3524	50	101	3610	109	100
Nonyl	3968	0	71	3532	119	200	3520	2	83	3562	1	15	3549	0	23	3565	0	4
Phenyl	.....	.....	.....	.....	.....	.....	3860	100	100	.....	.....	.....	3973	100	100	3863	100	104
o-Cl-Phenyl	3932	75	198	.....	.....	.....	3958	50	153	3859	100	114	3951	100	200	3856	50	145
p-Cl-Phenyl	3941	197	207	3858	200	200	3852	55	200	3880	215	209	3891	100	200	3876	200	247
p-Me-o-Phenyl	3942	100	143	3970	100	100	3595	100	106	3857	100	100	3535	50	137	3585	100	100
Styryl	3872	200	200	4221	187	200	3938	200	200	4188	150	175	3952	167	200	3883	172	200
2-Furyl	3851	67	83	.....	.....	.....	3853	101	101	3854	100	100	3862	100	100	3868	102	100
Part of ring	4063	32	76	3586	103	78	3963	62	57	3801	193	104	3931	5	50	3800	194	101

It should be noted, however, that the only really toxic nonyl compound is a dioxolane (No. 3532 in Table 6).

Perhaps the big difference between the 5- and 6-membered ring compounds is to be found in the specificity between organisms. Only one in 99 of the dioxanes was more fungitoxic to *Stemphylium* than to *Monilinia*. Seven out of the 54 dioxolanes were more toxic to *Stemphylium* (Nos. 745, 3586, 3799, 3800, 3801, 3944, 4004, 4180). This is 1 per cent of the dioxanes and 13 per cent of the dioxolanes.

The dioxolanes that are more toxic to *Stemphylium* seem to make pattern. None contains a benzene ring attached at the 2-position. In the hexyl (No. 3799), the propyl (No. 4004), and the propenyl (No. 4180) analogues, an alkyl group at the 2-position is relatively more potent to *Stemphylium* than to *Monilinia*. The nonyl group at position 2- does not fit this pattern (see group 1). It is more potent on *Monilinia*.

Compounds 3586, 3800, and 3801 are more toxic to *Stemphylium* than to *Monilinia* (see the next to the last line in Table 6). In these compounds the number 2- carbon occurs as a part of a 6-membered carbocyclic saturated ring that is itself unsubstituted. These three differ from each other only in the methyl groups on the dioxolane ring (see Table 6). This 6-membered saturated ring containing the number 2-carbon acts like the 6-carbon (hexyl) tail on No. 3799 in the third line in Table 6, which is also more toxic to *Stemphylium* than to *Monilinia*.

It is striking indeed, however, that compounds in the last line of Table 6 show no real toxicity to either organism. The ring is the same as for those in the line above. The substituents on the dioxane ring are the same. The difference is that the ineffective ones are substituted with 1 or 2 methyl groups on the carbocyclic ring in a position *para* to the number 2-carbon.

It is probable that these extra methyl groups overload the lipophilic quality of the molecule and, thereby, depress its potency. They act more like the 2-nonyl compounds in line 4 than like the 2-hexyl compounds in line 3 of Table 6.

Effect of Substituents in the 2-Position

The data on the 2-substitution in Table 6, show that here, as in the dioxanes, the *p*-chlorophenyl and styryl substitutions give the most toxic compounds of the lot. Presumably they drive the compound into the cell.

The activity of the hydrocarbon substitution in the 2-position (see groups 1, 4, and 5) is probably in the order propyl<hexyl>nonyl as in the dioxane series.

We are reminded here also to examine the effect of mono- and di-substitution in the 2-position because here, as in the dioxanes, hydrolysis of a single substituted carbon would give an aldehyde, a doubly substituted carbon a ketone. We may compare group 1 with 2 in the table, 5 with 4, and 5 with 6. What few data there are suggest that double substitution does not reduce activity and this suggests that the singly substituted compounds do not hydrolyze to aldehydes.

In the dioxolanes we have five compounds that differ from anything in the dioxane series. These contain a $-CH_3-COOC_2H_5$ group in the 2-position. These are Nos. 3927, 3944, 4196, and 4198 (see Appendix B).

None of these (but note No. 3944) exhibits any toxicity at all, possibly because of the weakly hydrophilic properties of the esters.

There is another small series of five compounds (Nos. 2835, 3350, 3948, 3953, and 4060, see Appendix B). These are hydroxyethyl substitutions at position 4- or acetic acid esters of them. The hydroxyethyl dioxolanes are essentially bland (Nos. 2835 and 3350), but the corresponding esters possess slightly higher toxicity indexes for *Monilinia*, presumably because the ester quenches or reduces the hydrophilic properties of the alcohol group.

Effect of Substituents in the 4- and 5-positions

We showed above that attaching hydrocarbons to the dioxane ring would improve or reduce fungitoxicity depending chiefly on the hydrophobic/hydrophilic balance. There is a very small amount of evidence for this in the dioxolanes. The compounds in group 3 in Table 5 have a methyl group at position 4-. Those in group 5 have methyl groups at both positions 4- and 5-. The potency tends to be smaller in group 5 than in group 3.

The compound 2-methyl-4-oxo-5-phenyldioxolane (No. 745, Appendix B) initially sparked our interest in the dioxolane series. With a toxicity index of 210 on *Stemphylium*, it is more toxic to this fungus than to *Monilinia*. The phenyl group is probably responsible for activating it.

Effect of Nitrogen and Sulfur Analogues

Analogous to the dioxolanes are the corresponding heterocyclic rings containing nitrogen or sulfur. The data on a few selected analogues appear in Table 8.

No. 854 in Table 8 is glyodin, the commercial fungicide introduced by Wellman and McCallan (1946). This is more potent than any 1,3-dioxolane, at least 100 times more fungitoxic than 2-nonyl-1,3-dioxolane, (No. 3532). This comparison more than any other shows why dioxolanes have never reached commercial adoption.

If only one of the dioxolane oxygens is substituted with nitrogen, we have No. 1174, 2-hendecyl-2-oxazoline. It is about as toxic to *Monilinia* as the 2-nonyl-1,3-dioxane (No. 3532). If the remaining oxygen is substituted with sulfur, we have No. 1734, a thiazole. The toxicity of this single available thiazole is about equivalent to the oxygen analogue. Dimond and Davis (1953) reported the activity of this compound and numerous analogues.

Table 8 reveals another interesting series. No. 1933 may be considered for our purposes here as a dioxolane in which the 2-position is a carbonyl group. It is not toxic, but, of course, it has no lipophilic group to drive it into the cell either. In the 4-position a $-CH_2-OH$ (No. 3350) group adds no potency.

The direct sulfur analogue is No. 3716 and this one is fungitoxic. It is as fungitoxic as any dioxane despite the absence of a lipophilic tail. In fact the addition of a methyl group at position 4- (No. 5496) does not improve potency. A 6-carbon tail at that position (No. 5501) weakens activity and a 10-carbon tail (No. 5497) quenches it altogether. No. 3716 needs further study.

Table 8. Comparative fungitoxicity of 1,3-dioxolanes with certain nitrogen and sulfur analogues

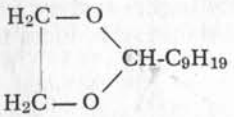
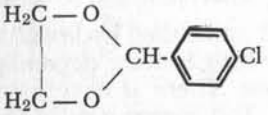
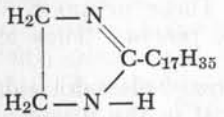
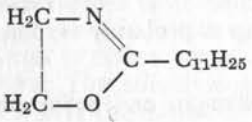
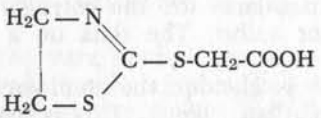
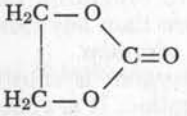
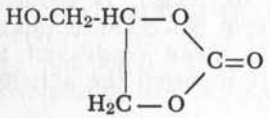
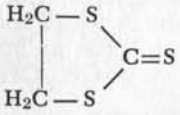
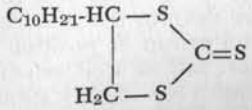
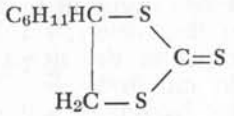
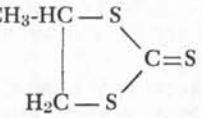
Number	Source	Structure	Toxicity Index St.	Toxicity Index Mo.
3532	USDA		121	200
3858	USDA		200	200
854	C & C		325	400
1174	CBCC		273	216
1734	R & H		261	235
1933	Jefferson		36	22
3350	CBCC		46	0
3716	CBCC		200	200
5497	Bobbio		0	0

Table 8. Comparative fungitoxicity of 1,3-dioxolanes with certain nitrogen and sulfur analogues (continued)

Number	Source	Structure	Toxicity Index St.	Toxicity Index Mo.
5501	Bobbio		101	100
5496	Bobbio		177	180

TOXICITY OF METHYLENEDIOXYBENZENES

As we have said earlier, methylenedioxybenzenes can be considered as analogues of 1,3-dioxolane compounds in which positions 4- and 5- are incorporated in a benzene ring. The structure of the methylenedioxybenzene nucleus is given above in Figure 1.

Methylenedioxybenzene nuclei occur in many natural products such as blackpepper, sesame, sassafras, celery, dill, and nutmeg. There is a methylenedioxybenzene nucleus in the newly discovered phytoalexin known as pisatin (see Perrin and Bottomley, 1962). The entomologists have intensively studied the biological activity of these compounds as synergists for the insecticidal activity of pyrethrum (see Metcalf, 1955).

The data on the fungitoxicity of methylenedioxybenzenes are given in Table 9 and in Appendix C.

Unfortunately, we do not have any analogues that are substituted in the 2-position between the two oxygen atoms as in the dioxanes or dioxolanes. This would enable a better comparison between these compounds and their dioxolane analogues. All of the analogues that we have are substituted on the number 6-carbon of the condensed benzene ring. For that reason the compounds are arranged in accordance with the substituents in that position on the ring.

Of the 32 analogues tested only one is more toxic to *Stemphylium* than to *Monilinia*. This is No. 2417, an analogue of phenyl acetic acid, which itself is more toxic to *Stemphylium* than to *Monilinia*. Hence, the differential activity of No. 2417 may well be due to the carboxyl group. This is the case for many other carboxyl compounds.

Isosafrole Derivatives

Isosafrole (No. 4703) is an ingredient of sassafras oil, an old folk remedy. This is the 6-propenyl derivative. It is an isomer of safrole (No. 4711) which is the 6-allyl derivative. Neither can be called really toxic. We regret that we do not have the 6-styryl analogue to compare with the 6-propenyl derivative.

If we substitute a nitro group for the terminal carbon of isosafrole we have number 1947, which is by far the most fungitoxic compound in the whole paper. It is about 100 times as toxic as any other compound in the series. This compound is an analogue of β-nitrostyrene which we showed many years ago to be highly fungitoxic (Horsfall, 1945). It was effective for us as a foliage protectant, but it was not a commercially feasible product because it burns the skin.

The nitro group is almost surely the activating group of No. 1947 because it is so much more toxic than isosafrole. Since No. 1947 is the most toxic compound in the entire paper, it needs a bit more discussion. It appears in this case that the $-NO_2$ group has a greater role than merely balancing the polarity of the molecule to improve permeation, as it seems to do when attached to the 5-position of the dioxanes. In compound No. 1947, the $-NO_2$ group participates in a resonating system.

It is capable of exerting an electronegative inductive effect on the mobile hydrogens on the α and β carbons of the chain causing the hydrogens to become reactive. Compounds 3276 and 4122 provide further evidence. Each contains an electronegative structure, a carbonyl for

Table 9. Toxicity index of methylenedioxybenzenes

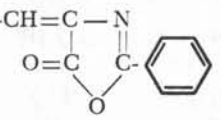
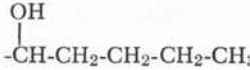
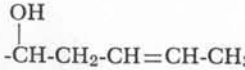
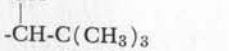
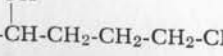
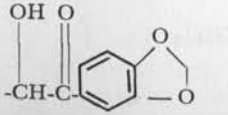
No.	The 6-substitution	Toxicity Index St.	Mo.
	Isosafrole derivatives		
4703	-CH=CH-CH_3	97	100
2410	-CH=CH-COOH	127	139
1947	-CH=CH-NO_2	347	350
3276		0	0
4122	-CH=C(CN)-COO-C_2H_5	4	16
4711	-CH_2-CH=CH_2	75	38
	Piperonaldehyde derivatives		
5428	-CH=O	104	110
2909	-CH-NOH	150	162
3092	-CH=N-NH-C(O)- 	0	0
3100	-CH=N-NH-C(O)-  -NO_2	0	0
4117	-CH=N-NH-C(S)-NH_2	10	17
	Piperonyl alcohol derivatives		
3919		118	200
4035		122	158
3994		182	200
3988		121	214
2376		0	5

Table 9. Toxicity index of methylenedioxybenzenes (continued)

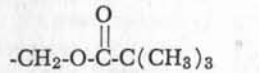
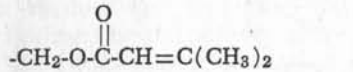
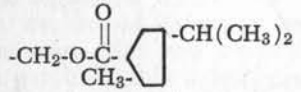
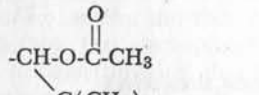
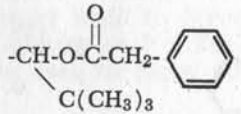
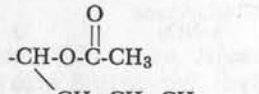
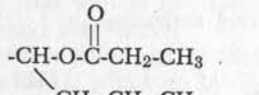
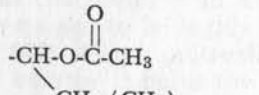
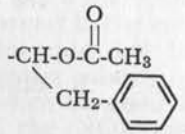
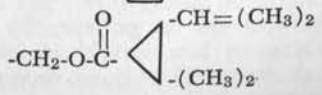
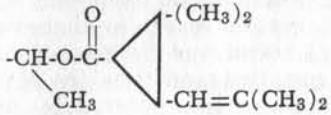
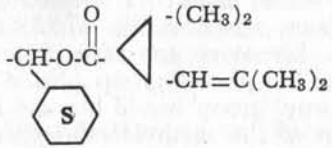
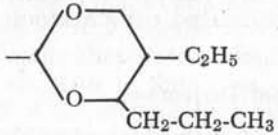
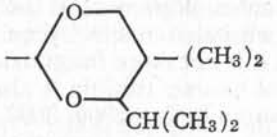
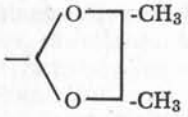
No.	The 6-substitution	Toxicity Index St.	Mo.
	Piperonyl alcohol esters		
3920		0	180
3923		107	238
3910		102	100
3989		85	149
4131		0	0
4008		24	200
4019		14	133
4022		50	175
4032		1	220
4010		3	19

Table 9. Toxicity index of methylenedioxybenzenes (continued)

No.	The 6-substitution	Toxicity Index St.	Mo.
4239		107	122
4241		9	51
	Miscellaneous analogues		
2417	$-CH_2-COOH$	240	179
3904		0	21
3996		1	76
3908		100	142

3276 and a cyano group for 4122. Despite this activating group, neither is toxic. Please note that there is no hydrogen on the nearer carbon of the chain and the hydrogen on the other carbon is boxed in by the steric effects of the ring structure on the one hand and the ester group on the other.

Since the electronegative structures on compounds 3276 and 4122 are to no avail, the toxicity should revert to that of the methylenedioxyphenyl nucleus itself, but because of their configuration, the molecules are off balance for permeation and thus are bland.

The reactive hydrogen hypothesis is supported by data on No. 2410 in which the carboxyl group would exert some electronegative induction on the α-β-unsaturated carbons, while in No. 4703 the methyl group would exert little induction. Removing the unsaturated carbons away from the resonating system of the phenyl group (No. 4711), the hydrogens of the unsaturated propenyl group would become less reactive and toxicity would rather be that of the methylenedioxybenzene itself.

In short, in compound No. 1947 toxicity seems to reside largely in the reactive hydrogens on the α- and β, unsaturated carbons. Toxicity for the other isosafrole derivatives is largely from the methylenedioxybenzene, modified to varying degrees by the slightly induced reactivity of the hydrogen attached to the α-β-unsaturated carbon atoms of the propenyl group.

Piperonal Derivatives

When the R- group at position 6- is an aldehyde (No. 5428) we have piperonal, an ingredient of perfumes. Piperonal is related to vanillin, which is also sweet-smelling. Piperonal is made by oxidizing isosafrole (No. 4703) mentioned above, but it is not more fungitoxic than isosafrole. It is less toxic than vanillin, but, of course, vanillin is also a phenol.

We have four analogues of piperonal, Nos. 2909, 3092, 3100, and 4117. No. 2909 is the oxime. It is a little more toxic than piperonal. Nos. 3092 and 3100 are carbazones and No. 4117 is a thiosemicarbazone. None is significantly fungitoxic.

Piperonyl Alcohol Derivatives

Piperonal, just discussed, can be reduced to piperonyl alcohol. Pisatin, the phytoalexin, can be considered as an analogue of piperonyl alcohol. We have five piperonyl alcohols in Table 9. Interestingly enough, except for No. 2376, they are all reasonably fungitoxic, at least to *Monilinia*. The *α-tert* butyl derivative seems to be pretty toxic to *Stemphylium* as well. The only really weak analogue is piperonyloin (No. 2376).

Apparently they are all provided with a big enough alkyl group to offset the hydrophilic property of the -OH group.

Piperonyl Alcohol Esters

We have 12 esters of piperonyl alcohol. Three of the really weak ones on both organisms are esters of chrysanthemum carboxylic acid (Nos. 4010, 4239, and 4241). These contain a 3-membered ring in the acid moiety. The other weak one is the phenylacetic ester (No. 4131).

Generally the esters seem to be pretty weak on *Stemphylium*. The only ones that are even slightly toxic (i.e. an index of 100) are Nos. 3923, 3910, and 4239. On the other hand, five of the esters (Nos. 3920, 3923, 4008, 4022, and 4032) show toxicity indexes of 200 more or less for *Monilinia*. Thus, except for the β-nitrostyrene derivative (No. 1947) they are about as toxic as any compounds mentioned in this paper.

The only direct comparison between an alcohol and its corresponding ester is that between the alcohol No. 3994 and its ester No. 3989. Making an ester did not affect the toxicity to *Monilinia*, but it did reduce activity to *Stemphylium*.

The differential effects of esterification on the two test organisms agree with the results on dithiocarbamic acids. There, too, esterification reduced potency to *Stemphylium* more than to *Monilinia* (see Rich and Horsfall, 1961).

Effect of Nitrogen and Sulfur Analogues

We have on hand analogues for this series also in which one or both oxygens have been replaced by nitrogen or sulfur. The data on a few selected analogues appear in Table 10.

Unfortunately, these compounds are all substituted in the 2-position, none in the 6-position; whereas the reverse is the case for the methylenedioxybenzenes. Besides there is one double bond in the 5-membered ring of these compounds, none in the other.

Nevertheless, it is interesting that when one nitrogen is substituted for one oxygen, (benzoxazole) activity is quenched whereas when one sulfur is substituted (benzothiazole) or two nitrogens are substituted (benzimidazole) activity is not quenched.

DISCUSSION AND SUMMARY

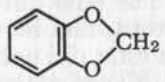
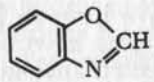
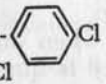
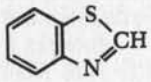
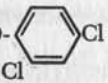
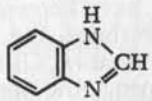
We have investigated the relation of structure to fungitoxic activity in a series of dioxanes, dioxolanes, and methylenedioxyphenyl derivatives as representatives of heterocycles containing two oxygen atoms separated by one carbon atom. We have discovered only one new fungitoxicant, but we have found interesting structure-activity relations especially for permeation.

The most toxic compound is 3,4-methylenedioxy-β-nitrostyrene. This cannot be considered an altogether new fungitoxicant, however, because we have known for two decades that β-nitrostyrene is fungitoxic.

Perhaps the most significant findings deal with the problem of permeation. Rich and Horsfall (1952) have shown that alkyl chains improve the permeation of such heterocycles as imidazoline and pyrazole. The commercial fungicide, glyodin, depends upon a 17 carbon tail attached to the number 2-carbon. Similarly, hexahydropyrimidine (Rader *et al*, 1952) is activated by a 17 carbon tail on the number 2-carbon.

It is interesting, therefore, to find that a carbon tail attached to the number 2-carbon also activates dioxanes and dioxolanes. Lacking some of the homologues, we could not determine precisely what the optimum length of tail should be, but it approximates six carbons. This is only a third as many carbons as is required for optimum activity for the imidazoline or hydropyrimidine nuclei, however.

Table 10. Comparative fungitoxicity of methylenedioxyphenyl derivatives with certain nitrogen and sulfur analogues

No.	Source	Substituent	Toxicity Index St.	Mo.	Structure of nucleus
		Methylenedioxyphenyl derivatives			
4703	CBCC	-6-CH=CH-CH_3	100	100	
1947	CBCC	-6-CH=CH-NO_2	395	400	
3994	CBCC	-6-CH(OH)$C(CH_3)_3$	200	200	
		Benzoxazole derivatives			
2438	EK	No Substitution	0	0	
2803	CBCC	-2-Phenyl	0	0	
2642	R&H	-2-CH_2-O- 	0	0	
		Benzothiazole derivatives			
499	EK	No substitution	100	100	
502	EK	-2-Cl	100	200	
2625	R&H	-2-phenyl	226	300	
2676	R&H	-2-CH_2-O- 	0	100	
		Benzimidazole derivatives			
2089	EK	No substitution	102	105	
2626	R&H	-2-Phenyl HCl	243	100	
2640	R&H	-2-CH_2-O- 	123	208	
2661	R&H	-2-CH=CH- 	262	286	

Presumably, the reason for the shorter length is that the dioxane or dioxolane nuclei are less hydrophilic than the nitrogen bases.

The evidence is a little fuzzy, but it appears that the optimum tail length for a dioxolane is longer than for a dioxane, probably because it has one fewer carbon atom in the ring.

Then, too, the optimum tail length is affected by the number of carbons in alkyl groups attached elsewhere on the ring. In fact, the activity seems to be roughly proportional to the number of carbons in the compound.

It is well known that activity generally follows a U-shaped curve for number of carbons in a straight chain. It is worthwhile to note here, then, that too many carbons in the tail on the number 2-carbon reduces activity. And more importantly, extra carbons in positions 4, 5, or 6 may reduce activity, presumably by making the molecule too lipophilic.

Still another observation on permeation is that the length of the tail (or the total number of carbons) should be shorter for *Stemphylium* than *Monilinia*. This is in line with numerous other compounds we have studied. The semipermeable membrane of *Stemphylium* apparently transmits compounds that are more polar than the membrane of *Monilinia*. We conclude from this that its membrane comprises less fat.

Miller and Richter (1960) debate this conclusion, but in this chess game if they are to checkmate, they must advance a hypothesis that fits the data better.

Another observation seems significant. *p*-Chlorophenyl and styryl substituents on the number 2-carbon seem to impart more activity to the dioxanes and dioxolanes than do the alkyl substituents there. The reasons for this are not very clear. Both, of course, are conjugated structures, but even this offers few clues.

Even so, one wonders what would happen to the toxicity of imidazoline or hydropyrimidine if a *p*-chlorophenyl or styryl substitution were to be made on the number 2- carbon. The paper of Rader *et al.* (1952) provides a partial answer. The 2-(*p*-methylphenyl) derivative of hydropyrimidine was 1000 times weaker than the heptadecyl derivative.

Here, then, seems to be an unresolved anomaly in structure-activity relationships. It is this that keeps us interested in the subject.

Dioxanes and dioxolanes are made from aldehydes and alkyl diols. Many toxicologists have a fondness for explaining the chemistry of action in terms of conversion to more toxic compounds. We feel that one must have strong evidence for conversion. Otherwise it is preferable to assume that the molecule under discussion is toxic in its own right.

We measured the rate of hydrolysis of the dioxanes to aldehydes and got wide variability when this was related to toxicity. To be sure, the aldehydes are toxic, but there seems to be no valid excuse for invoking aldehydes to explain the toxicity of the dioxanes or dioxolanes. The diols are too weak to be considered.

The methylenedioxyphenyl derivatives must be considered separately from dioxolane even though they may be thought of as dioxolanes condensed at positions 4- and 5- to a benzene ring. We have to consider them separately because all of our dioxolanes are substituted on the number 2-carbon. None of our methylenedioxybenzenes is. The methylenedioxybenzenes are all substituted on the number 6- carbon on the benzene ring.

Apparently, the most toxic are the piperonyl alcohol derivatives. In fact, the only compound that reached a toxicity index of 300 for *Stemphylium* was a piperonyl alcohol derivative.

We were pleased to note that in this context pisatin, the phytoalexin, can be considered as a derivative of piperonyl alcohol. Since the methylenedioxyphenyl nucleus occurs rather widely in nature, other investigators might well look for it in cases of natural resistance.

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Appendix A. Number, name, source, and toxicity index of dioxanes

Code No.	Name	Source	Toxicity Index St.	Mo.
3512	1,3-Dioxane,2-hexyl	USDA	201	114
3520	1,3-Dioxane,4-methyl-2-nonyl	USDA	1	83
3524	1,3-Dioxane,2-hexyl-4,6-dimethyl	USDA	50	101
3529	1,3-Dioxane,5-ethyl-2-hexyl-4-propyl	USDA	0	0
3533	1,3-Dioxane,5-ethyl-2-(2-furyl)-4-propyl	USDA	50	207
3535	1,3-Dioxane,2-(4-methoxyphenyl)-4,6-dimethyl	USDA	50	137
3536	1-3-Dioxane,5-ethyl-2-(4-methoxyphenyl)-4-propyl	USDA	0	53
3537	1,3-Dioxane,5-ethyl-2-methyl-2,4-dipropyl	USDA	24	92
3549	1,3-Dioxane,4,6-dimethyl-2-nonyl	USDA	2	6
3554	1,5-Dioxaspiro-[5.5]-undecane,2-isopropyl-3,3-dimethyl	USDA	1	187
3556	1,3-Dioxane,2-hexyl-2,4-dimethyl	USDA	1	0
3560	1,5-Dioxaspiro-[5.5]-undecane,3-ethyl-2-propyl	USDA	34	176
3564	1,3-Dioxane,2-hexyl-4,4,6-trimethyl	USDA	0	100
3588	1,3-Dioxane,2-ethyl-4-isopropyl-2,5,5-trimethyl	USDA	0	1
3595	1,3-Dioxane,2-(4-methoxyphenyl)-4-methyl	USDA	100	106
3652	1,3-Dioxane,5-ethyl-2-nonyl-4-propyl	USDA	0	0
3802	1,5-Dioxaspiro-[5.5]-undecane,2-methyl	USDA	0	0
3803	1,5-Dioxaspiro-[5.5]-undecane-3-ol,8-methyl	USDA	124	121
3804	1,5-Dioxaspiro-[5.5]-undecane-3-ol,9-methyl	USDA	133	120
3805	1,3-Dioxane,4,4,6-trimethyl-2-nonyl	USDA	0	0
3851	1,3-Dioxane,2-(2-furyl)	USDA	67	83
3852	1,3-Dioxane,2-(4-chlorophenyl)-4-methyl	USDA	55	200
3853	1,3-Dioxane,2-(2-furyl)-4-methyl	USDA	100	101
3855	1,3-Dioxane,2,4-dimethyl-2-propyl	USDA	100	100
3860	1,3-Dioxane,4-methyl-2-phenyl	USDA	100	100
3861	1,3-Dioxane,2-(2-furyl)-5,5-dimethyl	USDA	98	101
3862	1,3-Dioxane,2-(2-furyl)-4,6-dimethyl	USDA	100	100
3866	1,3-Dioxane-2,4,6-trimethyl-2-propyl	USDA	0	83
3869	1,3-Dioxane,2-ethyl-2,4-dimethyl	USDA	0	9
3870	1,3-Dioxane,2-ethyl-2,4,6-trimethyl	USDA	13	47
3872	1,3-Dioxane,2-styryl	USDA	200	200
3874	1,3-Dioxane,2-hexyl-2,4,6-trimethyl	USDA	50	142
3882	1,3-Dioxane,2-(2-chlorophenyl)-5,5-dimethyl	CBCC	0	223
3884	1,3-Dioxane,2-ethyl-2,5-dimethyl-5-nitro	CBCC	50	103
3891	1,3-Dioxane,2-(4-chlorophenyl)-4,6-dimethyl	CBCC	100	200
3905	1,3-Dioxane,2-(2-methoxyphenyl)-4,6-dimethyl	USDA	100	100
3911	Guaiacol,4-(4-methyl-2-*m*-dioxanyl)	USDA	103	243
3926	1,3-Dioxane,2,5-diethyl-2-methyl-5-nitro	CBCC	30	100
3928	1,5-Dioxaspiro-[5.5]-undecane,3-methyl-3-nitro	CBCC	0	0
3929	1,3-Dioxane,2-(4-chlorophenyl)-4-isopropyl-5,5-dimethyl	CBCC	0	30
3930	1,3-Dioxane,2-(4-methoxyphenyl)-4,4,6-trimethyl	USDA	0	98
3931	6,10-Dioxaspiro-[4.5]-decane,7,9-dimethyl	USDA	5	50
3932	1,3-Dioxane,2-(2-chlorophenyl)	USDA	75	198
3933	1,3-Dioxane,5-ethyl-5-nitro-2-phenyl	USDA	0	110
3934	1,3-Dioxane,5-methyl-5-nitro-2-phenyl	CBCC	0	0
3935	1,3-Dioxane,2-ethyl-2,5,5-trimethyl	USDA	1	5
3936	1,3-Dioxane,2-(4-chlorophenyl)-5,5-dimethyl	CBCC	9	111
3937	1,3-Dioxane,4,4,6-trimethyl-2-styryl	USDA	68	46
3938	1,3-Dioxane,4-methyl-2-styryl	USDA	200	200

Appendix A. Number, name, source, and toxicity index of dioxanes (continued)

Code No.	Name	Source	Toxicity Index St.	Mo.
3939	1,3-Dioxane,2-(4-methoxyphenyl)-5-methyl-5-nitro	CBCC	0	0
3941	1,3-Dioxane,2-(4-chlorophenyl)	USDA	196	207
3942	1,3-Dioxane,2-(4-methoxyphenyl)	USDA	100	143
3943	1,3-Dioxane,2,5,5-trimethyl-2-propyl	CBCC	29	29
3945	1,3-Dioxane,5-ethyl-2-phenyl-4-propyl	USDA	18	92
3946	1,3-Dioxane,5,5-dimethyl-2-phenyl	USDA	65	154
3947	1,5-Dioxaspiro-[5.5]-undecane,2,2,4-trimethyl	USDA	50	105
3949	1,3-Dioxane,2-(4-chlorophenyl)-5-ethyl-4-propyl	CBCC	0	23
3951	1,3-Dioxane,2-(2-chlorophenyl)-4,6-dimethyl	USDA	100	200
3952	1,3-Dioxane,4,6-dimethyl-2-styryl	USDA	167	200
3954	1,3-Dioxane,2-(2-chlorophenyl)-5-ethyl-4-propyl	CBCC	0	3
3955	1,3-Dioxane,5-butyl-5-ethyl-2-methyl-2-propyl	USDA	0	130
3956	6,10-Dioxaspiro-[4.5]-decane,8-butyl-8-ethyl	USDA	0	161
3957	1,3-Dioxane,2-(4-chlorophenyl)-4,4,6-trimethyl	USDA	25	193
3958	1,3-Dioxane,2-(2-chlorophenyl)-4-methyl	CBCC	53	153
3959	1,3-Dioxane,5-butyl-5-ethyl-2-hexyl	USDA	0	0
3960	1,3-Dioxane,2,5-diethyl-2-methyl-4-propyl	USDA	2	81
3961	1,3-Dioxane,2-(2-chlorophenyl)-4-isopropyl-5,5-dimethyl	CBCC	0	0
3962	1,3-Dioxane,5-butyl-5-ethyl-2-(4-methoxyphenyl)	USDA	0	0
3963	6,10-Dioxaspiro-[4.5]-decane,7-methyl	USDA	62	57
3964	1,3-Dioxane,2-(2-chlorophenyl)-4,4,6-trimethyl	USDA	5	196
3965	1,3-Dioxane,5-ethyl-4-propyl-2-styryl	USDA	5	0
3966	6,10-Dioxaspiro-[4.5]-decane,8-ethyl-8-nitro	CBCC	101	127
3967	6,10-Dioxaspiro-[4.5]-decane,8-ethyl-7-propyl	USDA	0	100
3968	1,3-Dioxane,2-nonyl	USDA	0	71
3969	1,3-Dioxane,5-ethyl-5-nitro-2-propenyl	CBCC	101	161
3971	1,3-Dioxane,5-methyl-5-nitro-2-propenyl	USDA	101	164
3973	1,3-Dioxane,4,6-dimethyl-2-phenyl	USDA	100	100
3974	1,3-Dioxane,4,4,6-trimethyl-2-propenyl	USDA	50	69
4002	1,3-Dioxane,5-methyl-5,nitro-2-propyl	CBCC	100	140
4005	1,3-Dioxane,5-butyl-5-ethyl-2-propyl	USDA	0	100
4006	1,3-Dioxane,2-(4-methoxyphenyl)-5,5-dimethyl	USDA	0	61
4009	1,3-Dioxane,5-ethyl-2-methyl-5-nitro-2-propyl	CBCC	74	94
4016	1,3-Dioxane,5-ethyl-5-nitro-2-propyl	CBCC	128	171
4036	1,3-Dioxane,4,4,6-trimethyl-2-propyl	USDA	0	22
4040	1,3-Dioxane,2-(chloromethyl)-5,5-dimethyl	CBCC	0	52
4042	1,3-Dioxane,5-butyl-5-ethyl-2-phenyl	USDA	36	33
4043	1,3-Dioxane,2-benzyl-2,4,6-trimethyl	USDA	53	139
4045	1,3-Dioxane,5-ethyl-2,4-dipropyl	USDA	2	79
4046	1,3-Dioxane,2,5-dimethyl-5-nitro-2-propyl	CBCC	81	100
4047	1,3-Dioxane,2,5,5-trimethyl-2-phenyl	USDA	0	100
4048	1,3-Dioxane,2-benzyl-2-methyl	USDA	100	100
4050	6,10-Dioxaspiro-[4.5]-decane,8-methyl-8-nitro	USDA	65	149
4051	1,3-Dioxane,2-(acetic acid)-2,5,5-trimethyl-ethyl ester	USDA	0	122
4053	1,3-Dioxane,2-benzyl-2,4-dimethyl	USDA	67	100
4054	1,3-Dioxane,5,5-dimethyl-2-nonyl	USDA	0	5
4056	1,3-Dioxane,5,5-dimethyl-2-propenyl	USDA	73	92
4058	1,3-Dioxane,5,5-dimethyl-2-propyl	USDA	9	0

Appendix A. Number, name, source, and toxicity index of dioxanes (continued)

Code No.	Name	Source	Toxicity St.	Index Mo.
4059	1,5-Dioxaspiro-[5.5]-undecane,3-butyl-3-ethyl	USDA	7	214
4061	1,3-Dioxane,2-benzyl-5-butyl-5-ethyl-2-methyl	USDA	6	0
4062	1,3-Dioxane,4,6-dimethyl-2-propyl	USDA	155	167
4063	1,5-Dioxaspiro-[5.5]-undecane,9-methyl	USDA	32	76
4064	1,3-Dioxane,2-benzyl-2,5,5-trimethyl	USDA	100	101
4066	1,3-Dioxane,2-(chloromethyl)-5-ethyl-4-propyl	CBCC	9	125
4067	1,3-Dioxane,2-(chloromethyl)-4-methyl	USDA	57	50
4068	1,5-Dioxaspiro-[5.5]-undecane,3,3-dimethyl	USDA	53	64
4069	1,3-Dioxane,2-benzyl-5-ethyl-2-methyl-4-propyl	USDA	34	31
4165	1,3-Dioxane,2,4,6-trimethyl-2-phenyl	CBCC	0	32
4171	1,3-Dioxane,4-methyl-2-propenyl	CBCC	50	157
4186	1,3-Dioxane,5-ethyl-2-methyl-2-phenyl-4-propyl	CBCC	0	45
4187	1,3-Dioxane,5-butyl-5-ethyl-2-methyl-2-phenyl	CBCC	5	7
4189	1,3-Dioxane,2-(acetic acid),2,4,6-trimethyl-, ethyl ester	CBCC	7	14
4192	1,3-Dioxane,2,4,4,6-tetramethyl-2-phenyl	CBCC	54	50
4195	1,3-Dioxane,2-(acetic acid),5-ethyl-2-methyl-4-propyl, ethyl ester	CBCC	3	122
4197	1,3-Dioxane,2-(acetic acid),2,4-dimethyl, ethyl ester	CBCC	83	79
4206	1,3-Dioxane,5-ethyl-2-propenyl-4-propyl	CBCC	36	101
4210	1,3-Dioxane,5-butyl-5-ethyl-2-(1-propenyl)	CBCC	8	184
4211	1,3-Dioxane,2-(acetic acid),2,4,4,6-tetramethyl-, 2-methyl,ethyl ester	CBCC	8	159
4212	1,3-Dioxane,2-(acetic),2,4,4,6-tetramethyl-, ethyl ester	CBCC	23	119
4214	1,3-Dioxane,4,6-dimethyl-2-propenyl	CBCC	0	27
4215	1,3-Dioxane,5-butyl-5-ethyl-2-styryl	CBCC	20	3
4222	1,3-Dioxane,5-butyl-5-ethyl-2-hexyl-2-methyl	CBCC	0	0
4223	1,3-Dioxane,5-butyl-2,5-diethyl-2-methyl	CBCC	0	50
4229	1,3-Dioxane,5-ethyl-2-(4-methoxyphenyl)-5-nitro	CBCC	0	0

Appendix B. Number, name, source, and toxicity index of dioxolanes

Code No.	Name	Source	Toxicity St.	Index Mo.
745	1,3-Dioxolane,2-methyl-4-oxo-5-phenyl	Monsanto	210	100
1933	1,3-Dioxolane,2-oxo	Jefferson	36	22
2835	1,3-Dioxolane,4-methanol-2,2-dimethyl	CBCC	50	92
3350	1,3-Dioxolane,2-oxo-4-hydroxyethyl	CBCC	46	0
3532	1,3-Dioxolane,2-nonyl	USDA	121	200
3545	1,3-Dioxolane,2-hexyl-4-methyl	CBCC	105	151
3562	1,3-Dioxolane,4-methyl-2-nonyl	USDA	1	15
3565	1,3-Dioxolane,4,5-dimethyl-2-nonyl	USDA	0	4
3585	1,3-Dioxolane,2-(4-methoxyphenyl)-4,5-dimethyl	USDA	100	100
3586	1,4-Dioxaspiro-[4.5]-decane	CBCC	103	78
3601	1,3-Dioxolane,2,4,5-trimethyl-2-propyl	CBCC	0	0
3610	1,3-Dioxolane,2-hexyl-4,5-dimethyl	USDA	109	100
3716	Carbonic acid, trithiocyclic ester with ethylene glycol	CBCC	200	200
3799	1,3-Dioxolane,2-hexyl	USDA	130	100
3800	1,4-Dioxaspiro-[4.5]-decane,2,3-dimethyl	USDA	194	101
3801	1,4-Dioxaspiro-[4.5]-decane,2-methyl	CBCC	193	104
3854	1,3-Dioxolane,2-(2-furyl)-4-methyl	USDA	100	100
3856	1,3-Dioxolane,2-(2-chlorophenyl)-4,5-dimethyl	USDA	50	145
3857	1,3-Dioxolane,2-(4-methoxyphenyl)-4-methyl	USDA	100	100
3858	1,3-Dioxolane,2-(4-chlorophenyl)	USDA	200	200
3859	1,3-Dioxolane,2-(2-chlorophenyl)-4-methyl	USDA	100	114
3863	1,3-Dioxolane,4,5-dimethyl-2-phenyl	USDA	100	104
3868	1,3-Dioxolane,2-(2-furyl)-4,5-dimethyl	CBCC	103	100
3873	1,3-Dioxolane,2-hexyl-2-methyl	CBCC	100	200
3876	1,3-Dioxolane,2-(4-chlorophenyl)-4,5-dimethyl	CBCC	200	247
3880	1,3-Dioxolane,2-(4-chlorophenyl)-4-methyl	CBCC	216	209
3883	1,3-Dioxolane,4,5-dimethyl-2-styryl	USDA	172	200
3886	1,3-Dioxolane,2-(2-chlorophenyl)	CBCC	100	200
3906	1,3-Dioxolane,2-(2,3-dimethoxyphenyl)	USDA	100	100
3907	1,3-Dioxolane,2-(2-methoxyphenyl)-4,5-dimethyl	USDA	100	100
3927	1,3-Dioxolane-2-propionic acid,2,4,5-trimethyl, ethyl ester	USDA	50	0
3944	1,3-Dioxolane-2-propionic acid,2,4-dimethyl, ethyl ester	USDA	136	67
3948	1,3-Dioxolane,4-methanol-2-propenylacetate	USDA	100	123
3953	1,3-Dioxolane,4-methanol-2-(*o*-chlorophenyl)-acetate	CBCC	100	100
3970	1,3-Dioxolane,2-(4-methoxyphenyl)	CBCC	100	100
4004	1,3-Dioxolane,4-methyl-2-propyl	CBCC	153	91
4034	1,3-Dioxolane,4,5-dimethyl-2-propyl	CBCC	75	17
4041	1,4-Dioxaspiro-[4.5]-decane,2,3,8-trimethyl	CBCC	0	0
4044	6,10-Dioxaspiro-[4.5]-decane-8,8-dimethyl	USDA	0	43
4052	1,3-Dioxolane,2-benzyl-2,4-dimethyl	USDA	100	100
4055	1,3-Dioxolane,2-benzyl-2-methyl	USDA	100	100
4057	1,4-Dioxaspiro-[4.5]-decane,2,8-dimethyl	USDA	30	27
4060	1,3-Dioxolane-4-methanol,2-(4-chlorophenyl)-acetate	CBCC	37	106
4065	1,3-Dioxolane,2-benzyl-2,4,5-trimethyl	CBCC	100	104
4168	1,3-Dioxolane,4,5-dimethyl-2-propenyl	CBCC	0	77
4180	1,3-Dioxolane,4-methyl-2-propenyl	CBCC	127	16

Appendix B. Number, name, source, and toxicity index of dioxolanes (continued)

Code No.	Name	Source	Toxicity Index St.	Mo.
4188	1,3-Dioxolane,4-methyl-2-styryl	CBCC	150	175
4196	1,3-Dioxolane-2-acetic acid,2,4-dimethyl-, ethyl ester	CBCC	3	0
4198	1,3-Dioxolane-2-acetic acid,2,4,5-trimethyl-, ethyl ester	CBCC	16	0
4213	1,3-Dioxolane,2,4,5-trimethyl-2-phenyl	CBCC	0	3
4218	1,3-Dioxolane,2,4-dimethyl-2-phenyl	CBCC	2	0
4219	1,3-Dioxolane-2-acetic acid, 2-methyl-,ethyl ester	CBCC	100	100
4221	1,3-Dioxolane,2-styryl	CBCC	194	200
4228	1,3-Dioxolane,2-methyl-2-phenyl	CBCC	0	25

Appendix C. Number, name, source, and toxicity of methylenedioxybenzenes

Code No.	Name	Source	Toxicity Index St.	Mo.
1947	3,4-Methylenedioxy-(β-nitro)styrene	CBCC	347	350
2376	Piperonyloin	CBCC	0	5
2410	3,4-Methylenedioxy cinnamic acid	CBCC	127	135
2417	Homopiperonylic acid	CBCC	240	179
2419	5-H-Indeno-[5.6]-1,3-dioxol-5-one-6,7-dihydro	CBCC	106	8
2909	Piperonal oxime	CBCC	150	162
3092	Benzoic acid, piperonylidenehydrazide	CBCC	0	0
3100	*p*-Nitrobenzoic acid, piperonylidene hydrazide	CBCC	0	0
3276	2-Oxazoline-5-one,2-phenyl-4-piperonylidene	CBCC	0	0
3904	Methylenedioxybenzene,6-[2-(1,3-dioxane)-5-ethyl-6-propyl]	USDA	0	21
3908	Methylenedioxybenzene,6-[2-(1,3-dioxolane)-4,5-dimethyl]	CBCC	100	142
3910	Piperonyl alcohol, fencholic acid ester	CBCC	102	100
3919	Piperonyl alcohol, α-butyl	CBCC	118	200
3920	Piperonyl alcohol, pivalic acid ester	CBCC	0	180
3923	Piperonyl alcohol, senecioic acid ester	CBCC	107	238
3988	Piperonyl alcohol, α-pentyl	CBCC	121	214
3989	Piperonyl alcohol, α-*tert*-butyl-,acetate	CBCC	85	149
3994	Piperonyl alcohol, α-*tert*-butyl	CBCC	182	200
3996	Methylenedioxyphenyl,6-[2-(1,3-dioxane)-4-isopropyl-5,5-dimethyl]	USDA	1	79
4008	Piperonyl alcohol α-propyl acetate	CBCC	47	200
4010	Piperonyl alcohol, chrysanthemum monocarboxylic acid ester	CBCC	4	19
4019	Piperonyl alcohol, α-propyl propionate	CBCC	21	100
4022	Piperonyl alcohol, α-iso-propyl acetate	CBCC	50	150
4032	Piperonyl alcohol, α-benzyl acetate	CBCC	2	220
4035	Piperonyl alcohol, α-(2-propenyl)	CBCC	122	158
4117	Piperonal thiosemicarbazone	CBCC	10	17
4122	3,4-Methylenedioxy-α-cyanocinamic acid, ethyl ester	CBCC	4	16
4131	α-*tert*-butylpiperonyl alcohol, phenylacetic acetate	CBCC	0	0
4239	α-Methylpiperonyl alcohol, chrysanthemum-monocarboxylic acid ester	CBCC	107	122
4241	α-Cyclohexylpiperonyl alcohol, chrysanthemum-monocarboxylic acid ester	CBCC	9	51
4703	Isosafrole	CBCC	97	100
4711	Safrole	CBCC	75	38
5428	Piperonal	EK	104	110

Appendix D. Sources of test compounds

Code	Source
Bobbio	Dr. F. O. Bobbio, Instituto Zimotecnia Universidade de Sao Paulo, Sao Paulo, Brazil
C & C	Union Carbide Corporation, New York, N. Y.
CBCC	Chemical Biological Coordination Center, National Academy of Sciences, Washington, D. C. (not now extant)
Com'l Solvents	Commercial Solvents Corporation, New York, N. Y.
EK	Eastman Kodak Co., Rochester, N. Y.
Jefferson	Jefferson Chemical Co., Inc., Houston, Texas
Koppers	Koppers Company, Inc., Verona, Pa.
Monsanto	Monsanto Chemical Co., St. Louis, Mo.
R & H	Rohm & Haas Co., Philadelphia, Pa.
Shell	Shell Development Co., Modesto, Calif.
USDA	Entomology Research Division, U. S. Dept. of Agr., Beltsville, Md.

We express our thanks to these suppliers of our test compounds.

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