Degradation of Matacil by the Ascorbic Acid Oxidation System[1]

by

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Many attempts have been made in the past to develop chemical oxidation methods that would simulate biological oxidation processes (BUHLER and MASON 1961, HAMILTON et al. 1966, MERZ and WALTERS 1949). Fenton's reagent, consisting of hydrogen peroxide and ferrous ions, is known to give hydroxylation products from aromatic substrates similar to those obtained with liver microsomal systems (MERZ and WALTERS 1949). However, the "ascorbic acid system" developed by BRODIE et al. (1954) and UDENFRIEND et al. (1954) is considered to be closest to biological systems. It consists of L-ascorbic acid, ferrous ions, ethylenediaminetetracetate (EDTA) and soluble oxygen. It has been used successfully to oxidize aromatic substrates to give products similar to those observed in biological systems (BRODIE et al. 1954, UDENFRIEND et al. 1954).

Oxidation reactions such as hydroxylation and dealkylation are frequently observed in metabolism of pesticides (FUKUTO and SIMS 1971). These reactions occur in the metabolism of carbamate insecticides such as carbaryl, matacil, etc. by plants, insects, and animals (ABDEL-WAHAB et al. 1966, KRISHNA and CASIDA 1966, KUHR and CASIDA 1967, OONNITHAN and CASIDA 1968, TSUKAMOTO and CASIDA 1967). It was of interest to investigate whether the ascorbic acid oxidation system would give products similar to those observed in the bio-degradation of carbamate insecticides containing aromatic rings, as degradation of any carbamate insecticide by the ascorbic acid oxidation system has not been reported. This paper reports on the degradation of one carbamate insecticide, matacil (4-dimethylamino-3-tolyl-N-methylcarbamate), by the ascorbic acid oxidation system.

MATERIALS AND METHODS

Mass spectra were recorded with an AEI Model MS12 mass spectrometer. Infrared spectra were recorded in micro KBr discs with a Beckman IR10 instrument equipped with a beam condenser and beam attenuator.

Chemicals.- All reagent grade chemicals and solvents were used. Matacil, m.p. 93-4°, was obtained from Chemagro Corp., Kansas City, U.S.A. Formaldehyde-^{14}C (sp. act. 16 mCi/mmole) was

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obtained from Amersham/Searle Corp., Toronto, Canada and used as such.

Synthesis of 4-amino-3-tolyl-N-methylcarbamate (4-amino matacil).- 3-Methyl-4-nitrophenol (Aldrich Chemical Corp., Milwaukee) was reacted in ether solution with methyl isocyanate to give 4-nitro-3-tolyl-N-methylcarbamate (4-nitromatacil), m.p. 102-04°C, reported m.p. 96-8°C (ABDEL-WAHAB and CASIDA 1967). The nitro compound was reduced with hydrogen in the presence of 10% palladium-on-charcoal at room temperature and pressure to give 4-amino matacil, m.p. 128-130°C, reported m.p. 130°C (ABDEL-WAHAB and CASIDA 1967). Yield was about 100%.

Synthesis of 4-methylamino-3-tolyl-N-methylcarbamate (4-methylamino matacil).- 4-Nitro matacil was reduced with hydrogen in the presence of an equimolar amount of formaldehyde and 10% palladium-on-charcoal at room temperature and pressure. The products were purified by preparative thin layer chromatography (TLC) to give 4-methylamino matacil, m.p. 105-07°C, reported m.p. 105-07°C (ABDEL-WAHAB and CASIDA 1967). Yield was about 50%.

4-Dimethylamino-3-methyl phenol (matacil phenol).- Matacil was hydrolyzed with aqueous sodium hydroxide solution under nitrogen to give matacil phenol, m.p. 84-6°C. Yield was nearly 100%.

4-Dimethylamino-^{14}C-3-tolyl-N-methylcarbamate (4-dimethylamino-^{14}C-matacil).- It was prepared according to the method of ABDEL-WAHAB and CASIDA (1967). A mixture of 4-amino matacil (0.015 mmole), formaldehyde-^{14}C (0.03 mmole) and 50 mg of 10% palladium-on-charcoal in 10 ml of methanol was shaken under hydrogen at room temperature and pressure for 1 hour. The product was purified by preparative TLC to give 4-dimethylamino-^{14}C-matacil. Yield was about 50%. The purity of the isolated material was examined by TLC and autoradiography and found to be more than 99% pure. The purified carbon-14-labeled matacil was mixed with non-labeled matacil to give sp. act. 8.84 uCi/mmole.

Determination of radioactivity.- A Packard Tri-Carb-Model 3320 liquid scintillation spectrometer operating at 4°C was used for determining radioactivity. Aqueous samples were counted in 10 ml of Aquasol (New England Nuclear, Boston, Mass.). Non-aqueous samples (0.5-2 ml) were counted in 10 ml of scintillation fluid containing 0.25 g dimethyl POPOP and 4 g PPO per 1 litre of toluene. Radioactivity content of the TLC spots was determined by counting the silica gel representing the spots in Cab-O-Sil (Beckman Inst. Co., Fullerton, Calif.) suspension.

Oxidation of matacil.- The oxidation mixture consisted of L-ascorbic acid (2.47 g, 14 mmole), ferrous sulfate heptahydrate (0.36 g, 1.3 mmole), and EDTA (2.42 g, 6.5 mmole) in 300 ml of 0.1 mole phosphate buffer (pH 6.7). Matacil (1.04 g, 5 mmole) was dissolved in the oxidation mixture and shaken in an oxygen atmosphere for 2 hours in a water bath at 37 $\pm$ 0.5°C. The reaction mixture was extracted three times with ethyl acetate.

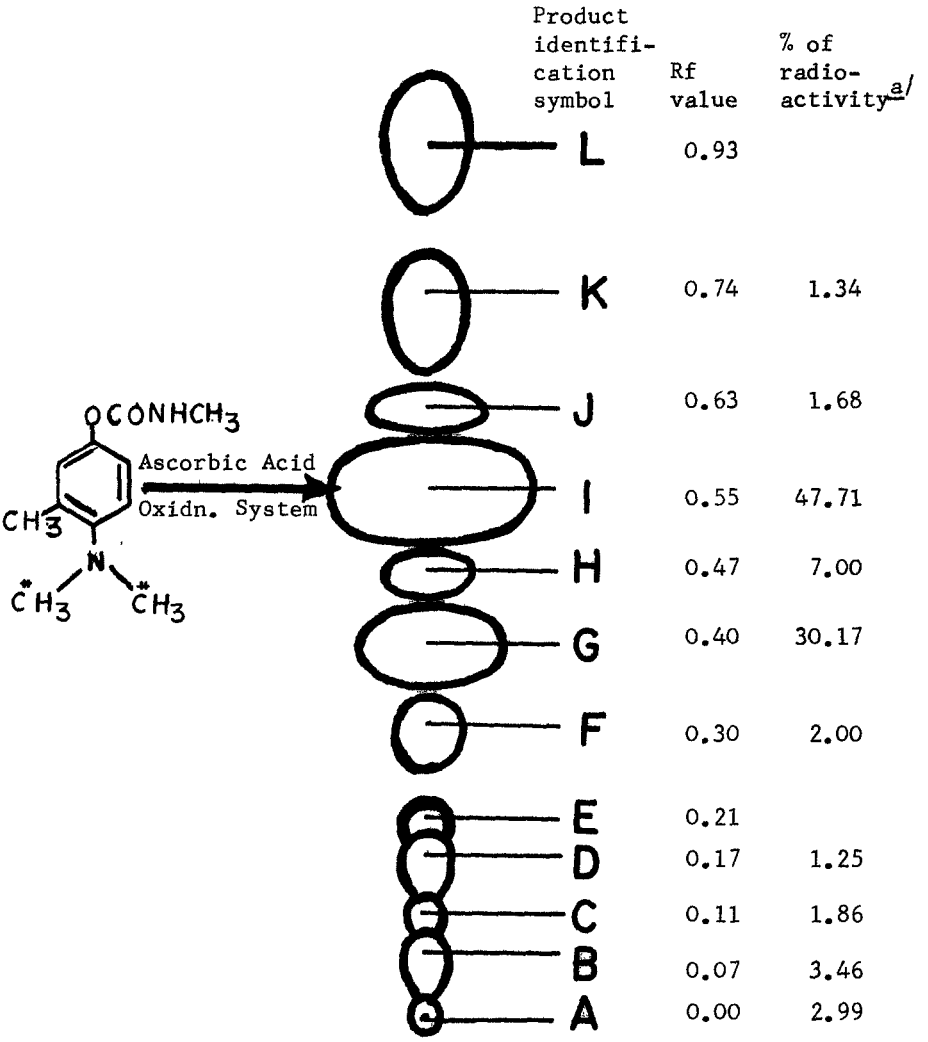


Figure 1. Thin layer chromatogram of the organic soluble oxidation products of matacil by the ascorbic acid system.

a/ Based on the total radioactivity in the organic soluble fraction.

The pH of the reaction mixture was adjusted to 8 with NaOH solution and re-extracted four times with ethyl acetate. The combined ethyl acetate extract was concentrated to about 5 ml and examined by thin layer chromatography, using diethyl ether as the developing solvent. Matacil and its degradation products were visualized by spraying with Gibbs reagent according to the method of KRISHNA et al. (1962). Ninhydrin reagent was used to detect degradation products containing carbamoyl groups (KRISHNA et al. 1962). The degradation products were isolated by preparative TLC. The isolated compounds were identified by co-chromatography with reference compounds and/or by infrared and mass spectrometry.

For quantitative determination of the relative abundances of the products, 4-dimethylamino-^{14}C-matacil (0.5 mmole, sp. act. 8.84 uCi/mmole) was oxidized in a similar manner, using one-tenth of the reagents used in the above experiment. The reaction mixture was extracted with ethyl acetate as described above. The concentrated reaction mixture was separated on TLC and the carbon-14 labeled degradation products were detected by autoradiography. The degradation products were also visualized by spraying with chromogenic reagents as described above. The relative abundance of the carbon-14 labeled degradation products was obtained by determining the ^{14}C-content of the radioactive spots (as shown by the autoradiogram) by scintillation counting.

RESULTS AND DISCUSSION

There were at least 12 products formed in the oxidation of matacil by the ascorbic acid oxidation system (A-L, Fig. 1). All these products were visible when the thin layer plates were sprayed with different chromogenic reagents. In the experiment with 4-dimethylamino-^{14}C-matacil, ten of these products were detected by autoradiography, indicating that there were at least ten products containing either one or both the N-$^{14}CH_3$ groups. Products E and L (Fig. 1) were not indicated in the autoradiogram but they were visible on spraying with chromogenic reagents and these could not have any of the $^{14}CH_3$ groups.

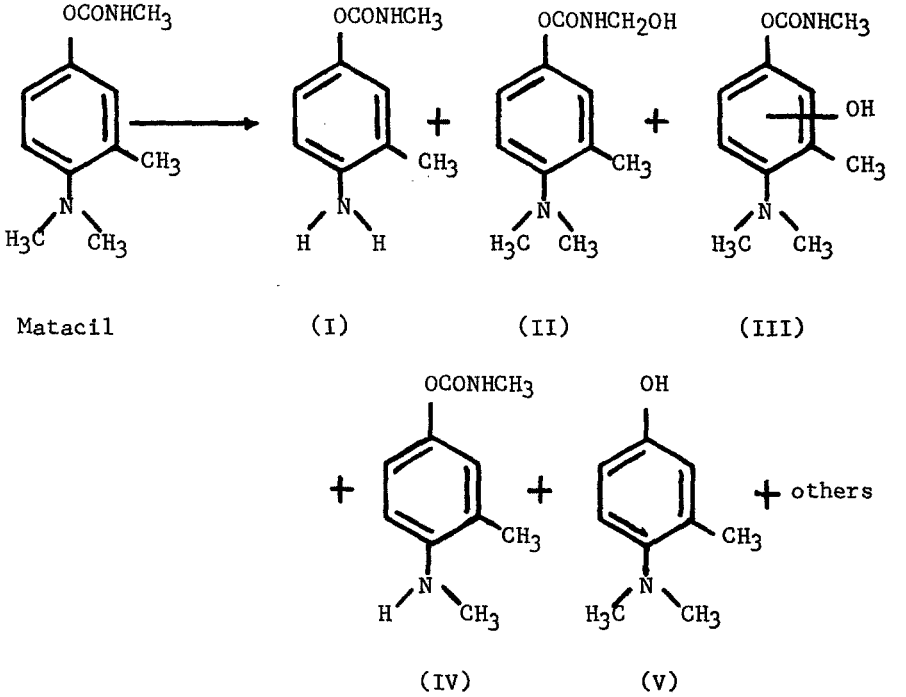


Figure 2. Products from the oxidation of matacil with the ascorbic acid system.

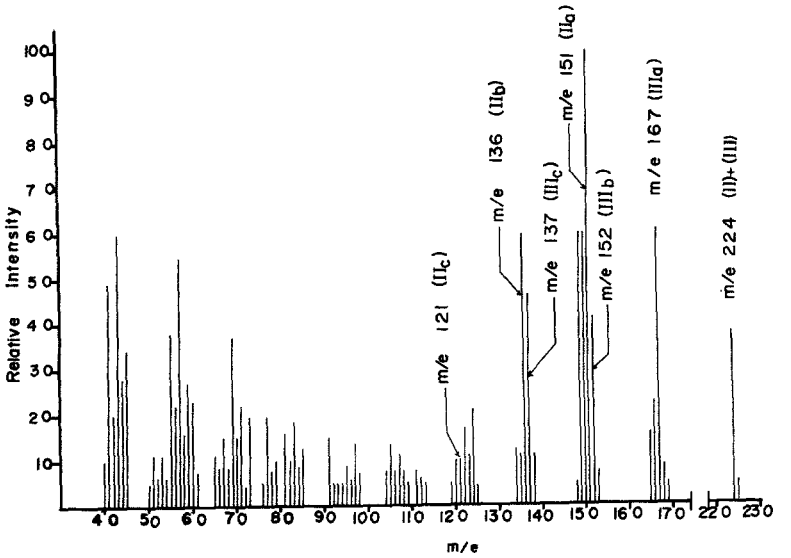


Figure 3. Mass spectrum of the compounds representing TLC spot F (Fig. 1).

Seven of the 12 oxidation products (A,B,C.D,H.K, and L) could not be identified while the five remaining products were tentatively or positively identified.

Product E was tentatively identified to be 4-amino-3-tolyl-N-methylcarbamate (Fig. 2,I) as this product co-chromatographed with the authentic sample. Furthermore, as mentioned before, it did not contain any radioactivity, indicating that both the $-^{14}CH_3$ groups were lost from the molecule and resulting in 4-amino-3-tolyl-N-methylcarbamate.

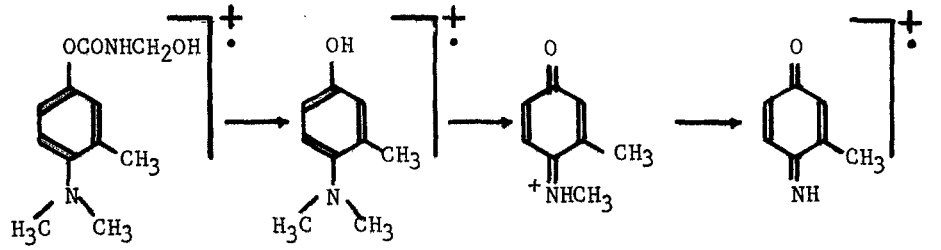
Product F gave violet color with chromotropic acid in sulfuric acid, indicating the presence of $-N-CH_2OH$ moiety in its structure (MENZER and CASIDA 1965). It also gave positive reaction to Gibbs reagent, indicating the presence of phenolic moiety. The infrared spectrum of the product(s) showed the presence of carbonyl, hydroxyl and -NH- groups. The mass spectrum (Fig. 3) of the product(s) strongly indicated the presence of two compounds having the same molecular weight. The mass spectrum showed molecular ion at m/e 224, in agreement with structures II or III (Fig. 4). The fragmentation patterns strongly indicated that two compounds having structures II and III are represented by this spot on TLC. The compound having structure II (Fig. 4) would be expected to give major fragments at m/e 151 (IIa), 136 (IIb), and 121 (IIc) in accordance with the general fragmentation pattern of similar carbamates (BIROS 1971). The mass spectrum indeed showed all these major fragments (Fig. 3). On the other hand, the compound having structure III would be expected to give major fragments at m/e 167, 152, and 137 representing species IIIa, IIIb, and IIIc (Fig. 4), respectively. It should also give a strong peak at m/e 57 representing $CH_3-N=C=O$ which resulted from the decomposition of

III to the corresponding phenol (IIIa). The mass spectrum, however, does not indicate the position of the OH group in the aromatic nucleus of III. These two proposed structures are in agreement with the infrared and mass spectra of the isolated material and its reactions to chromogenic reagents on TLC. Structure II would respond to chromotropic acid while structure III would give positive reaction with Gibbs reagent. Thus spot F is probably represented by both these compounds. All attempts, using different developing solvent(s) in TLC, failed to separate these two compounds.

Product G was suspected to be 4-monomethylamino matacil (IV, Fig. 2), as it co-chromatographed with the reference compound. Further proof of its identity was obtained from its infrared and mass spectra which were identical with those of the reference compound.

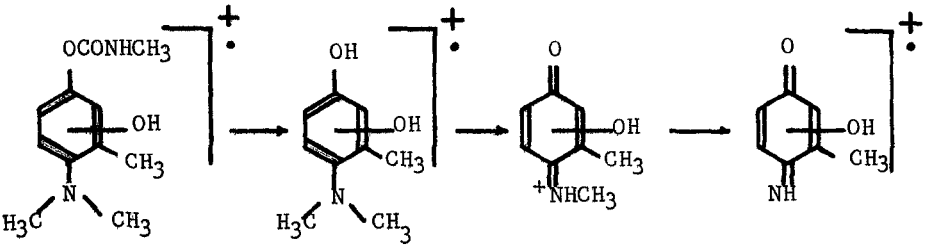
Compound I was unreacted matacil. It had the same infrared and mass spectra as that of the starting material.

Compound J was matacil phenol (V, Fig. 2). It co-chromatographed with the reference compound and had the same infrared and mass spectra as that of the reference compound.



m/e 224 m/e 151 m/e 136 m/e 121

(II) (IIa) (IIb) (IIc)



m/e 224 m/e 167 m/e 152 m/e 137

(III) (IIIa) (IIIb) (IIIc)

Figure 4. Assignment of structures to the major fragments in the mass spectrum of products F, tentatively identified as a mixture of 4-dimethylamino-3-tolyl-N-hydroxymethylcarbamate (II) and hydroxy matacil (III).

About 54% of the initial radioactivity in 4-dimethylamino-^{14}C-matacil was present in the ethyl acetate extract of the reaction mixture, 26% remained in the aqueous layer and the remaining 20% was lost, perhaps due to oxidation of the $^{14}CH_3$ groups to volatile products.

About 48% of the radioactivity in the organic soluble fraction was due to unreacted matacil. The major degradation product, 4-methylamino matacil (IV, Fig. 3), accounted for 30% of the radioactivity, matacil phenol (V) 1.7%, the ring hydroxylated matacil III and the hydroxymethyl derivative (II) accounted for another 2% of the radioactivity. Thus all identified compounds accounted for about 82% of the radioactivity in the organic soluble fraction of the reaction mixture. The relative abundance of 4-amino matacil (I) was not determined, as it did not contain any radioactivity. But, judging from the intensity of the TLC spot, it was considered to be a minor product.

The major products in the biodegradation of matacil by plants, insects and rat liver microsomes are 4-methylamino matacil (IV), 4-dimethylamino-3-tolyl-N-hydroxymethyl carbamate (II) and 4-amino matacil (I) (ABDEL-WAHAB et al. 1967, KRISHNA and CASIDA 1966, KUHR and CASIDA 1967, OONNITHAN and CASIDA 1968, TSUKAMOTO and CASIDA 1967). These were among the products obtained in the chemical oxidation of matacil by the ascorbic acid oxidation system (Fig. 3). The ring hydroxylated derivative (III), tentatively identified in the present study, and matacil phenol (V) have not been reported in any of the above-mentioned biodegradation studies involving matacil, although such products have been reported with other carbamate insecticides. However, in the studies with matacil all the metabolites were not identified and it is possible that they were formed but were not identified.

The results obtained in the present study indicate that the ascorbic acid oxidation system is capable of effecting demethylation, hydroxylation of aromatic ring, oxidation of $-NHCH_3$ group to $-NHCH_2OH$, and cleavage of the carbamate to the corresponding phenol. These reactions are similar to those observed in the biodegradation of matacil and other carbamate insecticides (FUKUTO and SIMS 1971). These results were obtained with matacil and should not be extrapolated to all other carbamates. But these results strongly indicate the versatility and usefulness of the chemical method for studying degradation of other carbamate insecticides and possibly indicate biodegradative products to be expected from such compounds.

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