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Analytical Studies on Isoxazoles. VI.[1] Colorimetric Determination of Some Isoxazole Herbicides with *p*-Dimethylaminocinnamaldehyde

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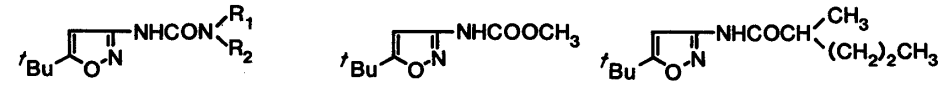
Some isoxazole herbicides were determined by a colorimetric method with *p*-dimethylaminocinnamaldehyde (DACA) as the reagent. The compounds (**2**, **3** and **4**) having various substituents at the 3-position were hydrolyzed to 3-amino-5-*tert*-butylisoxazole (**5**). The assay method was based on the coloration of **5** with DACA, which was reported previously. The optimum hydrolysis conditions were determined. Compound **5** was obtained from **2**, **3** and **4** with sufficient recoveries under neutral, alkaline and acidic conditions, respectively. The raw materials could thus be evaluated effectively by simple assay methods.

Keywords——isoxazole herbicide; 5-*tert*-butylisoxazole-3-substituted; colorimetric assay method; *p*-dimethylaminocinnamaldehyde; Schiff base; 3-amino-5-*tert*-butylisoxazole; hydrolysis condition

In previous studies on isoxazoles, several compounds, **1**, **2**, **3** and **4**, were found to have herbicidal activity. Isouron (**1**) showed the highest activity.[2] The other compounds, although their potencies for weed control were inferior to that of **1**, showed less phytotoxicity to the crops.[3] Thus, in order to develop effective herbicides to be used selectively in croplands, investigations have been continued.

In the previous paper, we reported a colorimetric method for the assay of **1**.[1] This method was based on the Schiff base formation of 3-amino-5-*tert*-butylisoxazole (**5**) with *p*-dimethylaminocinnamaldehyde (DACA). It is also possible to analyze **2**, **3** and **4** by this method when they are hydrolyzed to **5**. This paper describes simple assay methods for these compounds and the establishment of the optimum conditions.



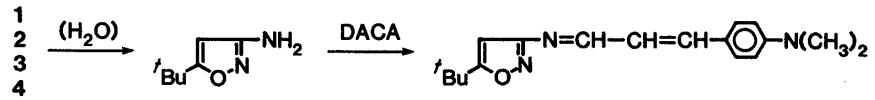
$R_1=R_2=CH_3$ **1** (isouron)
$R_1=CH_3$, $R_2=H$ **2**

3

4

^{t}Bu = *tert*-butyl

Chart 1



5

6

^{t}Bu = *tert*-butyl

Chart 2

Experimental

Apparatus and Reagent——The same apparatus and reagents as described in the previous paper[1] were used.

Reagent Solution——0.8% DACA Solution: Dissolve 0.8 g of DACA in $CHCl_3$ to make 100 ml.

1% DACA Solution: Dissolve 1 g of DACA in a mixture of acetone and MeOH (1 : 1) to make 100 ml.

Methanolic HCl Solution: Dilute 6 ml of hydrochloric acid with MeOH to make 100 ml. Dilute 1 ml of the solution with MeOH to make 25 ml (*ca.* 0.03 N).

Assay Procedure of 2——Accurately weigh about 25 mg of the sample into a 200-ml volumetric falsk, dissolve it in and dilute to the mark with $CHCl_3$. Transfer exactly 0.5 ml of the solution to a hydrolysis tube[4] and evaporate the solvent under reduced pressure. Add exactly 2 ml of 10% DMF aqueous solution, seal the tube and heat it at 157—163 °C for 1 h. After cooling the tube to room temperature, open it and transfer exactly 1 ml of the solution to a 12-ml centrifuge tube. Add exactly 2.5 ml of $CHCl_3$, shake with a shaker for 3 min and centrifuge for 3 min. Discard the aqueous layer and transfer exactly 1 ml of the $CHCl_3$ layer to a 10-ml volumetric flask. Proceed as directed in the general method for the assay of **5**.

Assay Procedure of 3——Accurately weigh about 15 mg of the sample into a 100-ml volumetric flask, dissolve it in and dilute to the mark with 0.1 N NaOH. Transfer exactly 2 ml of the solution to a 10-ml volumetric flask, stopper loosely and heat it at 100 °C for 40 min. After cooling, add exactly 2 ml of 0.1 N HCl and dilute to the mark with H_2O. Pipet 1 ml of the solution into a 12-ml centrifuge tube. Add exactly 2.5 ml of $CHCl_3$, shake with a shaker for 3 min and centrifuge for 3 min. Discard the aqueous layer and transfer exactly 1 ml of the $CHCl_3$ layer to a 10-ml volumetric flask. Proceed as direcred in the general method for the assay of **5**.

Assay Procedure for 4——Accurately weigh about 35 mg of the sample into a 100-ml volumetric flask, dissolve it in 20 ml of DMF and dilute to the mark with H_2O. Transfer exactly 1 ml of the solution to a 10-ml volumetric flask and add 1 ml of hydrochloric acid. Stopper loosely and heat the flask at 100 °C for 40 min. After cooling, dilute the reaction solution with H_2O to make exactly 10 ml. Pipet 1 ml of the solution into a 12-ml centrifuge tube. Add exactly 2.5 ml of $CHCl_3$, shake with a shaker for 3 min and centrifuge for 3 min. Discard the aqueous layer and transfer exactly 1 ml of the $CHCl_3$ layer to a 10-ml volumetric flask. Proceed as directed in the general method for the assay of **5**.

General Method for the Assay of 5——After the hydrolysis, add 5 ml of $CHCl_3$ to each sample solution, then 1 ml of 0.8% DACA solution and 1 ml of methanolic HCl solution, mix, and dilute to the mark with $CHCl_3$. Separately, take 6 ml of $CHCl_3$ into a 10-ml volumetric flask, add 1 ml of 0.8% DACA solution and 1 ml of methanolic HCl solution, mix, and dilute to the mark with $CHCl_3$. Use this solution as the blank solution. After allowing each solution to stand for 15 min, read the absorbance at 533 nm using the blank solution as the blank.

Calibration Curve——Calibration curves were made according to the standard assay procedures. Linear relationships between the concentration of each compound and the absorbance were obtained in the ranges of 40—200 μg/ml for **2**, 50—250 μg/ml for **3** and 110—550 μg/ml for **4**.

Identification of the Hydrolysis Product——1) Spectrophotometry: Measure the visible absorption spectrum of the final colored solution obtained by the standard assay procedure. Compare the spectrum with that of the colored solution obtained from **5** in the same manner.

2) Thin Layer Chromatography: Proceed with **2**, **3** and **4** as directed in each assay method. After the hydrolysis, extract the reaction solution with $CHCl_3$ (1 ml). Spot 10 μl of the $CHCl_3$ solution on a TLC plate (E. Merck, Silica gel F_{254}, Art. 5715). Develop the chromatogram with a mixture of benzene and acetonitrile (1 : 1). Spray 1 N HCl and 1% DACA solution (detection limit: 1 μg for **2**, **3** and **4**). Separately, perform the hydrolysis in the same manner using samples of 10-fold greater concentration. After the hydrolysis, extract the reaction solution with $CHCl_3$ (1 ml). Spot 10 μl of the $CHCl_3$ solution on another TLC plate. Develop the chromatogram with the same solvent and dry it in air. Spray H_2O, 0.1 N NaOH and H_2SO_4 for **2**, **3** and **4**, respectively, and heat the plate at 180—190 °C for 5 min. Cool and spray 1 N HCl, and then 1% DACA solution (identification of unreacted **2**, **3** and **4**).

Results and Discussion

Compounds **2**, **3** and **4** were hydrolyzed under the optimum conditions. After the hydrolysis, the resulting **5** was extracted with $CHCl_3$ and determined *via* the coloration reaction with DACA. Each product was identified as **5** by comparison of the absorption spectrum with that of authentic **5** and by thin layer chromatography. No spot other than that of **5** was found on the TLC plate after the hydrolysis reactions of **2** and **3**. In the case of **4**, a small amount of unreacted material was found together with **5**.

The hydrolysis conditions of these compounds were examined, as described below.

Hydrolysis Conditions for Compound 2

Compound **2** was hydrolyzed by heating the aqueous solution, as described for **1**[1] (in a

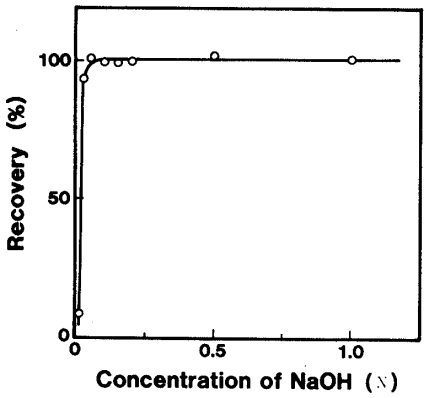


Fig. 1. Effect of NaOH Concentration in the Hydrolysis of **3**

NaOH solutions of **3** were heated at 100 °C for 40 min.

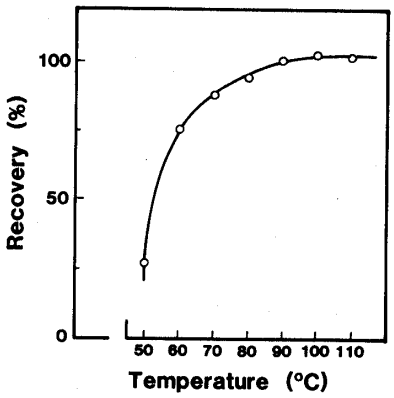


Fig. 2. Effect of Temperature on the Hydrolysis of **3**

A 0.1 N NaOH solution of **3** was heated at various temperatures for 40 min.

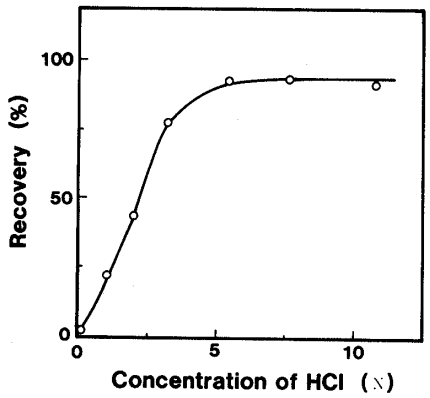


Fig. 3. Effect of HCl Concentration on the Hydrolysis of **4**

HCl solutions of **4** were heated at 100 °C for 40 min.

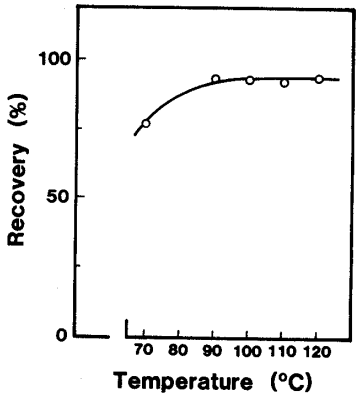


Fig. 4. Effect of Temperature on the Hydrolysis of **4**

A 5.5 N HCl solution of **4** was heated at various temperatures for 40 min.

sealed tube at 160 °C), and **5** was formed quantitatively. The hydrolysis yield remained constant over 1 h.

Hydrolysis Conditions for Compound 3

Compound **3** was hydrolyzed in the presence of alkali; an NaOH solution of **3** was heated at 100 °C. Figure 1 shows the effect of NaOH concentration on the hydrolysis. The reaction was nearly complete at NaOH concentrations over 0.1 N. Figure 2 shows the recoveries at 50—110 °C. Compound **3** was completely hydrolyzed at 90 °C or above. More than 30 min were required for the reaction. The hydrolysis yield was insufficient in an acid solution; when **3** was heated in 5.5 N HCl, only 20% of **3** was hydrolyzed.

Hydrolysis Conditions for Compound 4

Compound **4** was hydrolyzed in the presence of acid; an HCl solution of **4** was heated at 100 °C. Figures 3 and 4 show the effects of HCl concentration and temperature, respectively, on the hydrolysis. The hydrolysis occurred in about 90% yield with over 5.5 N HCl at 90—120 °C. More than 30 min were required for the reaction.

The hydrolysis yield was insufficient in alkaline solution; when **4** was heated in 1 N NaOH, 47% of **4** was hydrolyzed.

Characteristics of the Assay Method

Samples of **2**, **3** and **4** were assayed with coefficients of variation of 2.19, 1.10 and 0.83%, respectively ($n=7$ each). Previously we established colorimetric and fluorometric methods for the determination of **1**.[4,5] Since both methods are based on diazo coupling, they have the disadvantages that the procedure is complex and that speed is essential, because of the lability of the diazonium salt of **5**. The present method is simple, and does not require speed of execution or ice-cooling. It is suitable for routine analyses.

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References and Notes

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