Degradation of N'-(4-Chloro-o-tolyl)-N,N-dimethylformamidine in Six Different Fruit

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Samples from plants which had been treated with varying rates of N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine (I) (Galecron or Fundal) were analyzed for residues of the parent compound and three anticipated degradation products at various intervals after the last application. Only I and one major degradation product, 4'-chloro-o-formotoluidide (III), were detected in any of the samples. Fruit having low residues of I (apples, pears, and cherries) also had low residues of III, while plums and strawberries had higher residues of both chemicals. Peaches were an exception to either case since they had the highest amount of I but very low residues of III. No correlation existed between the amounts of I and III to such variables as amount of chemical applied or sampling interval. The nature of the fruit and environmental factors were accredited as the governing factors affecting the formation and retention of III in the fruit samples. Both I and III disappeared readily from treated fruit, thus resulting in less than 1 ppm of total residue in all crops at harvest, except peaches, which had approximately 2 ppm of total residue. The chief factors which appeared to account for these decreases were weathering and growth dilution, rather than chemical or enzymatic degradation.

Formulations of N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine, (chlordimeform) (I), bearing the trade names of Galecron and Fundal, have recently been introduced as an agricultural pesticide for the control of mites on some fruit crops. The efficacy of this material for the control of many species of mites, including a number which are resistant to some of the organophosphorus acaricides, either as an adulticide or an ovicide, is described in research reports by Batiste and Berlowitz (1969), Batiste *et al.* (1970), Cone (1968), Dittrich (1966a,b, 1967a,b, 1969), Furr and Davis (1969), Jeppson *et al.* (1969), Shaw *et al.* (1968), Stafford (1968), Westigard (1969), Westigard and Berry (1970), and Wilson and Oliver (1969). This chemical has also demonstrated usefulness for the control of some insects affecting cole crops (Harris and Svec, 1970a,b; Judge and McEwen, 1970), corn (Klostermeyer, 1968), and cotton (Harding, 1970; Mitri and Kamel, 1970; Zeid *et al.*, 1968). The chemical, physical, and biological properties of chlordimeform are thoroughly described in technical bulletins supplied by the manufacturers (CIBA, 1967; NOR-AM, 1970). Excellent publications have appeared recently which pertain to the analysis of chlordimeform and its major degradation products in plants and soil (Geissbühler *et al.*, 1971; Kossmann *et al.*, 1971) and its metabolic fate in animals and plants (Knowles, 1970).

Sen Gupta and Knowles (1969) discovered that when ^{14}C-chlordimeform was injected into the stems of apple seedlings the chemical was slowly translocated into the leaves of these plants. Twenty days after the injection was made these workers identified four metabolites—N'-(4-chloro-o-tolyl)-N-methylformamidine (II), 4'-chloro-o-formotoluidide (III), 4-chloro-o-toluidine (IV), and N-(2-chlorophenyl-D-glucosylamine) (V)—as well as the unchanged parent compound in the stems and leaves of the treated plants. They observed the same organosoluble degradation products of I in leaves of apple seedlings when the chemical was applied topically to the leaves.

Ehrhardt and Knowles (1970) observed differences in the behavior of I when it was applied to the leaves of grapefruit seedlings as compared to its fate on apple seedling leaves. They recovered much less of the undegraded parent compound from treated citrus leaves during a similar time interval of exposure of the chemical to these leaves. A much higher concentration of unextractable, thus unidentified, radio-labeled residue occurred in the leaves of citrus, while the identified organosoluble radioactive metabolites in grapefruit leaves were generally similar to those isolated from apple seedlings. However, several minor unidentified metabolites were detected in grapefruit leaves which they speculated to be the salts of the corresponding identified bases. Notwithstanding certain variables such as environmental conditions, these researchers concluded that I is apparently more persistent when applied to apple leaves than to grapefruit leaves. They attributed this to morphological and physiological differences in the leaves of these two plant species which affect partition, volatility, solubility, and absorbability of the active ingredient I.

No information appeared in the literature regarding the persistence or fate of I on edible fruit produced from trees treated with this chemical for mite control until the recent report by Ercegovich *et al.* (1972). They reported that when this acaricide was applied according to good agricultural practices following the suggested label recommendations to crop plants of apples, cherries, peaches, pears, plums, and strawberries, residues of I decreased to a very low amount by the time the fruit was harvested and ready for consumption. Their findings revealed that the amount of total residue on these fruit crops was directly related to the amount of chemical applied, indirectly related to the function of the number of days the fruit was sampled after the last application of the chemical, and was influenced by the nature of the fruit surface. The results of their investigation were based on an analytical procedure which was not specific for the parent compound but was capable of measuring a number of possible degradation products in addition to the parent compound. This investigation, therefore, was conducted to determine more critically the nature of the chemical composition of the total residue derived through the use of I on apple, cherry, peach, pear, plum, and strawberry fruit at various intervals prior to and at harvest. The emphasis of this investigation was directed primarily to those degradation products which could be extracted by organic solvents since other workers have shown that the organosoluble degradation products overwhelmingly exceed the aqueous-soluble degradation products in concentration in several species of plant seedlings (Sen Gupta and Knowles, 1969; Ehrhardt and Knowles, 1970).

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Table I. Details of Spray Program to Which Fruit Samples Were Subjected

Crop	Rate of application, oz a.i./100 gal	Number of applications	Total oz a.i. applied per acre[a]	Orchard location
Apple	8	3	96	Pa.
Cherry	16	2	128	New York
Peach	16	2	64	New York
Pear	16	3	96	New York
Plum	8	2	48	Pa.
Strawberry	8	1	8	New Jersey

[a] Calculated in terms of rate of chemical used, number of applications, and total gallonage of spray applied during the season.

Table II. Recovery of Chlordimeform and Its Degradation Products from Fortified Fruit Samples

Crop	Average percent recovery[a] I[b]	II[b]	III[b]	IV[b]	Number of analyses
Apples	87.7	103.5	88.0	83.3	5
Cherries	93.1	98.9	90.8	81.3	4
Peaches	93.2	98.9	83.0	76.2	4
Pears	93.0	98.9	84.0	88.9	3
Plums	84.9	96.6	90.0	80.4	6
Strawberries	90.4	98.9	84.0	86.4	4

[a] Ten μg of each compound added to 60 g of fresh tissue. [b] I = *N'*-(4-chloro-*o*-tolyl)-*N*,*N*-dimethylformamidine; II = *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine; III = 4'-chloro-*o*-formotoluidide; IV = 4-chloro-*o*-toluidine.

Table III. ppm of Chlordimeform and Its Degradation Products Found in Whole Apples at Various Intervals after a Total Seasonal Application of 96 oz a.i./Acre

Interval, days	ppm found[a] I[b]	III[b]	ppm of total acaricidal residue[c]
30	0.75	0.04	0.80
45	0.50	0.12	0.64
60	0.41	0.12	0.55
90	0.23	0.11	0.36
107[d]	<0.04	0.28	0.33

[a] Compounds II and IV were not detected in any of the samples. [b] See footnote a, Table II. [c] ppm Total residue = [(ppm$_{I}$ × 1.0) + (ppm$_{II}$ × 1.27) + (ppm$_{III}$ × 1.15) + (ppm$_{IV}$ × 1.37)]. [d] Typical harvesting interval for mature fruit.

Table IV. ppm of Chlordimeform and Its Degradation Products Found in Whole Peaches at Various Intervals after a Total Seasonal Application of 64 oz a.i./Acre

Interval, days	ppm found[a] I[b]	III[b]	ppm of total acaricidal residue[c]
14	6.63	1.07	7.90
28	6.55	0.38	7.01
56	1.83	0.23	2.10
70[d]	1.60	0.20	1.84

[a] Compounds II and IV were not detected in any of the samples. [b] See footnote b, Table II. [c] See footnote c, Table III. [d] Typical harvesting interval for mature fruit.

MATERIALS AND METHODS

Source and Nature of Fruit Samples. Samples of apples, cherries, peaches, pears, plums, and strawberries were collected at various intervals after the last application from orchards and plants which had been treated with aqueous sprays of Galecron 50 EC, 4 lb a.i./gal. In each case the sprays were applied to established orchard plants by conventional high pressure or hydraulic orchard sprayers to thoroughly cover the crop plants to run off. Additional information regarding the rates of application, number of applications, total amount of active ingredient applied per acre, and the location of the application is included in Table I. All of the fruit samples were placed in a freezer immediately after collection and stored in a frozen state at −20 ±5°C until they were analyzed. Prior to analysis the stones or seeds were removed from the fruit and then the fleshy parts, including the fruit skin, were macerated in a Hobart food chopper. Sixty-gram aliquots of the macerates were removed for analyses, which were conducted in triplicate by the procedure described below.

Analytical Method. In addition to the apparatus and chemical reagents normally used for the analysis of total residue of **I** in crops (Geissbühler *et al.*, 1971) these investigations required thin-layer chromatography equipment, fluorescent indicator, green, M. (Woelm Co., New Orleans, La.), redistilled chloroform, benzene, and isopropyl alcohol, and reference standards of chemicals **II**, **III**, and **IV**. The latter materials were supplied by CIBA Agrochemical Co., Vero Beach, Florida. The purity and the chemical and physical properties of these chemicals are described by Kossmann *et al.* (1971).

The principle of the method used involved the recovery of the parent compound and its degradation products from the plant tissue by means of extraction with organic solvents. The parent compound and its degradation products were separated from each other by partitioning **III** into an organic phase and then extracting **I**, **II**, and **IV** from an acidified aqueous phase by partitioning them into chloroform. Compounds **I**, **II**, and **IV** were then isolated and purified by thin-layer chromatography. Fractions containing the three respective chemicals **I**, **II**, and **III** were then subjected to hydrolysis, diazotization, coupling, and colorimetric measurement as previously described (Ercegovich *et al.*, 1972). The hydrolysis step was omitted for the fraction containing **IV**.

The data reported here were obtained through the use of methods as supplied by the manufacturer (CIBA, 1967). These methods have since been modified and were recently reported in detail by Kossmann *et al.* (1971). The primary difference between our method and that of Kossmann *et al.* was the manner by which the parent compound and degradation products were recovered from the fruit samples. In our method 60 g of macerate were extracted with 160 ml of isopropyl alcohol for 2 min in a Waring Blendor. Benzene (80 ml) was then added and the extraction was continued for an additional 5 min in the blender. The plant residue was then separated from the organic solvent mixture by filtering through glass wool. The blender jar and the plant residue were washed with five successive portions of 2:1 mixture of isopropyl alcohol and benzene. The filtrate was then transferred to a 1000-ml separatory funnel, 100 ml of saturated sodium chloride solution were added, and the pH was adjusted to 8 by adding 2 *N* ammonium hydroxide. Following pH adjustment, 150 ml of distilled water were added; the funnel was then shaken vigorously for 2 min and set aside until the two phases separated. The aqueous phase was collected into another separatory funnel and again extracted with 160 ml of benzene. The benzene extract was combined with the original solvent phase, but the aqueous phase was discarded.

To separate degradation product **III** from the parent compound and other degradation products, **II** and **IV**, the benzene phase was extracted four times with 10-ml portions of 1 *N* sulfuric acid. The benzene phase was retained for the later recovery of compound **III**, while the combined acid extract was made alkaline with 5 ml of 10 *N* sodium hydroxide and

partitioned three times against 25-ml amounts of chloroform. The chloroform phase was handled in the same manner for the ultimate purification and recovery of the compounds **I**, **II**, and **IV** by thin-layer chromatography as described by Kossmann *et al.* (1971), as was the benzene phase which contained compound **III**. Quantification of the compounds in the respective fractions was achieved by subsequent hydrolysis, diazotization, color development, and spectrophotometric determinations as reported by Kossmann *et al.* (1971) and Ercegovich *et al.* (1972).

Samples of fruit not treated with the acaricide were fortified with appropriate amounts of technical grade of the respective chemicals to determine the efficiency of the recovery procedure. A summary of data demonstrating the efficiency of recovery of the parent compound and its major degradation products is presented in Table II. In addition to the recovery determination, a control to monitor the efficiency of the diazotization, color development, and column separation steps was performed with each series of analyses by reacting known amounts of 4-chloro-*o*-toluidine by the same procedures used for the unknown samples. The average recovery of the 4-chloro-*o*-toluidine standard for 26 determinations was 97.2%. In calculating the ppm of the respective compounds it was not necessary to make any correction for the check values since, in all cases, these values were below the sensitivity of the method. However, all of the data in this report have been corrected for recovery efficiency and are based in terms of fresh weight of tissue. The smallest amount of 4-chloro-*o*-toluidine which was routinely detected in 60 g of crop sample by this method, using a Bausch & Lomb Spectronic 600 spectrophotometer with a 1-cm light path, was 2 μg, or equivalent to 0.04 ppm of compounds **I**, **II**, and **III** and 0.03 ppm for compound **IV**.

RESULTS AND DISCUSSION

The most obvious findings of this investigation are that only the parent compound and one major degradation product, **III**, were found in any of the six different types of fruit at any time during the various intervals after the last application and sampling date. Neither of the other two anticipated degradation products, **II** nor **IV**, was detectable within the limits of the sensitivity of the analytical method used (Tables III through VIII).

The data in Tables III, V, and VI disclose that fruit which have low residues of **I** (apples, pears, and cherries, respectively) also had very low amounts of the major degradation product, **III**. On the other hand, plums and strawberries which had higher amounts of the parent compound at various intervals between the last application and harvest also had higher levels of **III** (Tables VII and VIII). Peaches are an exception to either of these two cases in that this fruit had the highest residual amounts of **I**, but very low residues of **III** by comparison with the other fruit (Table IV). The conversion of **I** to **III** was observed to be most rapid in strawberries, slightly slower in plums, and still less rapid and of about the same magnitude in apples, cherries, peaches, and pears.

These results do not show any understandable correlation between the amounts of **I** and **III** to such variables as amount of chemical applied or sampling interval after the last application of the acaricide. This is clearly illustrated by the fact that at the same interval of 14 days, strawberries, which were treated with only 8 oz. a.i./acre, 34% of the total residue was composed of **III**, whereas only 7.4% of the total residue in cherries consisted of **III**, though the latter crop was treated with 16 times as much of the active ingredient. On the other hand, during a comparable interval of 30 days, pears contained over three times as much **III** than apples, though both crops were treated with the same amount of acaricide. It would therefore appear that the type of fruit, and especially the nature of the fruit surface, may play an important role in the formation and retention of **III**. Though this experiment was not designed to specifically test for the effects of environmental factors on the degradation of **I**, it would appear that such factors as rainfall, temperature, and sunlight may be predominant factors affecting the conversion of **I** to **III** and their persistence on fruit.

Table V. ppm of Chlordimeform and Its Degradation Products Found in Whole Pears at Various Intervals after a Total Seasonal Application of 96 oz a.i./Acre

Interval, days	ppm found[a] I[b]	ppm found[a] III[b]	ppm of total acaricidal residue[c]
30	1.38	0.31	1.74
45	0.96	0	0.96
60[d]	0.64	0.18	0.85

[a] Compounds **II** and **IV** were not detected in any of the samples. [b] See footnote b, Table II. [c] See footnote c, Table III. [d] Typical interval for harvesting of mature fruit.

Table VI. ppm of Chlordimeform and Its Degradation Products Found in Whole Cherries at Various Intervals after a Total Seasonal Application of 128 oz a.i./Acre

Interval, days	ppm found[a] I[b]	ppm found[a] III[b]	ppm of total acaricidal residue[c]
14	1.23	0.10	1.35
21	0.96	0.12	1.11
28	0.66	0.09	0.77
35[d]	0.57	0.13	0.73

[a] Compounds **II** and **IV** were not detected in any of the samples. [b] See footnote b, Table II. [c] See footnote c, Table III. [d] Typical interval for harvesting of mature fruit.

Table VII. ppm of Chlordimeform and Its Degradation Products Found in Whole Plums at Various Intervals after a Total Seasonal Application of 48 oz a.i./Acre

Interval, days	ppm found[a] I[b]	ppm found[a] III[b]	ppm of total acaricidal residue[c]
0	5.25	0	5.25
14	2.97	0.35	3.38
21	2.15	0.40	2.63
28	0.69	0.69	1.51
32	0.54	0.61	1.27
51[d]	0.50	0.25	0.80

[a] Compounds **II** and **IV** were not found in any of the samples. [b] See footnote b, Table II. [c] See footnote c, Table III. [d] Typical interval for harvesting of mature fruit.

Table VIII. ppm of Chlordimeform and Its Degradation Products in Whole Strawberries at Various Intervals after a Total Seasonal Application of 8 oz a.i./Acre

Interval, days	ppm found[a] I[b]	ppm found[a] III[b]	ppm of total acaricidal residue[c]
3	2.97	1.21	4.41
7	2.47	0.88	3.52
14	1.92	1.13	3.27
21	1.83	0.57	2.27
41[d]	0.04	0	0.04

[a] Compounds **II** and **IV** were not found in any of the samples. [b] See footnote b, Table II. [c] See footnote c, Table III. [d] Typical interval for harvesting of mature fruit.

Table IX. Comparison of ppm of Chlordimeform and Major Degradation Product Found, Calculated Total Residues, and Determined Total Chlordimeform-Derived Residues Found in Fruit Crops with Respect to Increasing Interval Between Last Application of the Insecticide and Sampling

Crop	Days	Total oz a.i. applied	ppm			
			I[a]	III[b]	I + III[c]	I[d]
Plum	0	48	5.25	0.00	5.25	4.77
Strawberry	3	8	2.97	1.21	4.41	5.70
Strawberry	7	8	2.47	0.88	3.52	4.02
Strawberry	14	8	1.92	1.13	3.27	3.54
Plum	14	48	2.97	0.35	3.38	3.38
Peach	14	64	6.63	1.07	7.91	7.99
Cherry	14	128	1.23	0.10	1.35	1.32
Strawberry	21	8	1.83	0.57	2.27	2.58
Plum	21	48	2.15	0.40	2.63	2.71
Cherry	21	128	0.96	0.12	1.11	1.14
Peach	28	64	6.55	0.38	7.01	6.96
Plum	28	48	0.69	0.69	1.51	1.50
Cherry	28	128	0.66	0.09	0.77	0.80
Apple	30	96	0.75	0.04	0.80	0.82
Pear	30	96	1.38	0.31	1.74	1.70
Plum	32	48	0.54	0.61	1.27	1.42
Cherry	35[e]	128	0.57	0.13	0.73	0.74
Strawberry	41[e]	8	0.04	0.00	0.04	0.04
Apple	45	96	0.50	0.12	0.64	0.63
Pear	45	96	0.96	0.00	0.96	1.02
Plum	51[e]	48	0.50	0.25	0.80	1.02
Peach	56	64	1.83	0.23	2.10	2.15
Apple	60	96	0.41	0.12	0.55	0.52
Pear	60[e]	96	0.64	0.18	0.85	0.94
Peach	70[e]	64	1.60	0.20	1.84	1.92
Apple	90	96	0.23	0.11	0.36	0.33
Apple	107[e]	96	0.00	0.28	0.33	0.27

[a] Chlordimeform. [b] Degradation product III, 4′-chloro-*o*-formotoluidide. [c] Calculated ppm of total residue as determined by adding ppm of chlordimeform and ppm of degradation product III found by differential analysis. [d] ppm of total residue of chlordimeform found by nonspecific method of analysis (Ercegovich *et al.*). [e] Harvesting interval for mature marketable fruit.

In their investigations on the effects of light on the decomposition of I, Knowles and Sen Gupta (1969) found that it decomposed readily when exposed to ultraviolet radiation of 254 and 364 nm and to sunlight, but not upon exposure to fluorescent light. Their studies indicated that the degree of decomposition was directly related to the duration and intensity of exposure and that the composition of the degradation products varied with the quality of radiation. After exposure to the shorter wavelength of ultraviolet light there was considerably less III present than after exposure to the longer wavelength of light. However, the exposure to the shorter radiation resulted in a much greater amount of other types of degradation products. Also noted in their study was the fact that the stability of I was inversely related to the solvent polarity and acidity. In view of this evidence it is suggested that the degree of decomposition of I on fruit surfaces would be influenced by such factors as intensity, duration, and quality of exposure to light, color, pH, and chemical nature of the fruit surface, and the nature of the deposition of the chemical on the fruit.

A comparison of the data in columns C and D in Table IX shows excellent agreement for the total amounts of Galecron-derived residues in all six crops. These data confirm the reliability and reproducibility of the analytical methods used and indicate that neither II nor IV could have escaped detection if in fact they were present in any of the fruit samples. The reported values for the persistence of I and III on the various crops are of primarily comparative value because the applications were made at a number of different geographical locations and by four different co-operators. For a more reliable comparison of the fate of I on these fruit crops, it would be necessary to apply the chemical more critically and to seriously consider the environmental factors which greatly influence the persistence of any chemical on plants. On the other hand, the results of this investigation are of meaningful significance in that they more closely approximate expected residues of I when it is used by the fruit grower on a practical basis.

It can therefore be concluded that when I is applied for effective pest control on apples, cherries, peaches, pears, plums, and strawberries, the parent compound and organosoluble degradation products will disappear quite readily from the fruit. The total residue of I and III in all of the fruit was less than 1 ppm at harvest time, except in peaches, where the total residue was approximately 2 ppm. Though the parent compound disappeared at a more pronounced and continued rate throughout the season than did III, the latter compound accounted for approximately 11, 18, 21, and 31% of the total measurable residue in peaches, cherries, pears, and plums, respectively; whereas 100% of the residue in apples was determined to be III but only I was detected as the chemical residue in strawberries. On the basis that I disappeared at a significantly rapid rate in all of the various fruit while the rate of disappearance of III was decidedly slower, it can be concluded that there is a continued conversion of I to III and that the degradation product is slightly more persistent than the parent compound.

Although the degradation of I was not studied beyond III, the present information indicates that neither chemical nor enzymatic degradation of I occurred to a significant degree in any of the crops, with the possible exception of strawberries. The chief factors responsible for the decrease of I in these fruit appear to be weathering and growth dilution.

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Herbicide-Derived Chloroazobenzene Residues: Pathway of Formation

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In soil, the chloroaniline moieties of certain phenylamide herbicides are liberated by microbial acylamidases, and are subsequently transformed by peroxidases to stable chloroazobenzene residues. The intermediate steps of this transformation were studied by allowing 4-chloroaniline or 3,4-dichloroaniline to react under steady state conditions with peroxidase and H_2O_2. The results indicated that the initial attack of peroxidase produced a free chloroanilino radical. Formation of another labile intermediate, chlorophenylhydroxylamine, was detected by the spectrum of its trisodium pentacyanoaminoferroate complex. The obtained results are consistent with a proposed pathway involving the transformation of the chloroanilines by peroxidases to chlorophenylhydroxylamines, with or without involvement of free chloroanilino radicals. The chlorophenylhydroxylamines spontaneously condense with excess chloroanilines and form chloroazobenzenes. The last step may be indirect and may involve the rapid autoxidation of the respective chlorohydrazobenzene intermediates.

The fate of certain phenylamide herbicides in soil is unusual in the respect that the initial step of their biodegradation is followed by a combination of enzymatic and chemical reactions which are synthetic rather than degradative in nature. They give rise to azobenzenes and to polyaromatic products of higher complexity. This type of transformation affects primarily the acylanilides (Bartha and Pramer, 1970), but the fate of methyl *N*-(3,4-dichlorophenyl)-carbamate (swep) is similar (Bartha and Pramer, 1969). Recent studies on ^{14}C ring-labeled compounds (Chisaka and Kearney, 1970; Bartha, 1971) demonstrated that both polymerized and nonpolymerized chloroaniline moieties have extended residual lives in soil, the latter ones apparently persisting in the form of humic complexes. Sprott and Corke (1971) observed the disappearance of low 3,3′,4,4′-tetrachloroazobenzene (3,3′,4,4′-TCAB) concentrations from some Ontario soils within a few weeks, but Kearney *et al.* (1970) detected 3,3′,4,4′-TCAB residues in rice field soils that were treated with 3′,4′-dichloropropionanilide (propanil) 2 and 3 yr prior to sampling. The weight of the evidence indicates that chloroazobenzenes and other chloroaniline transformation products (Rosen and Siewierski, 1971) are relatively persistent environmental pollutants. An understanding of their formation mechanism is basic to any attempt to prevent or reduce their production.

Peroxidases have a wide distribution in nature and occur in soil (Galstyan, 1958, 1959; Kozlov, 1964; Bartha and Bordeleau, 1969). They are specific in respect to their primary substrate (H_2O_2 or some alkylperoxides) but are capable of using a wide range of electron donors, including substituted anilines (Saunders *et al.*, 1964). The mode of action of peroxidases was studied by Chance (1949a,b, 1952) and numerous other workers (George, 1953a,b; Yamazaki *et al.*, 1960; Yamazaki and Piette, 1961). From these studies the currently accepted mechanism of peroxidase action (Figure 1) has emerged. In the presence of H_2O_2 complex I is formed very rapidly (less than 1 sec), whereas its conversion to complex II requires a longer time (Saunders *et al.*, 1964; Cormier and Prichard, 1968).

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