Effect of Alcohols on the Stability of Iprodione in Solution

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Both primary and secondary alcohols degrade iprodione, 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide. Steric hindrance has been found to have an inverse effect on the rate of its decomposition, and a fully substituted alcohol, such as *tert*-butanol, does not degrade iprodione due to extreme steric hindrance. The instability of iprodione in alcohol was found to be a function of the structure of the alcohol. The product, N-(3,5-dichlorophenyl)-3-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide, is obtained from all of the reacting alcohols. Confirmation of this structure came from the consideration of its NMR, mass spectral, and elemental analysis data.

INTRODUCTION

Iprodione, or 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide is a widely used fungicide for the control of phytopathogenic fungi (*1, 2*). It is used (*1*) on vine and table grapes, fruit trees, almonds, berries, cereals, cotton, potatoes, vegetables, flowers, ornamentals, and oil seed plants as a foliar spray. It also has applications (*1*) for the treatment of seeds of cereals and vegetables and for crops such as sugar beets, rice, rapeseed, and sunflowers.

The residues of iprodione, as well as those of many other chemicals, in food items are being monitored by the United States Department of Agriculture (USDA). This monitoring program, known as the Pesticide Data Program (PDP), gathers valuable food safety-related data (*3–7*) through the collection of samples and their analyses in selected state laboratories (e.g. Ohio Department of Agriculture) and in several federal facilities.

Iprodione is a very useful agricultural chemical, and its residue is commonly found in plums (*3*), grapes (*3–5*), carrots (*4, 5, 7*), strawberries (*6*), nectarines (*5*), cherries (*5*), and other produces (*8*). This chemical has shown stability problems during analysis when it is in methanol-containing solvents, thereby requiring our laboratory to avoid alcoholic solvents for the extensive iprodione related analytical assays. A similar stability problem with methanol was also indicated by others (*9*). These observations have prompted the initiation of a comprehensive study to ascertain the effects of methanol

and several other alcohols on the stability of this chemical. In this study, primary, secondary, and tertiary alcohols have been included.

MATERIALS AND METHODS

General Methods. *Chemicals and Consumables.* Iprodione of 99% purity was purchased from Chem Service Inc. (West Chester, PA). Anhydrous grades of benzyl alcohol, 1-butanol, 2-butanol, *tert*-butanol, ethanol, methanol, 2-methyl-1-propanol, 1-propanol, 2-propanol, 1-hexanol, and 1-octanol were purchased from Sigma-Aldrich, Inc. (St Louis, MO). EM Science brand silica gel 60 for column chromatography and Silica Gel 60 F_{254} coated plates (EM-15327–1) for thin layer chromatography were purchased from VWR International (Bristol, CT).

Analytical Instruments. *(a) General.* Nuclear Magnetic Resonance (NMR) spectra were recorded in $CDCl_3$ on a BRUKER DRX-600 spectrometer operating at 600 and 150 MHz for ^{1}H and ^{13}C, respectively. The NMR-SCAPE software program, a product of Spectrum Research, LLC (Madison, Wisconsin), was used for ^{13}C chemical shift calculations. Electrospray ionization mass spectroscopy (ESI-MS) was performed with a Micromass Quattro triple-quadrupole mass spectrometer using 5% aqueous acetonitrile as the solvent. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Evaporation of reacting solvents was carried out under vacuum using a Buchi rotary evaporator at temperatures below 33 °C.

(b) Gas Chromatography−Mass Spectrometry (GC-MS) Instrument. GC Acquisition Parameters. Instrument: Agilent 6890N gas chromatograph; column: HP-1-MS; column dimension: 30 m, 0.25 mm ID, 0.25 μm film thickness; carrier: helium at constant flow 1 mL/min; oven temperature program: 70 °C initial temperature (hold 1 min), 70 to 230 °C at 10 °C/min (hold 6 min), 230 to 265 °C at 25 °C/min (hold 15 min); run time: 39.40 min; injection: splitless, 250 °C; injection volume: 1 μL.

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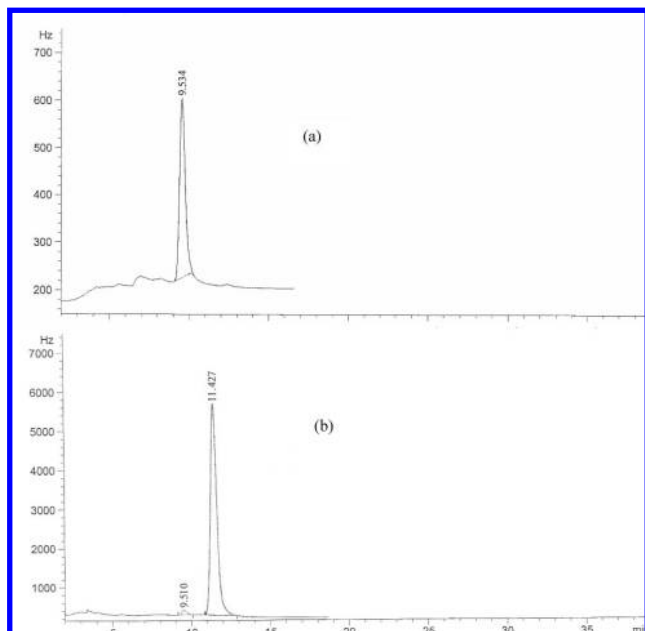† Ohio Department of Agriculture.
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Figure 1. (**a**) Chromatogram of an analytical standard (10 ng) of iprodione; (**b**) chromatogram of a iprodione (15 ng) in methanol after 192 h at 25 °C.

Table 1. Decomposition of Iprodione by Alcohols[a]

reactant	temp (°c)	reaction time (hour)	percentage of decomposition (%)
methanol	2	96	33
methanol	2	450	98
methanol	25	192	100
methanol	47	2	95
methanol + 1% water	25	96	98
ethanol	25	120	98
ethanol	45	7	95
ethanol + 1% water	25	144	95
2-propanol	25	120	19
2-propanol	47	4	4
2-propanol + 1% water	25	144	0
1-propanol	25	96	95
1-propanol + 1% water	25	168	61
1-butanol	25	168	100
1-butanol + 1% water	25	168	20
2-butanol	25	168	5
tert-butanol	25	168	0
1-hexanol	25	168	98
1-hexanol + 1% water	25	168	68
2-methyl-1-propanol	25	168	96
2-methyl-1-propanol + 1% water	25	168	25
1-octanol	25	360	92
1-octanol + 1% water	25	168	25
benzyl alcohol	25	120	8

[a] Percentage of decomposition was determined by GC analysis of an aliquot of the reaction mixture. No decomposition of iprodione occurred after 1 year if its solution in t-butanol was stored at 25 °C. The percentage of decomposition data is from single experiments. Complete decomposition of iprodione to its isomeric product occurred when its solution in benzyl alcohol was stored at 25 °C for one year.

MS Acquisition Parameters. Detector: Agilent 5973n mass selective detector; detector tune: maximum sensitivity (Atune.U), acquisition mode: scan; transfer line temp: 280 °C; Ms source temp: 230 °C; Ms quad temp: 150 °C; solvent delay: 3 min; mass scan range: 70 – 450 amu; scan threshold: 25; instrument control system: enhanced MSD Chemstation-D01.00 Build 75.

   (c) Gas Chromatogrpahy (GC) Instrument. GC Acquisiton Parameters. Instrument: Agilent 6890N gas chromatograph; column: JW DB-1; column dimension: 30 m, 0.53 mm ID, 1.5 μm film thickness; carrier:
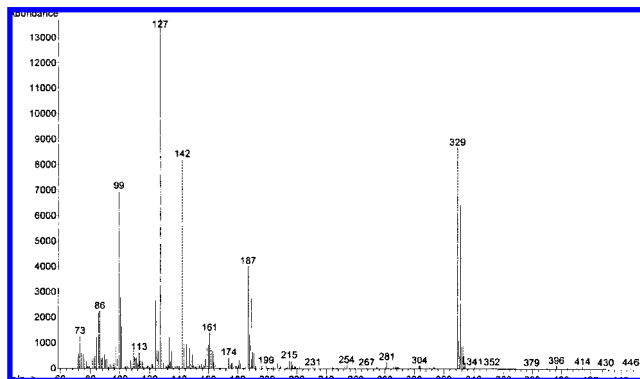

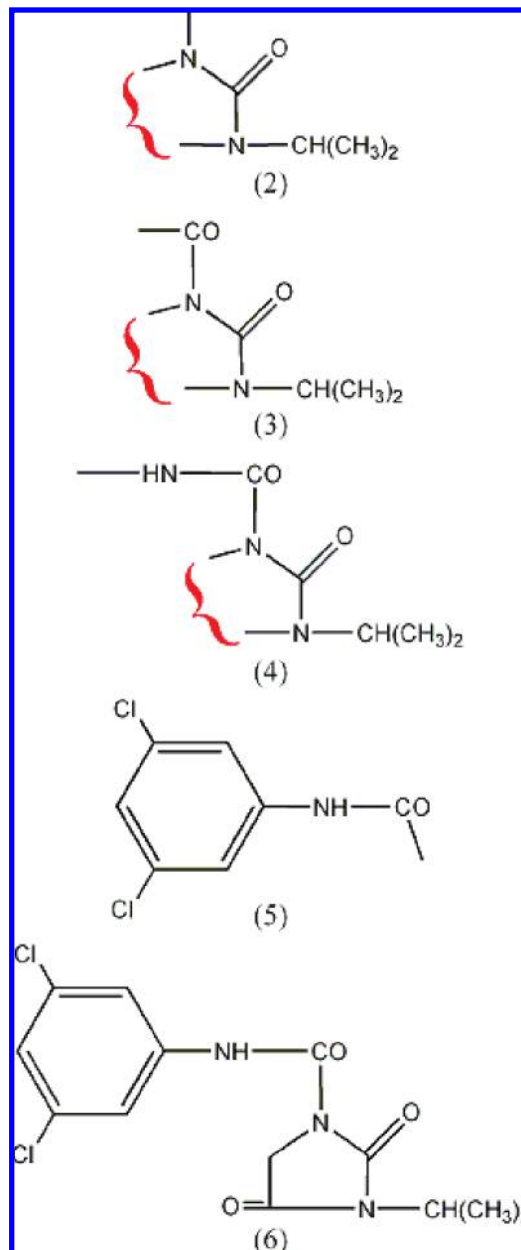**Figure 2.** Mass spectrum of methanol derived product of iprodione.

Figure 3. Structures consistent with mass spectral information of the product of iprodione with methanol.

helium at 18.8 mL/min measured at 140 °C; oven temperature program: 140 °C initial temperature, 140 to 220 °C at 30 °C/min, 220 °C (hold 20 min); detector: Agilent G2397A electron capture detector; detector

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Table 2. Proton Chemical Shifts of Compound **6**

	proton chemical shifts		
	chemical		coupling
proton	shift (δ, ppm)	multiplicity[a]	constant (J, Hz)
2 CH$_3$ (isopropyl)	1.45	d	J$_{CH3, CH}$ = 7
CH (isopropyl)	4.40	p	
H-5	4.30	s	
NH	9.99	s	
H-2′ or H-6′	7.46	d	J$_{2′,4′}$ = J$_{6′,4′}$ = 1.5
H-4′	7.10	d	

[a] Singlet, s; doublet, d; triplet, t; pentad, p.

Table 3. Carbon chemical shifts of compound **6**

	carbon chemical shift
carbon No.	chemical shift (δ, ppm)
2 CH$_3$ (isopropyl)	19.4
CH (isopropyl)	44.8
C-5	47.4
C-2	155.6
C-4′	124.4
C-4	167.26
NHC=O	148.0
C-2′ or C-6′	118.1
C-3′ or C-5′	135.4
C-4	167.2
C-1′	138.9

temperature: 300 °C; makeup gas: nitrogen at 60.0 mL/min; injection: splitless, 250 °C; injection volume: 2 μL.

Preparation of Alcoholic Solutions of Iprodione and Its Stability Monitoring. Iprodione (40 mg) was dissolved in 20 mL of an alcohol at the temperature of the study. For studies at elevated temperatures, solutions were made initially at 25 °C, after which the temperature was raised to the desired level. The occurrence of change of iprodione concentration in its solution was monitored by the analysis of aliquots with a gas chromatograph equipped with an electron capture detector (ECD) and also by thin layer chromatography (TLC) on silica gel plates. An aliquot's gas chromatographic and mass spectral data, as well as melting points of isolated products, were used to identify the product of a reaction.

Thin Layer Chromatography (TLC). Thin layer chromatography was performed on Silica Gel 60 F$_{254}$ precoated plates (size: 2.5 × 7.5 cm; layer thickness 250 μm). These plates were developed with hexane/ethyl acetate (2:1, v/v). The spots on the developed plates were located by exposure to UV light (254 nm). Iprodione and its products had R_f values of 0.48 and 0.70, respectively.

Column Chromatography. The column chromatography was performed with Silica Gel 60 (E. Merck) having a particle size of
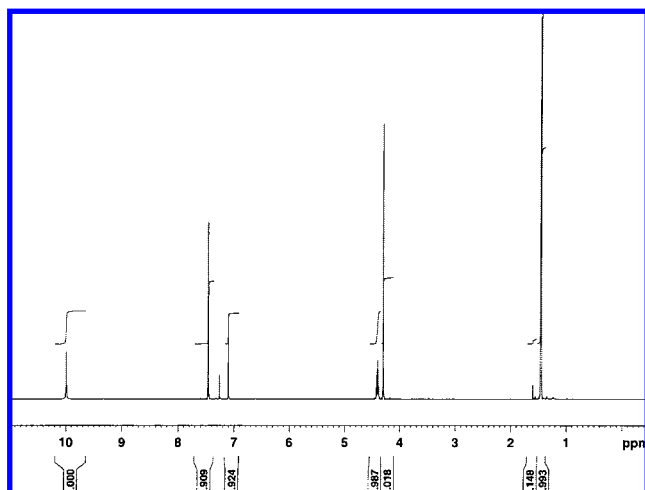
Figure 4. ^{1}H NMR spectrum of product from iprodione.
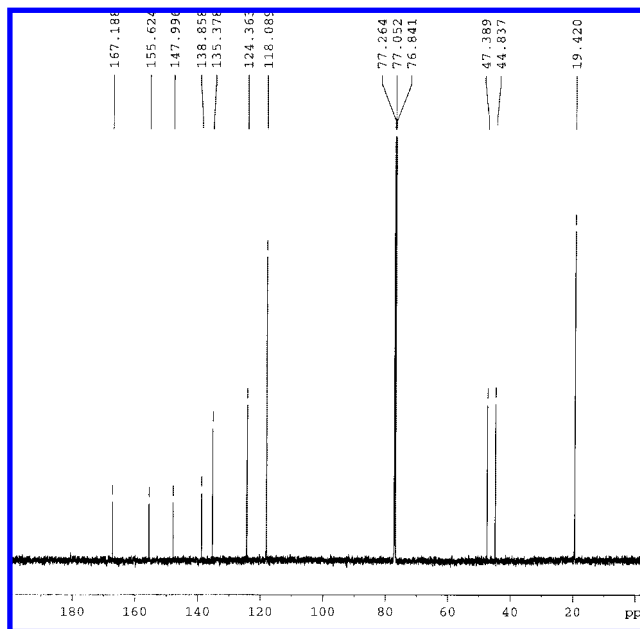
Figure 5. ^{13}C NMR spectrum of product from iprodione.
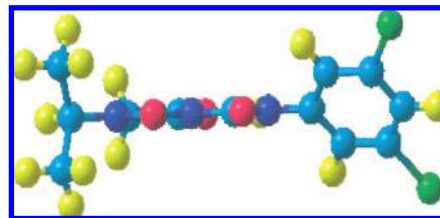
Figure 6. Molecular model of compound **6**.

70–230 mesh and with a glass column (40 × 1.5 cm) packed with this silica gel (35 g). The column was prewashed with hexane and was eluted with 300 mL of hexane/ethyl acetate (2:1 v/v). An automatic fraction collector (model CF-1, Spectrum Chromatography, Houston, TX) was used to collect the fractions. The presence of a chemical in various fractions was monitored by TLC and also by GC-ECD chromatography.
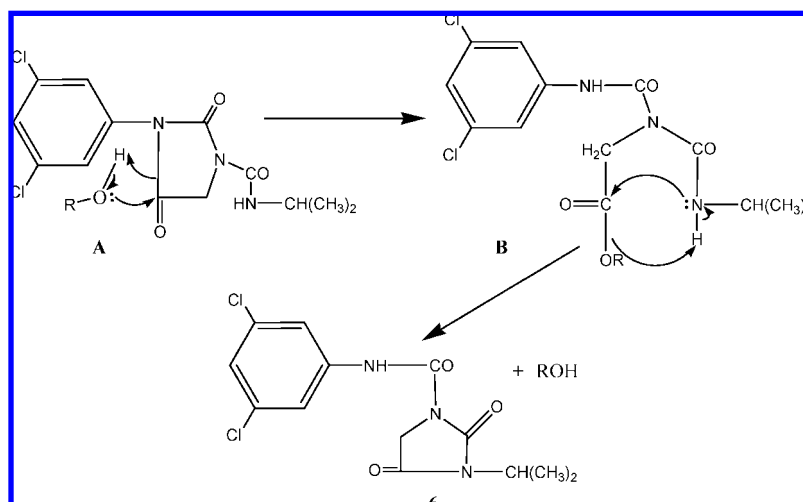
Isolation of Products. If a complete change of iprodione to a product took place, then the isolation of the product was accomplished simply by the evaporation of the solvent to a residue. In the event of an incomplete transformation, the purification step involved the evaporation of a solvent, except of benzyl alcohol, followed by column chromatography of the residue (200 mg) to yield pure product. The identification of the product from the reaction with benzyl alcohol was accomplished by gas chromatographic and mass spectral analysis of an aliquot of the reaction mixture. The retention times in the GC-ECD chromatogram and also in the GC-MSD total ion chromatogram for the benzyl alcohol generated product were found to be identical with corresponding retention times of the product from methanol. Similarly, these two products produced identical mass spectra. For column chromatography, a silica gel column was used. Methanol was used to crystallize the product.

RESULTS AND DISCUSSION

Degradation of Iprodione by Methanol. When a solution of iprodione in methanol was left at 25 °C, it gradually changed to a product having a longer gas chromatographic retention time (**Figure 1**). This change became complete or almost complete after 192 h at 25 °C or after 2 h at 47 °C. (**Table 1**).

The formation of the same product took place at a slower rate if the temperature was maintained at 4 °C. The product, after isolation and crystallization, had a melting point of 201

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Scheme 1. Mechanism of degradation of iprodione

°C and an ESI-MS ion at *m/z* 330.05 [M]$^+$. Anal. Calcd for C$_{13}$H$_{13}$Cl$_2$N$_3$O$_3$: C, 47.29; H, 3.97; Cl, 21.48; N, 12.73. Found: C, 47.40; H, 3.97; Cl, 21.48; N, 12.70.

Structures of the Product from Methanol. Insightful information regarding the structure of the product was derived from mass spectral and NMR data. Elemental analyses and ESI-MS-generated [M]$^+$ ion peak at *m/z* 330.05 established that the product is an isomer of iprodione.

Gas Chromatography–Mass Spectrometry. The GC-MS analysis was performed under electron impact (EI) mode. The mass spectrum (**Figure 2**) produced ions at *m/z* 99, 127, 142, and 188 that were consistent with the theoretical values for the partial structures **2**, **3**, **4**, and **5** (**Figure 3**), respectively. The appearance of these ions and another at *m/z* 329, due to [M−1]$^+$, lends conclusive support for the product to have structure **6** (**Figure 3**).

Nuclear Magnetic Resonance Spectrometry. Tables 2 and **3**, respectively, show the ^{1}H NMR and ^{13}C NMR data for the methanol-assisted decomposition product of iprodione. The assignment of chemical shift values for various hydrogen and carbon atoms was made on the basis of ^{1}H NMR spectrum, ^{13}C NMR spectrum, heteronuclear single quantum correlation (HSQC) experiment, heteronuclear multiple bond correlation (HMBC) experiment, and software-generated calculated values. Similar to the GC-MS data, the NMR data were consistent with the assignment of structure **6** for the product. As expected, the proton (**Figure 4**; **Table 2**) and carbon (**Figure 5**; **Table 3**) spectra showed signals for 13 hydrogen and 13 carbon atoms. The two methyl groups had identical proton and carbon resonances, indicating the magnetic equivalence of these two groups. Some other data (**Table 2**, **Table 3**) indicate that an identical NMR-related relationship also exists either between 2′ and 6′ or between 3′ and 5′ positions. The molecular model (**Figure 6**) of product **6** also predicts these three equivalences. The ^{1}H NMR signal due to the −NH− group of the product appeared as a singlet at δ 10.0, whereas the signal of iprodione, recorded under identical conditions, gives a doublet at δ 7.6. This downfield shift of the product's −NH− signal and its lack of coupling are in conformity with structure **6** for the product.

Thus, it is evident from the above that N-(3,5-dichlorophenyl)-3-(1-methylethyl)-2,4-dioxo-1-imidazolidine carboxamide (**6**) is the product from the reaction of iprodione with methanol. The same compound (**6**) was also reported to have been obtained from iprodione by its rearrangement in an ethanolic solution

(*9*) or by its chemical transformation in a sterile mineral medium (*10*). The cyclization of 3-(isopropylcarbamoyl)-5-(3,5-dichlorophenyl) hydantoic acid (*2*), the metabolism of iprodione in rats (*11*), the metabolism of iprodione in plants (*8*), or the reaction of 3-isopropylimidazolidine-2,4-dione with 3,5-dichlorophenylisocyanate (*9*) also produces **6**.

Additional Degradation Study. In addition to the methanol-related study, the solutions of iprodione in benzyl alcohol, 1-butanol, 2-butanol, *tert*-butanol, ethanol, 1-hexanol, 2-methyl-1-propanol, 1-propanol, 2-propanol, and 1-octanol were analyzed to determine its stability. The results of these analyses are summarized in **Table 1**. This table's data clearly indicate that, except for benzyl alcohol, all primary alcohols degrade iprodione quite rapidly. A much slower degradation was observed with benzyl alcohol. The presence of 1% water in methanol or ethanol did not slow down the degradation of iprodione. However, somewhat slower rates of reaction are observed if 1% water is present in 1-propanol, 1-butanol, 1-hexanol, 2-methyl-1-propanol, or 1-octanol. By comparison with the primary alcohols, the degradation by a secondary alcohol was found to be extremely slow, and no degradation occurred if 1% water is present in 2-propanol. *tert*-Butanol, the only tertiary alcohol available for the present study, did not show any adverse effect on the stability of iprodione. The lack of reaction of *tert*-butanol or very slow degradation by secondary alcohols is most likely due to the crowding or steric effects created by substituents on the hydroxyl group bearing a carbon atom.

Identification of Products. The identification of the products arising from iprodione's reaction with various alcohols was achieved by the comparison of their GC-ECD chromatograms and mass spectral information with the respective data of the product from methanol. Also, the melting points of the isolated products, as well as those of products' mixtures with the product from methanol, were considered for determining a product's identity. These comparisons did not show any discrepancy between the compared data, thereby proving that **6** is the common structure for all products, including the one from methanol.

Reaction Mechanism. The formation of a common product (**6**) from all of the reacting alcohols, the faster reaction rate of primary alcohols, except benzyl alcohol, the slower reaction rate of secondary alcohols, and the complete lack of reaction of *tert*-butanol point to a common reaction mechanism. The most likely mechanism of the transformation

of iprodione to **6** is outlined in **Scheme 1**, where the initial step is the formation of a transition state (A) involving the hydroxyl group of the reacting alcohol and a ring carbonyl group of iprodione. The ring opening from the transition state followed by simultaneous ring closure, as has been indicated in B, will then lead to the formation of **6**. The structure (B) must be transient in nature, because the chromatographic analyses of the reaction mixtures fail to detect its presence. The substitution of hydrogen atoms on the hydroxyl-bearing carbon atoms may create steric hindrance for the approach of an alcohol's hydroxyl group toward the carbonyl group of iprodione. The degree of this crowding, or steric, effect will be determined by the number and, to a lesser extent, by the size of the substituents. A highly substituted carbon will offer increased resistance toward the formation of the transition state (A) of **scheme 1**, thereby adversely affecting the change of iprodione to **6**. The above reasoning offers an acceptable explanation for all the experimental findings summarized in **Table 1**.

In conclusion, at the ambient temperature, both primary and secondary alcohols degrade iprodione, whereas it is stable in *tert*-butanol. The order of degradation rate is primary > benzyl > secondary. Also, the degradation rate is temperature dependent.

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