

# Diisopropylammonium Dichloroacetate I.

## Assay, Distribution Coefficient, and Solubility Studies

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Solubility and distribution coefficient values were determined for the drug, diisopropylammonium dichloroacetate, using a nonaqueous titration method. A second assay procedure is presented for the drug utilizing the modified Fujiwara colorimetric method.

IN RECENT YEARS, several papers have described the pharmacological properties of diisopropylammonium dichloroacetate.<sup>1</sup> The hypotensive properties of the drug have been reported (1-3). The anti-toxic action of diisopropylammonium dichloroacetate has been demonstrated against potassium cyanide and quinine hydrochloride (4). Biological studies with diisopropylammonium dichloroacetate have indicated its effect on the blood levels of galactose, glycine, and cholesterol (5), on the utilization of amino acids by the liver and other tissues (6), and on the interference with oxidation-reduction processes (7). Villari, Mazzacca, and Coraggio (8) observed the activation effect of diisopropylammonium dichloroacetate on mice infected with MHV-3 (mouse hepatitis virus).

During preliminary biopharmaceutical investigations of diisopropylammonium dichloroacetate, it was necessary to find suitable assay procedures for solubility and distribution coefficient studies. A colorimetric assay for diisopropylammonium dichloroacetate has been reported by Maruyama and Hasegawa (9). Their procedure is based on the color reaction involving naphthorescorcinol and glyoxalic acid, an alkaline degradation product of diisopropylammonium dichloroacetate. More recently, a colorimetric method for the determination of polyhalogenated organic compounds was reported by Leibman and Hindman (10). The method used by these authors is a modification of the Fujiwara alkaline pyridine reaction (11).

The purpose of this paper is to report on the application of two analytical procedures for the assay of diisopropylammonium dichloroacetate: a colorimetric method based on the modified Fujiwara technique (10) and a nonaqueous titration procedure (12). These assays have proven applicable for the determination of the solubility and the distribution coefficient of this agent.

### EXPERIMENTAL

#### Nonaqueous Titrimetry

**Analysis of Diisopropylammonium Dichloroacetate.**—The nonaqueous titration procedure described by Fritz (12) was utilized for the analysis of diisopropylammonium dichloroacetate. Each milliliter of 0.1 *N* potassium methoxide solution is equivalent to 23.014 mg. of the drug.

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<sup>1</sup> Marketed as Diedi by the Italsebar Co., Milan, Italy.

**Analysis of Solutions of Diisopropylammonium Dichloroacetate.**—Aliquots of diisopropylammonium dichloroacetate solutions (aqueous and nonaqueous) were pipeted into 100-ml. beakers and the solution allowed to evaporate to dryness. The residue was dissolved in 20 ml. of neutralized DMF and the solution titrated with potassium methoxide solution as previously described.

**Solubility Studies.**—An amount of diisopropylammonium dichloroacetate known to be in excess of that required for a saturated solution was weighed accurately and placed in a glass-stoppered bottle containing the solvent. The bottle was placed in a rotating constant-temperature bath and equilibrated for 2 hr. at  $26 \pm 0.1^\circ$ . After equilibration, aliquot samples of the supernatant liquid were removed and assayed for the drug by nonaqueous titration.

**Distribution Coefficient Studies.**—The distribution coefficients of diisopropylammonium dichloroacetate between benzene-water and chloroform-water systems were determined by nonaqueous procedures. Accurately weighed samples of the compounds were added to glass-stoppered bottles containing 5 ml. of distilled water and 5 ml. of the organic solvent. The bottles were placed on a rotating constant-temperature bath and equilibrated for 2 hr. at  $26 \pm 0.1^\circ$ . After equilibration, the 2 layers were separated and analyzed for diisopropylammonium dichloroacetate by nonaqueous titration.

**Colorimetric Procedure.**—Aqueous solutions of diisopropylammonium dichloroacetate were analyzed colorimetrically by the modified Fujiwara reaction (10). The proposed procedure differs from that reported by Leibman and Hindman (10) in that it is recommended that suitable standard solutions of this drug be run simultaneously. This general procedure was extended to the determination of the solubility of diisopropylammonium dichloroacetate in several solvents.

### RESULTS

Table I shows the results obtained from the analysis of diisopropylammonium dichloroacetate by a nonaqueous titration procedure. The method proved to be suitable for the crystalline salt as well as for aqueous and nonaqueous solutions of the drug. The nonaqueous procedure is accurate, rapid, and has many potential applications.

TABLE I.—ANALYTICAL DATA FROM NONAQUEOUS TITRATION STUDIES

Diisopropylammonium Dichloroacetate	mg. Taken	mg. Found	% Recovery <sup>a</sup>
Powder	118.35	118.24	99.9 $\pm$ 0.17
Aqueous soln.	100.00	100.3	100.3 $\pm$ 0.4
Chloroform soln.	99.87	100.87	101.0 $\pm$ 0.26

<sup>a</sup> Average of 5 or more determinations  $\pm$  standard deviation.

TABLE II.—SOLUBILITY OF DIISOPROPYLAMMONIUM DICHLOROACETATE<sup>a</sup>

Solvent	Solubility in Gm./100 ml. Soln. <sup>b</sup>
Distilled water	58.0 ± 0.32
Ethanol, 95%	46.3 ± 0.31
Chloroform	50.6 ± 0.21
Benzene	15.6 ± 0.22

<sup>a</sup> Average of 5 or more determinations ± standard deviation. <sup>b</sup> All data represent studies conducted at 26 ± 0.1°.

TABLE III.—DISTRIBUTION COEFFICIENTS OF DIISOPROPYLAMMONIUM DICHLOROACETATE<sup>a</sup>

Concn. of Diisopropylammonium Dichloroacetate, Gm. <sup>b</sup>	Benzene/Water	Chloroform/Water	
		$K = \frac{C_{CHCl_3}}{C_{H_2O}}$	$K = \frac{C_{CHCl_3}^c}{(C_{H_2O})^n}$
0.5029	0.017	...	...
1.0059	0.017	...	...
1.0019	...	0.22	0.345
1.5110	...	0.34	0.345
2.0034	...	0.44	0.348

<sup>a</sup> All data represent studies conducted at 26 ± 0.1°. Leaders indicate data were not determined. <sup>b</sup> Concentration of the drug partitioned between 5 ml. of organic phase and 5 ml. of aqueous phase. <sup>c</sup> The value of *n* was found to be 2.3.

TABLE IV.—COMPARATIVE SOLUBILITY STUDIES BY NONAQUEOUS AND COLORIMETRIC PROCEDURES

Solvent	Solubility in Gm./100 ml. Soln.	
	Nonaqueous	Colorimetric <sup>a</sup>
Distilled water	58.0 ± 0.33	60
Ethanol, 95%	46.3 ± 0.31	48.5
Chloroform	50.6 ± 0.21	<sup>b</sup>
Benzene	15.6 ± 0.22	15

<sup>a</sup> Standard deviations were not determined for the colorimetric assay. <sup>b</sup> The colorimetric procedure is not applicable with chloroform as the solvent (10).

Tables II and III, respectively, include data demonstrating the use of this method for the determination of solubilities and distribution coefficients.

In the solubility studies, solvents were selected to cover a broad range of dielectric constants (2.28–80). It was observed that, with the exception of chloroform, the solubility of diisopropylammonium dichloroacetate increased as the polarity of the solvent increased. The solubility in chloroform was much

greater than anticipated from the polarity of the solvent (Table II). It is proposed that the increased solubility of this drug in chloroform may be attributed to an association between solute and solvent molecules.

An examination of the distribution studies of diisopropylammonium dichloroacetate (Table III) reveals that in the partitioning between benzene and water, the expression of  $K = C_{org.}/C_{H_2O}$  is a constant value. This type of expression was not adequate for calculating the distribution coefficient between chloroform and water. However, a constant value was obtained from the use of the following expression:  $K = C_{org.}/(C_{H_2O})^n$  where the value of *n* is obtained from the slope of the line by plotting log  $C_{org.}$  against log  $C_{H_2O}$ . The data obtained suggest an association of the drug molecule in the organic phase.

The modified Fujiwara colorimetric procedure proved to be suitable as an assay method for this drug. However, reproducible results were obtained only if appropriate standards were run simultaneously with the test solutions. In analyzing 4 samples of the same concentration (0.4 mg./ml.) in 2 successive runs, the average deviations of the absorbance measurements were found to be ±0.003 and ±0.004. The utilization of a standard curve gave variable results. It was also found necessary to use a freshly prepared benzidine-formic acid reagent. Additional studies on the solubility of diisopropylammonium dichloroacetate in several solvents were conducted utilizing the modified Fujiwara colorimetric procedure. The data obtained from these studies are in agreement with those obtained in the nonaqueous titration experiments (Table IV).

This colorimetric procedure may prove valuable in studying the degradation of this drug and related molecules possessing the dichloroacetic acid moiety.

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