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SPECTROPHOTOMETRIC DETERMINATION OF QUINOLINIUM ACID ESTERS WITH 2, 4-DINITROPHENYLHYDRAZINE

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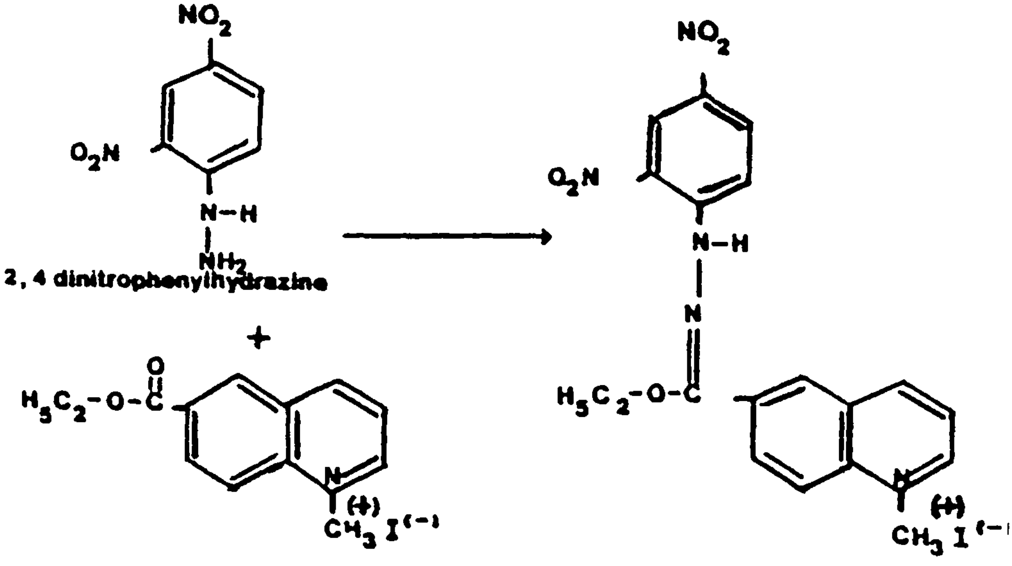
SUMMARY

Carbonyl compounds react with 2, 4-dinitrophenylhydrazine to form the corresponding hydrazones. These hydrazones in the presence of 1N NaOH give a reddish brown color which can be utilized for colorimetric analysis. This method is used for the spectrophotometric determination of quinolinium-6-carboxy-1-methyl-iodide. Furthermore 2, 4-dinitrophenylhydrazine is utilized to kinetic measurements of the rate of hydrolysis of the quinolinium acid esters.

INTRODUCTION

2, 4-dinitrophenylhydrazine (DNPH) has been utilized for the spectrophotometric analysis of different carbonyl compounds.

Seligson and Shapiro and El Hawary and Thompson used it for the determination of alpha-keto acids. Recently Gilver and Brond used it for the spectrophotometric determination of methyl glyoxal. In the present study DNPH Is used for quantitative assay of quinolinium-6-carboxyl-1-methyliodide ethyl ester (QME). This procedure utilizes the reactions of 2, 4-quinolinium-6-carboxy-1-methyliodide ethyl ester (III) which with 1N NaOH gives a reddish brown color whose absorbance is measured in the visible spectra. Furthermore DNPH is utilized for kinetic measurements of the rate of hydrolysis of QME.



II

III

Equation I

MATERIALS AND METHODS

Material - All chemicals used in this study were reagent grade and used as supplied, unless otherwise indicated. Absorbance measurements were made with a double-beam spectrophotometer[1], and pH measurements were made with a pH-meter[2].

Preparation of 2, 4-dinitrophenylhydrazone of QME (Comp III) -- Exactly 10 mg of QME was dissolved in 10 ml of water. To this solution was added 5 ml of 1% solution DNPH in 5N HCl. This mixture was allowed to stand for 30 minutes at 25°C, it was then transferred to a 50 ml separatory funnel, and the hydrazone was extracted from the aqueous solution with three 15 ml portions of twice-lasss-distilled ethyl acetate. The combined ethyl acetate extracts were evaporated, and the precipitated hydrazone was collected in the dried form. This was recrystallyzed from ethyl acetate m.p. 256-258° (M.W. found 400; Calc. 392.36).

A quantity of the dried hydrazone was dissolved in ethyl acetate and chromatographed on Whatmann #1 filter paper sheets using the mixture N-Butanol: Ethanol: 0.5N Ammonia (70:0:20) in an ascending technique as follows: Samples were applied to the filter paper sheets, using micropipettes, and as soon as the chromatogram was developed, it was allowed to dry in a current of dry air. The spots were marked and the R_f value was calculated

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[1] Spectronic 2000, Baush-Lamb Co. Fisher Scientific Co., 52 Fadem Road
Springfield, NJ 07081

[2] Corning pH meter model No.5. Fisher Scientific Co., 52 Fadem Road
Srpingfield, NJ 07081

to be 0.77 (±0.01). At the same time a chromatogram was run loaded with ethyl acetate solution of DNPH and under the same chromatographic conditions for comparison, it was found that the DNPH moved all the way up with the solvent front.

Quantitative Estimation of Hydrazone of QME (III -- The approach to the quantitative estimation of compound II was based on the fact that, compound II forms hydrazone with DNPH which then with 1N NaOH provides a reddish - brown color while the hydrolyzed product of compound II (Quinolinium-6-carboxyl-1-methyliodide, acid) does not form hydrazone. A solution of the QME (1.0 mg/ml) was prepared in a 2N HCl solution. 2.0 ml aliquots of the stock solution were distributed in nine test tubes, and the tubes were taken one by one at the following time intervals: 10, 20, 40, 60, 90, 120, 150, 180 minutes and the last tube was taken at the end of 24 hours, to represent the infinite time. To each tube was added 0.5 ml of DNPH sol. and allowed to stand for 30 minutes, and the 8 ml of 1N NaOH were added and after further standing of another 10 minutes, the absorbance of the solution was measured at 500 nm and the concentration of the residual unhydrolyzed QME was calculated from the standard curve.

RESULTS AND DISCUSSION

The reaction of DNPH (I) with QME (II) to form the hydrazone of QME (III) is given in Equ. I. The addition of DNPH to the carbonyl involves nucleophilic attack by the basic notrogen compound on the carbonyl carbon. Promotion of carbonyl oxygen makes carbonyl carbon more susceptible to nucleophilic attack; and this reaction is favored by high acidity.

Paper chromatography of cpd III showed a spot with an R_f value of 0.77 (±.01), while the simultaneously run DNPH moved with the solvent front. In general, no tailing or smearing were detected on the chromatograms which is an indication of the purity of the compound III. The dried hydrazone is an orange-brown crystalline compound with a melting point of 256-250°C. The m.w. of the hydrazone was found to be 400[1] (calc. 392.3) that is 101.94% of the calculated value. Furthermore, the formation of the hydrazone was followed and by IR spectra. Recovery determination for sample solutions spiked with known weights of compound II are tabulated in Table I. The average percentage recoveries were 100.02% and ranged from 99.2 - 100%.

When hydrazone reaction mixture is mixed with alkali, a reddish brown color develops: This color is stable, nevertheless, a reagent blank is advisable because it will reduce or eliminate background absorption. No error is encountered in the comparison of the absorbances of the sample and standard solutions, provided the concentrations of these solutions are approximately equal.

The fact that hydrazones give a reddish brown color when treated with 1N NaOH solution was utilized for the colorimetric determination of QME and for the study of the rate of the hydrolysis, in a 2N HCl.

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[1] Golbraith Laboratories: Knoxville, Tenn. 37921

TABLE I

Recovery Determinations of

Quinolinium-6-carboxy-1-methyl iodide, ethyl ester

Sample #	mls. of stock solu. 1.0 mg/ml	mls. of water	Concentration of Ester		
			Added mg.	Found mg.	Percent of Recovery
1	0.5	1.5	.500	0.505	101
2	0.5	1.5	.500	0.496	99.2
3	1.0	1.0	1.000	1.000	100
4	1.0	1.0	1.000	0.997	99.7
5	1.5	0.5	1.500	1.495	99.7
6	2.0	0.0	2.000	2.010	100.5
Average					100.02

The reaction was stopped at specific time intervals by immersing the tubes into ice. The hydrazone derivative of the unhydrolyzed ester was formed by adding DPNH which with 1N NaOH gave a reddish brown color whose absorbance was determined spectrophotometrically. The pseudo-first order rate constant was determined by utilizing the expression.

$$\log (C_o-x) = \log Co- kt/2.303 \text{ ------ Eq.2}$$

Where Co is the concentration at time zero and (C_o-x) is the concentration of the unreached ester at time t. The form k is the observed pseudo-first rate constant.

Kinetic runs were carried out in triplicate. The slope of the line was used in the determination of the rate constant which was found to be 7.6×10^{-3} min^{-1}, while the half-life was calculated to be 91 min.

In the light of these observations, studies are in progress with a series of quinolinium, carboxy-1-methliodide esters to elucidate some of the structural requirements and events occuring during their hydrolysis.

REFERENCE

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3. Seligson, D. and Shapiro, B., Anal. Chem., 24, 754 (1952)