posed to lindane at a concentration of 5 ppm. To study the potential effects of increasing insecticide concentrations in the root environment, eight nutrient solutions were treated with lindane at 0.25, 0.5, 1, 2, 4, 6, 8, and 10 ppm. Fifteen-day-old plants (three groups of six plants each) were then grown for 24 hr in each of the nutrient solutions, when plants were harvested, extracted, and analyzed as described.

Results based on glc are presented in Figure 2. The penetration of lindane into roots appeared to be proportional to insecticidal concentrations up to 6 ppm, which comes close to the limit of the water solubility of lindane. The translocation of the insecticide into the greens, however, increased at a much slower rate when roots were exposed to increasing concentrations of the insecticide. The penetration and translocation of lindane, therefore, is to some extent also a function of the concentration of the insecticide in the root environment.

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Dissociation Constants of Succinic Acid 2,2-Dimethylhydrazide

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Dissociation constants were determined for succinic acid 2,2-dimethylhydrazide. The pK_1 and pK_2 were found to be 2.81 and 4.63, respectively.

Succinic acid 2,2-dimethylhydrazide (SADH) is an important plant growth retardant commercially available as Alar 85 and B-9. SADH is reported in the manufacturer's technical data sheet (Uniroyal, Inc., 1965) as a weak acid having an ionization constant of 1.12×10^{-5}. However, there is indication that SADH is bipolar in nature; namely, the solubility in water is enhanced by strong acids or bases, at acid pH SADH is retained by a strong acid cation exchange resin (Martin *et al.*, 1964), and hydrazides generally are monoacidic bases if the substituent does not contain a second nitrogen atom capable of being protonated (Gyenes, 1968). Further, preliminary studies indicated that the dissociation constant of the carboxyl group was considerably different from that reported.

Because of extensive studies underway on absorption of SADH requiring knowledge of the ionic nature of the molecule and the importance and extensive use of this chemical in research and commercial agriculture, a reassessment of the dissociation constant was essential.

EXPERIMENTAL SECTION

Apparatus. Dissociation constants were determined by potentiometric titration using a glass electrode (Schott & Gen., Mainz, Germany, S 30050-15 C) and a calomel reference electrode (Beckman 39402) in conjunction with a Beckman Century SS pH meter, calibrated in 0.01 pH units.

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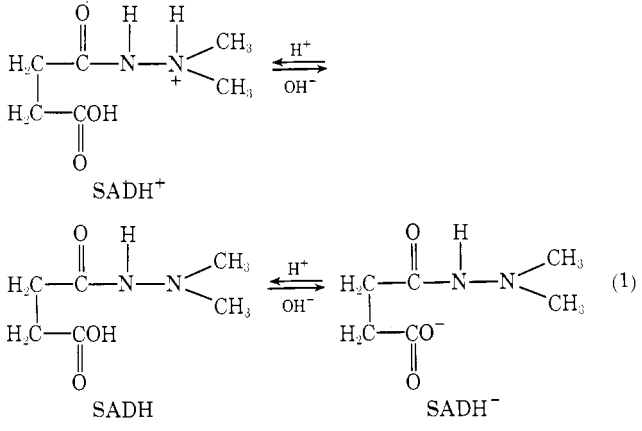
Chemicals. Technical grade SADH was Soxhlet extracted with acetone until a white solid precipitated. The precipitate was dissolved in hot isopropyl alcohol and cooled with magnetic stirring. The precipitate was filtered, air dried, and then vacuum dried at room temperature. Titration curves using this material coincided with those obtained with a reference sample (99.9% pure). C, H, and N analyses showed good purity. Theory: C, 44.99; H, 7.55; N, 17.49. Analysis: C, 44.82; H, 7.59; N, 16.38.

Procedure and Calculations. Titrations were carried out at 25 ± 1° in a nitrogen atmosphere. SADH concentration at half-neutralization was 0.01 *M*.

Dissociation equilibria may tentatively be written:



(1)

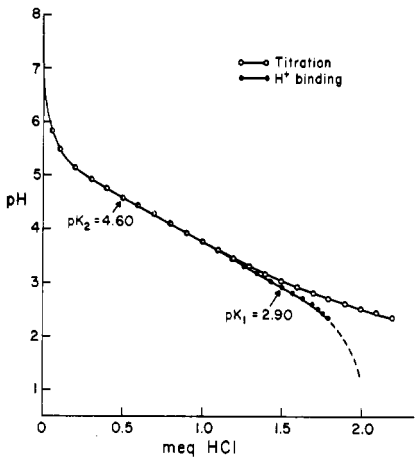


Figure 1. Titration curve for the sodium salt of succinic acid 2,2-dimethylhydrazide with HCl. 1 mM of SADH was first converted to the sodium salt by the addition of NaOH up to the equivalence point and then back titrated with 0.1 N HCl. SADH concentration at pK_1 and pK_2 was 0.0167 M and 0.01428 M, respectively. The H^- binding curve was calculated from the relationship: mequiv H^+ bound = mequiv HCl added − mequiv H^+ observed

Table I. Titration of SADH with NaOH. SADH Concentration at Half Neutralization was 0.02 M. Temperature, 25 ± 1°

ml NaOH (0.1044 N)	pH	pK_2'	−log $\gamma_\pm$	pK_2
0	3.77			
2.0	3.98	4.89	0.0225	4.91
4.0	4.15	4.72	0.0353	4.76
6.0	4.32	4.66	0.0364	4.69
8.0	4.48	4.62	0.0410	4.66
10.0	4.62	4.58	0.0447	4.63
12.0	4.81	4.58	0.0479	4.63
14.0	5.01	4.58	0.0507	4.63
16.0	5.29	4.59	0.0532	4.64
18.0	5.79	4.60	0.0554	4.65
20.0	10.85			

Apparent acid dissociation constants (pK_2') were calculated from the equation

$$pK_2' = pH + \log \frac{[HA]}{[A^-]} \quad (2)$$

$[A^-]$ and [HA] were calculated from the mass balance, $C = [A^-] + [HA]$, where C is the analytical concentration of SADH and, from the electroneutrality condition, $[A^-] + [OH^-] = [Na^+] + [H^+]$. Thus, for the titration of SADH with NaOH, $[A^-] = [NaOH] + a_{H^+}$ and $[HA] = C - [NaOH] - a_{H^+}$. p$K'$ values so calculated were converted into thermodynamic constants by means of the Debye–Hückel theory

$$pK_2 = pK_2' + \frac{0.509\sqrt{I}}{1 + 1.6\sqrt{I}} \quad (3)$$

where the ionic strength $I = \frac{1}{2}\Sigma c_i z_i^2$. The summation was carried out over all ionic species i of concentration c and valence z.

Apparent base dissociation constants (pK_1') were calculated from the equation

$$pK_1' = pH + \log \frac{[HB^+]}{[B]} \quad (4)$$

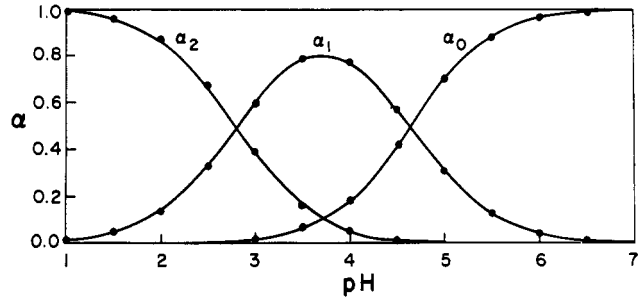


Figure 2. Diagram illustrating the distribution of various species of SADH as a function of pH. α_2, α_1, and α_0 represent $(SADH)^+/C$, (SADH)/C, and $(SADH)^-/C$ species, respectively

Table II. Titration of SADH with HCl. SADH Concentration at Half Neutralization was 0.02 M. Temperature, 25 ± 1°

ml HCl (1 N HCl)	pH	$\gamma_\pm$	pK_1
0	3.76		
0.2	3.57	0.9554	2.52
0.4	3.39	0.9388	2.70
0.6	3.24	0.9322	2.78
0.8	3.09	0.9165	2.80
1.0	2.95	0.9110	2.81
1.2	2.82	0.9025	2.81
1.4	2.70	0.8927	2.82
1.6	2.57	0.8929	2.81
1.8	2.45	0.8894	2.81
2.0	2.34	0.8866	2.81

Here HB^+ and B refer to the ionized and to the nonionized form of SADH, respectively. Because titration was carried out at low pH, activity corrections were made for stoichiometric concentrations of HB^+ (Albert and Serjeant, 1962).

$$pK_1 = pH + \log \frac{[BH^+]\gamma_\pm - a_{H^+}}{[B] + a_{H^+}} \quad (5)$$

Here $\gamma_\pm$ was calculated from $-\log \gamma\pm = 0.509\sqrt{I_1}(1 + 1.6\sqrt{I_1})^{-1}$, where $I_1 = [HCl]^- a_{H^+}$.

RESULTS AND DISCUSSION

The titration curve of Na–SADH with HCl shows that, for each mole of SADH, two equivalents of H^+ were bound (Figure 1). Estimated values for pK_1' and pK_2' were 2.90 and 4.60, respectively. Calculated pK values at various points during the titration were consistent toward the pH limits but varied considerably around the midpoint (Tables I and II). If one rejects those values obtained near the midpoint (pH 3.20 to 4.30) of the titration curve, a pK_1 of 2.81 ± 0.01 and pK_2 of 4.63 ± 0.03 are obtained. Using these values, the degree of dissociation of SADH was calculated as a function of pH and plotted (Figure 2). The two dissociation curves overlap between pH 2.63 ($SADH^-/C$ = 0.01) and pH 4.81 ($SADH^+/C$ = 0.01). This overlap is responsible for the variation in pK values calculated (Tables I and II) using data from this range of the titration curve. By selecting pK values from outside the region of overlap, the error due to the presence of mixed species should be minimal. Titrating the sodium salt of SADH with HCl between pH 8.30 and 4.40, where the $[SADH^+]$ species remains negligible, gave a pK_2 of 4.63 ± 0.02, which is identical with the value in Table I.

Comparing the pK values found with those for acethydrazide of 3.24 (Lindegren and Nieman, 1949) and succinic acid,

pK_1 = 4.19, pK_2 = 5.59 (Simms 1928), permits a tentative assignment of pK_1 to the hydrazide moiety and pK_2 to the carboxyl group.

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Gas Chromatographic Method for Residues of Baygon and Metabolites in Plant Tissues

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A gas chromatographic method for the determination of Baygon (*o*-isopropoxyphenyl *N*-methylcarbamate) and its metabolites in plants has been developed. The residues are removed from the plant sample by successive blending with acetone and chloroform. Baygon and the water-soluble conjugates are separated from each other and cleaned up separately. The conjugated metabolites are released by enzyme hydrolysis before the cleanup. Detection is by electron capture gas chromatography of the trichloroacetyl derivatives.

Baygon (*o*-isopropoxyphenyl *N*-methylcarbamate) is an insecticide being developed for agricultural use on cereal grains and pasture crops by Chemagro, Division of Baychem Corp., under license from Farbenfabriken-Bayer A.-G. It is presently used in pest control operations.

Metabolism studies on green beans (Everett and Gronberg, 1968; Kuhr and Casida, 1967) and corn (Everett and Gronberg, 1968; Gronberg, 1970) show that Baygon is partially converted to the carbamate-containing moieties, *o*-hydroxyphenyl *N*-methylcarbamate (hereafter called *o*-OH Baygon) and *o*-isopropoxyphenyl *N*-hydroxymethylcarbamate (hereafter called N-CH_2OH Baygon), and to a minor degree, other compounds of less toxicity, such as isopropoxyphenol. Structures of Baygon and the major metabolites are given in Figure 1. In plants, intact Baygon appears to be present in free form but the metabolites are primarily in the form of conjugated *O*-glycosides. The sugar portions of the glycosides may include mono-, di-, and trisaccharides (Kuhr and Casida, 1967).

The primary concern in the development of a suitable residue method was to account for the parent compound and for those toxic metabolites which metabolism studies showed might be formed from the parent compound. Since metabolism studies indicated that *o*-OH Baygon and N-CH_2OH Baygon were formed in plants, an analytical method was developed to account for residues of Baygon and these two metabolites.

The procedure is outlined in Figure 2. Residues of Baygon and conjugated metabolites are extracted from the plant tissues. Baygon is then separated from the conjugated metabolites and cleaned up by hexane–acetonitrile partitioning and column chromatography on Florisil. The metabolites are released from the conjugated form by enzyme hydrolysis and cleaned up by column chromatography on silica gel. Baygon and the metabolites are determined by gas chromatography after derivatization to obtain the desired sensitivity of detection with the electron capture detector.

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ANALYTICAL METHOD

Apparatus. A Hewlett-Packard Model 5750B gas chromatograph equipped with an electron capture detector was used. Explosion-proof blender motors were used to minimize the fire hazard from volatile organic solvents.

Reagents. Florisil (PR grade, 60–100 mesh) was heated in an oven at 130° for 24 hr to remove moisture. It was then deactivated by adding 2.5% H_2O (2.5 ml of H_2O + 97.5 g of dried Florisil) and allowed to equilibrate for at least 24 hr in a tightly stoppered bottle before use. β-Glucosidase enzyme, Emulsin from Almonds (Catalog No. G8625, Sigma Chemical Co.), was used as the enzyme. Phosphate buffer, pH 5, was prepared by adding 15 ml of 2/15 *M* $NaHPO_4 \cdot 7H_2O$ to 1000 ml of 2/15 *M* KH_2PO_4. Phosphate buffer, pH 11, was prepared by dissolving 27.2 g of KH_2PO_4 and 10 g of NaOH in water and diluting to 1000 ml. Silica gel, Fisher S-279, was heated in an oven at 130° for 24 hr to remove moisture. It was then deactivated by adding 10% H_2O (10 ml of H_2O + 90 g of dried silica gel) and allowed to equilibrate for at least 24 hr in a tightly stoppered bottle before use. All chemicals were analytical reagent grade; organic solvents were redistilled in glass stills prior to use. Trichloracetyl chloride (Eastman No. 2032) was used for derivatization reactions.

Sample Preparation. Grind the plant tissues in a Hobart food chopper in the presence of Dry Ice and place the samples in frozen storage overnight to allow the Dry Ice to sublime.

Sample Extraction. Weigh a 20-g sample into a Waring Blendor jar. Add 5 ml of H_2O and 200 ml of acetone and