Degradation of Zectran in Alkaline Water

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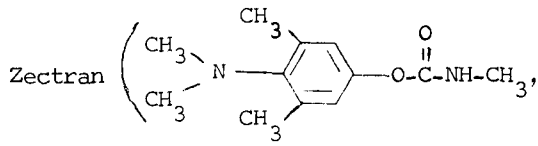

, 4-dimethylamino-3,5-xylyl methyl carbamate) has been chosen as a possible replacement for DDT in managing detrimental forest insects (GIBSON & CHAPMAN 1972). The toxicity of Zectran is presumed to be due to its action as a cholinesterase inhibitor. It is thus toxic to a wide spectrum of organisms and should be placed among those carbamates whose environmental levels are of potential concern (ALY & EL-DIB 1971). It has recently been found that fresh solutions of Zectran at pH 9-9.5 are several times more toxic to fish than are fresh solutions at pH 6.5, and upon aging for several days the level of toxicity increases at both pH's (PERSONAL COMMUNICATIONS).

Carbamates are known to hydrolyze rapidly in alkaline solutions (DITTERT & HIGUCHI 1963); but Zectran's immediate hydrolysis product, 4-dimethyl amino-3,5-xylenol, hereafter referred to as "xylenol", is a much less potent cholinesterase inhibitor than is Zectran itself (OONNITHAN 1968). In the solid state, Zectran is subject to photodecomposition on its 4-dimethyl end to some products which are slightly more toxic than Zectran, but no hydrolysis at the carbamate end is seen (ABDEL-WAHAB and CASIDA 1967). Studies of the photodecomposition of "xylenol" have not been reported. The photodecomposition of other carbamates and numerous other pesticides has been reported (SU & MATTHEW 1972; CROSBY 1972). The contribution of pH and of light intensity towards the instability of several carbamates in solution (excluding Zectran) has been reported (WAUCHOPE and HAQUE 1973).

It seems likely that Zectran would degrade in alkaline water, under the influence of sunlight, to products that have not been identified. Some of these products may be more toxic, perhaps by other mechanisms, than Zectran itself. This report describes a high pressure liquid chromatographic technique for resolving aged Zectran solutions into various components and is preliminary to biotoxicity studies of each component to be reported at a later date.

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Materials and Methods

The aqueous medium used for preparing solutions of Zectran or "xylenol" was distilled water reconstituted with $NaHCO_3$, $CaSO_4$, $MgSO_4$ and KCl (MARKING 1969). This is the same aqueous medium as used by the United States Department of Interior Fish Pesticide Research Unit, La Crosse, Wisconsin for conducting their toxicity tests. A borate buffer system was added to keep the medium at pH 9.5. The pH of all solutions was checked daily, and sodium hydroxide added whenever necessary to maintain the pH at 9.5. Solutions for aging were prepared by dissolving 0.15 gms of Zectran or of "xylenol" in 10 ml of acetone and then pipetting 0.15 ml of this stock into 100 ml of aqueous medium held in a 150 ml beaker. The final Zectran or "xylenol" concentration was thus 22.5 mg/l (0.10 mM Zectran or 0.14 mM "xylenol"). The beakers were covered loosely with watch glasses and stored in a cold water bath (12-13° C.). This temperature corresponded to that used for toxicity tests.

The laboratory lights (fluorescent) were on for 9 hours each day. The intensity of illumination at the bottom of the water bath measured 40 foot-candles which compared well with that present during toxicity studies.

A model 1240 Perkin-Elmer high pressure liquid chromatograph equipped with a UV 254 nm double-beam detector and a reverse phase, octa-decyl coated Sil-X-II column, 1/2 meter long x 2.6 mm dia. was used for analysis. The column was operated at room temperature and at a pressure of from 400 psi to 800 psi, as required to obtain a flow rate of approximately 1 ml/min, with the various solvents used.

Figure 1 shows tracings of typical elution patterns obtained with the three solvent systems employed. A solution of Zectran held at pH 9.5 and 12-13° C. for three days is exhibited here and six components can be resolved. After equilibrating the column with the eluting solvent, a 10 µl aliquot of the aged Zectran solution is injected. In Figure 1A the Zectran (peak #1) is seen to be retained for a time on the column and then to come off cleanly; other components elute too close together for analysis with this methanol/water mixture. The column is next equilibrated with the second solvent and a second 10 µl aliquot of the same aged Zectran solution injected. The pattern seen in Figure 1B results. Components #2, #3 and #4 elute cleanly; the Zectran (component #1) is retained on the column and elutes only very slowly after about 20 minutes (not shown). Finally, the column is equilibrated with pure deionized water and a third 10 µl aliquot of the same aged solution injected. The pattern seen in Figure 1C results. Components #4, #5 and #6 are seen clearly now with #3, #2 and #1 all eluting in very broad slowly formed peaks (not shown). The components are hereafter referred to by these numbers which represent their decreasing retention on the column and probably, given the non-polar nature of the column coating,

represent also their increasing polarity.

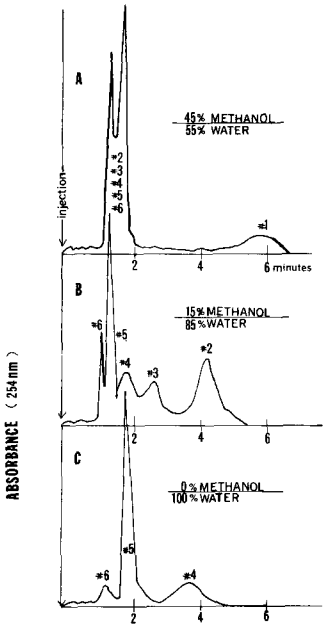


Figure 1. Elution pattern as a function of eluent composition. In (A), components 2 through 6 elute too close together for analysis; in (B), components 2 and 3 are cleanly resolved; in (C), components 4, 5 & 6 are resolved. The eluting solvent composition is given as percentages by volume.

The column data is rendered quantitative by using a planimeter to estimate the area of each isolated peak. Data for component #1 is taken from the pattern obtained using the solvent of Figure 1A; data for components #2 and #3 from that of Figure 1B; and data for components #4, #5 and #6 from that of Figure 1C.

Results and Discussion

The areas of Zectran or "xylenol" peaks are directly proportional to molar concentration (Figure 2). At this wavelength, Zectran apparently has a greater molar extinction coefficient than "xylenol". The wavelength of the detector could not easily be changed from 254 nm. The low concentrations of Zectran used for toxicity tests could easily be detected at this wavelength but "xylenol" could not be. Nevertheless, the concentrations used in this report were kept low, rather than raised to several times above those used in toxicity tests, to see what components could be detected.

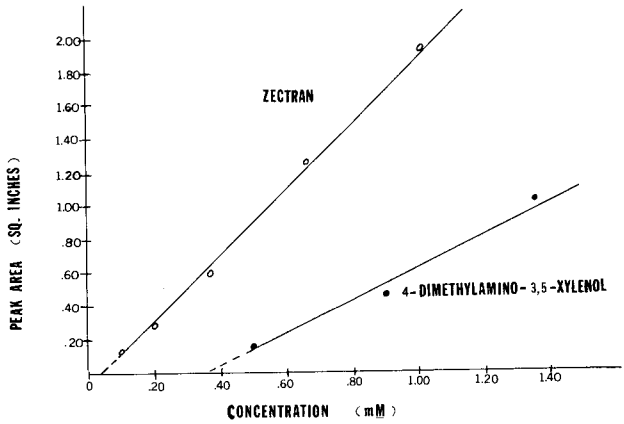


Figure 2. Peak areas (observed at 254 nm) vs concentration. Limit of detectability for Zectran is about 0.06 mM (13 mg/l) and for "Xylenol" is about 0.36 mM (59 mg/l).

Figure 3 shows the disappearance of Zectran from two solutions held at different pH's. The half-life at pH 9.5 is approximately two days, whereas at pH 7.4 the half-life is approximately two weeks. These results are comparable to those reported for the hydrolysis of other carbamates in alkaline vs neutral waters (ALY & EL-DIB 1971).

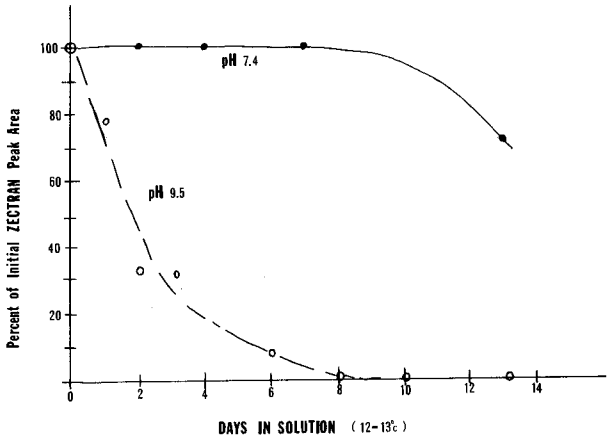
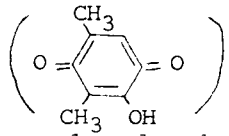


Figure 3. Zectran stability as a function of pH at 12-13° C.

Figure 4 shows the appearance of components #2 through #6 from Zectran with time at pH 9.5. The acetone used in the preparation of the solutions elutes where component #5 elutes.

However, the irregular shape of #5's time curve in each study suggests that there may be a significant product eluting here as well. Component #4 elutes where the standard, 2-hydroxy-3,5-dimethyl 1,4-benzoquinone  elutes. Water solutions of this standard are purple colored as are the aged Zectran or "xylenol" solutions. The time at which one notes these aged solutions becoming colored corresponds to the time at which a substantial amount of component #4 appears. Component #6 has been isolated and is presently being subjected to IR, NMR and mass spectroscopic examination.

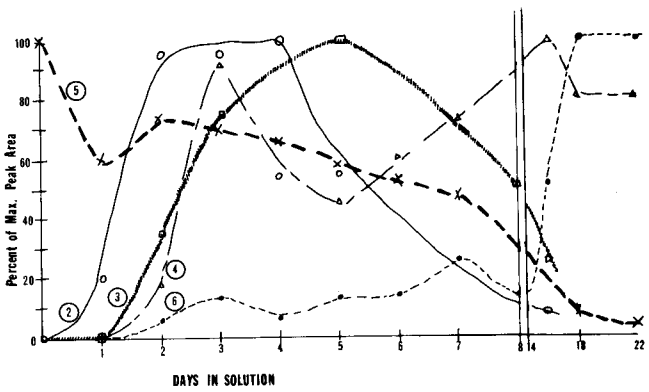


Figure 4. Zectran solution components as a function of time @ pH 9.5 (12-13° C).

The only components to persist after several weeks are #4 and #6. Measurements show the maximum peak area of #6 to be twice the maximum peak area of #4, but without information on molar extinction coefficients one cannot draw conclusions as to which one represents the more abundant final product. The order of appearance of the components is #2, #3, #4 and #6 as can be seen by examination of the initial slopes of the curves. The appearance of #5, if indeed it is an interesting component, is masked too much by acetone to describe. This order of appearance suggests degradation to ever more polar components.

Components #4 and #6 each show two or more times at which they reach peak concentration. This phenomenon is peculiar, but it is quite reproducible. Once their chemical identities are learned, perhaps the reasons for such multiphasic behavior will be understood.

When "xylenol" solutions rather than Zectran solutions are examined, the same components are seen to arise and in the same sequence (Figure 5). However, the rates of appearances are

greatly accelerated. Component #2 reaches a peak concentration in 24 hours as compared to over 48 hours required when formed from Zectran. Component #3 peaks in two days as compared with five days, etc. The great similarity seen in Figures 4 and 5 suggests that the components being observed are derivatives of Zectran's hydrolysis product and not of Zectran itself.

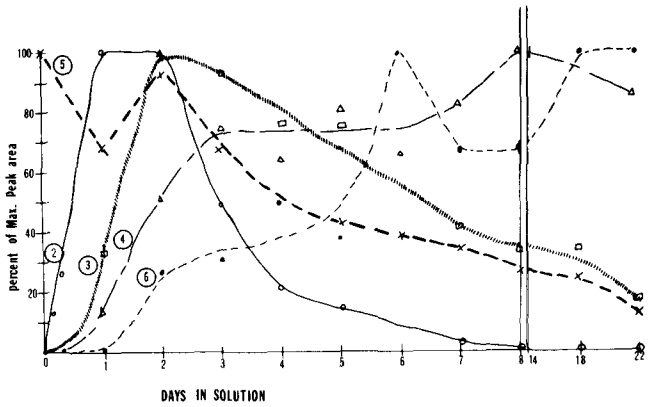


Figure 5. "Xylenol" solution components as a function of time @ pH 9.5 (12-13° C.).

The role of pH in the formation of these components can be seen by comparing Figure 5 and Figure 6. When "xylenol" solutions are aged at pH 7.4 only components #4 and #5 are seen to be present. Aging Zectran at pH 7.4 for nearly two weeks gives no detectable sign of any of these products other than some #5 (presumably acetone).

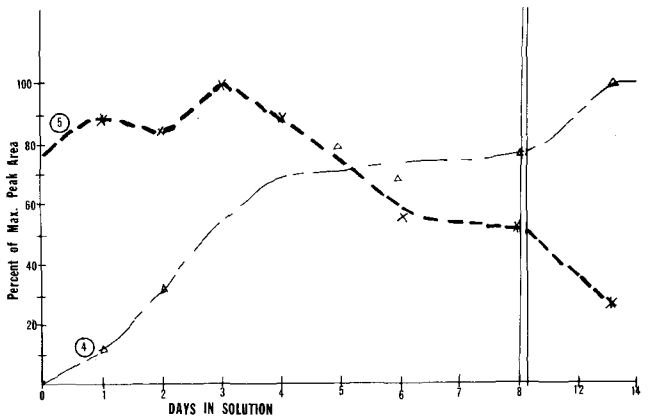


Figure 6. "Xylenol" solution components as a function of time @ pH 7.4 (12-13° C.).

In conclusion, I would suggest that at alkaline pH (9-9.5), Zectran hydrolyzes rapidly to "xylenol"; that the "xylenol" then converts to its "xylenoxide" ion form because of this high pH; and that this form is very sensitive to photooxidation. Components #2, #3 and #4 may then represent the most immediate products of this photooxidation. A similar suggestion for the enhanced photooxidation of the 1-naphthol derived from carbaryl at high pH has recently been made by WAUCHOPE & HAQUE (1973). The identification of and toxicity tests on these degradation products of Zectran are the subject of the author's continued concern.

Acknowledgements

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