Determination of the pK_a Values of Metribuzin and Three of Its Metabolites: A Comparison of Spectrophotometric and Potentiometric Methods

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The pK_a values for metribuzin and its three known metabolites [DA (deaminated metribuzin), DK (diketometribuzin), and DADK (deaminated diketometribuzin)] were determined by potentiometric titrimetry and by UV spectrophotometry. The values obtained potentiometrically, by measurement between pH 2 and pH 12 for these four compounds, were 7.1, 7.3, 10.0, and 8.3, respectively. The apparent pK_as determined spectrophotometrically for these four compounds were 1.0, 7.3, 9.9, and 8.4, respectively. The pK_a determined for DA by the spectrophotometric method was in agreement with that obtained by potentiometric titrimetry. The observed spectral shift for DK may involve tautomerization as well as deprotonation. The UV spectra of DADK at various pH values could not be analyzed directly to obtain a pK_a value, but the value obtained after deconvolution of the UV spectra into component Gaussians agreed with the titrimetric pK_a. The spectrophotometrically determined pK_a value of approximately 1.0 obtained for metribuzin may be associated with acid-catalyzed decomposition during protonation of the molecule. The potentiometrically determined pK_a of DK corresponded to protonation of the amino group, but the potentiometrically determined pK_a of metribuzin did not.

Ionization constants of *s*-triazines have been shown to be important in studies of soil adsorption [for reviews, see Bailey and White (1970) and Weber (1970)] and aqueous solubilities (Ward and Weber, 1968). Ionic binding to soil constituents, which decreases effective herbicidal activity, has been postulated to be related in a predictable manner to the pK_a values of the herbicides (Weber, 1966). In addition, ionization constants and their relation to lipophilicity are important to the study of membrane transport and metabolism (Goldstein et al., 1974).

Spectrophotometrically determined pK_a values have already been reported for several 1,3,5-triazine herbicides (Weber, 1967, 1977) and for metribuzin (Ladlie et al., 1976a; Weber, 1980). Studies of the herbicidal activity of metribuzin, a 1,2,4-triazine, have been performed at various pH values (Ladlie et al., 1976a–c, 1977a,b). The present paper describes the redetermination of the ionization constants for metribuzin and is the first, to our knowledge, to report the ionization constants for metribuzin's three known metabolites. As part of this study, a comparison was done of the results obtained from two different methods of determining the pK_a values, spectrophotometry and potentiometric titrimetry. In addition, experiments were done to determine whether or not the observed pK_a values corresponded to ionization of the amino group.

MATERIALS AND METHODS

Standards and Solvents. Analytical standards of metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)one: Bay 943371], DA [deaminated metribuzin, 6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one], DK [diketometribuzin, 4-amino-6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione], and DADK [deaminated diketometribuzin, 6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2*H*,4*H*)-dione] were obtained from Mobay Chemical Corp., Agricultural Chemicals Division, Kansas City, MO. Stated purities for these compounds were 99.6%, 95%, 95%, and 95%, respectively, and these values were not inconsistent with purity analyses by HPLC, using the method described by Parker et al. (1983). Structures of these compounds, as given by Smith and Wilkerson (1974), among others, are shown in Figure 1.

Solvents used were HPLC-grade methanol (Fisher Scientific Co., Fairlawn, NJ) and deionized water (Darco Water Systems, Durham, NC). The formaldehyde used was from Fisher Scientific Co., as were the concentrated hydrochloric acid and sodium hydroxide used for making the pH adjustments.

Instrumentation. Spectrophotometric titrations were recorded on a Beckman DB-GT UV/vis spectrophotometer (Beckman Instrument Co., Irvine, CA), connected to a Beckman 10-in. recorder. Measurements of pH were made with a Fisher Accumet pH meter, Model 620, and a Fisher combination pH electrode.

Potentiometric titrations were performed using a Radiometer autoburet, Model ABU 12 (Radiometer America, Inc., Westlake, OH), an Orion pH meter, and an Orion pH electrode (Models 407A and 91-05, respectively, Orion Research, Cambridge, MA). The pH meter was continuously monitored with a PE56 recorder (Perkin Elmer Corp., Norwalk, CT). The recorder span was set so that pH 7.0 was centered, with pH 2–12 contained between zero and full scale.

The UV absorbance spectra of DK with and without formaldehyde in water–methanol (9:1 v/v) were scanned in a 1-cm quartz cell against water–methanol (9:1 v/v) by using a Cary 17 spectrophotometer (Varian, Palo Alto, CA). Scan conditions were 0.2 nm/s, slit 0.1 nm (automatic), and 0.2 nm bandwidth.

The high-performance liquid chromatographic (HPLC) system used for the analysis of metribuzin solutions consisted of a Waters 6000A pump (Waters Associates, Milford, MA), a 25-cm Du Pont Zorbax ODS column (E. I. Du Pont de Nemours, Inc., Wilmington, DE), a Bio-Rad variable-wavelength UV detector, set at 254 nm (Bio-Rad Laboratories, Richmond, CA), and a Beckman 10-in. recorder.

Deconvolution of the UV spectra (separation of the spectra into component Gaussians) was done on a Radio Shack TRS-80 computer (Tandy Corp., Fort Worth, TX) equipped with a Radio Shack printer, Model VI.

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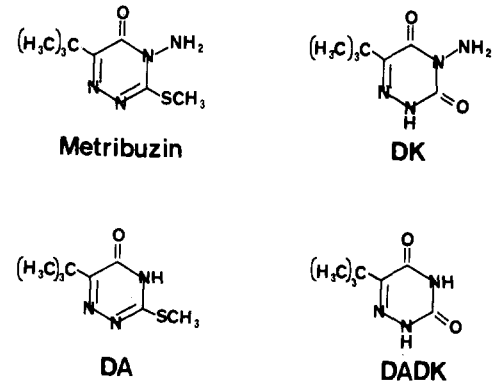

Figure 1. Structures of metribuzin, DA, DK, and DADK.

Procedures. Spectrophotometric titrations were done according to the method of Weber (1977), which is based on the pioneering studies of Flexser et al. (1935). Approximately 1 mg of each standard was dissolved in 1 mL of methanol. Appropriate dilutions were made with deionized water to give final (known) concentrations of approximately 5×10^{-5} M. Microliter volumes of concentrated HCl or 10% NaOH were added to a 25-mL portion of the solution until the desired pH was reached, at which point a UV spectrum was taken over the range 360–190 nm, at a scan rate of 25 nm/min. The aliquot was then returned to the solution for further pH adjustment. Separate portions of the 5×10^{-5} M solution were used for the acidic and basic portions of the titration curve.

For the titrimetric experiments, 20 μmol of each compound was dissolved in 0.22 mL of methanol or *p*-dioxane and 2.0 mL of 0.010 N HCl (20 μequiv) was added. The resulting solution was then titrated with 0.1 N NaOH. Under these conditions, pHs between 2.3 and 12 could be scanned.

Control experiments were done by titrating 2.0 mL of 0.01 N HCl with 0.1 N NaOH at a constant rate of 0.042 mL/min. The addition of up to 70 μL of 37% formaldehyde had no effect on the titration curve if the formalin was first neutralized over marble chips. The effect of methanol was checked by titrating pyridine (20 μmol) with and without methanol (10% v/v) present. Methanol had no detectable effect on the apparent pK_a of pyridine. Reported pK_a values represent the pHs corresponding to the minimum slopes of the titration curves following a clear inflection.

RESULTS AND DISCUSSION

General Considerations. For the spectrophotometric titration to be successful, the absorbance spectra of the protonated and neutral species must be different enough for the absorbance at selected wavelengths to be used to determine the concentrations of these species. Then

$$pK_a = pH - \log [(A - A_{MH^+})/(A_M - A)] \quad (1)$$

where A is the absorbance at a particular pH, at a given wavelength, A_M is the absorbance of the molecular species at that wavelength, and A_{MH^+} is the absorbance of the protonated species at that wavelength.

To determine the individual absorbances of M and MH^+, pHs where each form is the only one present in the solution must be achievable. An additional assumption implicit in this method is that the only process occurring with changing pH is protonation or deprotonation and not decomposition, chemical reaction, isomerization, or tautomerization (Jaffe and Orchin, 1962).

A difficulty encountered with both methods is the possibility of the compound having more than one pK_a (leading to complex and perhaps uninterpretable titration curves) or the possibility of precipitation during the titration. Presumably this would occur, if at all, during the transition from ionized to unionized states and, in the titrimetric experiment, would have the effect of shortening the time before the sharp rise in pH that accompanies completion of the titration. Thus, precipitation would lower the apparent pK_a very slightly (probably a fraction of a pH unit).

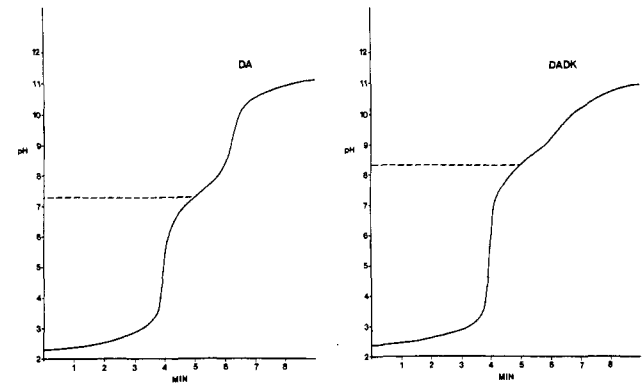

Figure 2. Potentiometric titration curves for DA and DADK (20 μequiv of each compound plus 20 μequiv of HCl, titrated with 0.1 N NaOH at a titration rate of 42 μL/min).

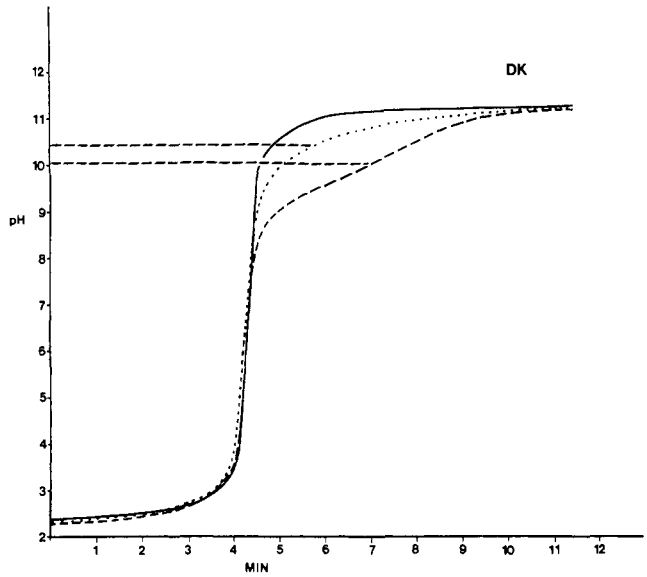

Figure 3. Potentiotentimetric titration curves for DK. All titrated with 0.1 N NaOH at 42 μL/mm: (– – –) 20 equiv of DK plus 20 μequiv of HCl; (- - -) 20 μequiv of DK plus 20 μequiv of HCl plus formaldehyde; (—) 20 μequiv of HCl plus formaldehyde.

Should a compound have more than one titratable group, and more than one pK_a between 2.5 and 11.5, a potentiometric titration under these conditions would probably not resolve the pK_as unless they differed by at least 2 pH units. If they were, for example, 1 pH unit apart, one would expect to see only one inflection with an apparent pK_a midway between the two. Such an occurrence would, however, be detectable by the amount of base consumed in the titration.

Potentiometric Titration Experiments. Each of the metribuzin metabolites showed a clear inflection within the pH 2.3–12 range. Apparent pK_as were as follows: for DA, 7.3 ± 0.1 (2σ, $N = 3$); for DK, 10.0 ± 0.15; for DADK, 8.3 ± 0.1. Potentiometric titration curves are shown in Figures 2 and 3. It was necessary to titrate at the slowest speed of the autoburet in order to achieve the standard deviations shown; however, there were no indications that

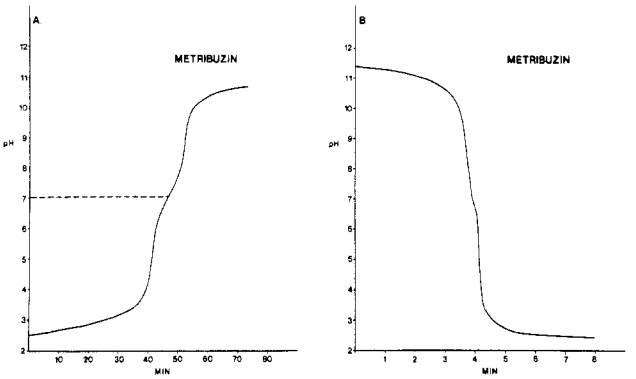

Figure 4. Potentiometric titration curves for metribuzin: (A) 20 equiv of metribuzin plus 20 μequiv of HCl, titrated with 0.01 N NaOH at a titration rate of 42 μL/min; (B) 20 μequiv of metribuzin plus 2 mL of 0.01 N NaOH, titrated with 0.1 N HCl at a titration rate of 42 μL/min.

the mean apparent pK_as were titration rate dependent. The amount of NaOH required for the titrations indicated that 1 equiv of OH^- was neutralized per mol of DA and DADK but 2 equiv were needed per mol of DK. Thus the reported value for DK (10.0) was actually the mean of unresolved pK_as for two ionizable sites.

Formol titration, titration in the presence of formaldehyde (Sorenson, 1907; Levy and Silverman, 1937), of DK eliminated the contribution from one of the ionizable states to the potentiometric titration curve but had no effect on the pK_a determined spectrophotometrically (see below). The most probable explanation for this occurrence is that ionization of the amino group, which is not conjugated to the double-bond system comprising the UV chromophore, was suppressed by the formation of an amine–aldehyde derivative, while the spectrophotometrically detectable proton/deprotonation involved the ring system directly and was not altered by the formaldehyde. That DK did react with formaldehyde was evidenced by an increase in the UV absorbance from 0.185 to 0.193 at 261 ± 1 nm (pH 2.1) and from 0.179 to 0.200 at 292 nm (pH 12.5) after addition of HCHO.

The exact sites of protonation/deprotonation of the metabolites cannot be determined simply from potentiometric titration curves. Unfortunately, as will be discussed later, the UV spectra also do not unequivocally answer these questions.

Unexpected results were obtained in the potentiometric titration of metribuzin. As shown in Figure 4A, during titration with 0.01 N NaOH there was a clear break in the titration curve near pH 7.1. The break corresponded to the utilization of less than 0.5 equiv of NaOH/mol of metribuzin. When 20 equiv of metribuzin, in 10% methanol, was treated with 20 μequiv of NaOH and back-titrated with 0.1 N NCl, an even smaller break in the curve was seen (Figure 4B), and obvious precipitation occurred. Replacing the methanol with 1,4-dioxane eliminated both the break in the curve and the precipitation. An infrared spectrum of the material precipitated at pH 2.5 from methanol–water gave no evidence of an amine hydrochloride but simply differed from the spectrum of stock metribuzin in suggesting involvement of the $-NH_2$ and C=O groups in intermolecular hydrogen bonding (Bellamy, 1958). A detailed spectral study involving IR, NMR, UV, and mass spectra of these compounds will be presented elsewhere (Albro et al., 1984). Metribuzin reacted rapidly with formaldehyde as evidenced by the infrared and NMR spectra of the product; again, neither precipitation nor a break in the titration curve occurred during the formol titration of metribuzin. Metribuzin hydrochloride was formed by bubbling HCl through a benzene solution of metribuzin; an infrared spectrum of the product bore little resemblance to that of the material precipitated from aqueous methanol with HCl at pH 2.5. There was no change in the UV spectrum of dilute (10^{-4} M) metribuzin in the pH 2.5–11 range (see below). It appears, then, that metribuzin forms a dimer (or higher polymer) as the pH drops below 7, and the increased solubility above pH 7 may involve dissociation to the monomer. When titration is very slow, the effect can be detected as a slight inflection on the titration curve. At higher rates of titration the dissociation may occur too slowly to be observed, and when a hydrogen bond disrupting solvent (dioxane) is present, the phenomenon is also not seen.

Spectrophotometric Titrations. UV absorbance spectra and plots of absorbance versus pH at selected wavelengths for the four compounds of interest are shown in Figures 5 and 6. The apparent pK_a values obtained from these curves are as follows: for metribuzin, 1.0 ± 0.1; for DA, 7.3 ± 0.2; for DK, 9.9 ± 0.1; for DADK, 8.4 ± 0.1. The apparent pK_a value obtained for metribuzin agrees within experimental error with literature values of 0.99 (Ladlie et al., 1976a) and 1.1 (Weber, 1980). Both of these earlier values were obtained by using the spectrophotometric method.

As can be seen in Figure 6, the UV absorption spectra of DADK could not be used directly to determine the pK_a, because the shift in absorbance between the protonated and unprotonated forms of these molecules was too slight. In order to obtain a spectrophotometric pK_a for this compound, a computer program (listings available on request) was written to deconvolute the absorbance peaks, and the amplitudes of the component Gaussians were plotted against pH in order to arrive at a pK_a value. For the deconvolution, a self-consistent set of Gaussians was determined by fitting the UV spectra at the pH extremes, such that the same set of Gaussians could fit all pHs by varying only the amplitude factors. The reconstructed UV spectra were of the form

$$f(\lambda) = \sum_{i=i}^{n} A_i e^{-K_i(\lambda_u - O_i)^2} \qquad (2)$$

where n is the total number of Gaussians, λ_u is the highest wavelength scanned, A_i is the amplitude of the Gaussian, O_i is the offset, and K_i is the peak width. In the case of DADK, a minimum of five Gaussians was needed to fit the UV spectrum. Absorbance maxima for these five peaks were at 198, 209, 229, 249, and 263 nm. Deconvoluted spectra, at pH 3.2 and pH 9.5, showing the contributions of the five component gaussians, are shown in Figures 7 and 8, respectively. The absorbance vs. pH curves from which the pK_a value was determined are shown in Figure 9. The absorbance values (i.e., the peak heights) of the peaks at 249 and 263 nm were used to construct these curves.

The complex set of spectral shifts for DADK not associated with a detectable isosbestic point suggests that protonation of the ring is accompanied by the establishment of a dynamic equilibrium between tautomeric forms. Tautomeric shifts would not be expected for metribuzin because of the ring substitutions, and indeed, a clear isosbestic point is seen for this compound.

Formol titrations of metribuzin and DK were also performed using the spectrophotometric method. The titration curve for metribuzin was essentially unchanged from that without formaldehyde, indicating that the break in the titration curve at 1.0 does not correspond to protonation of the amino group.

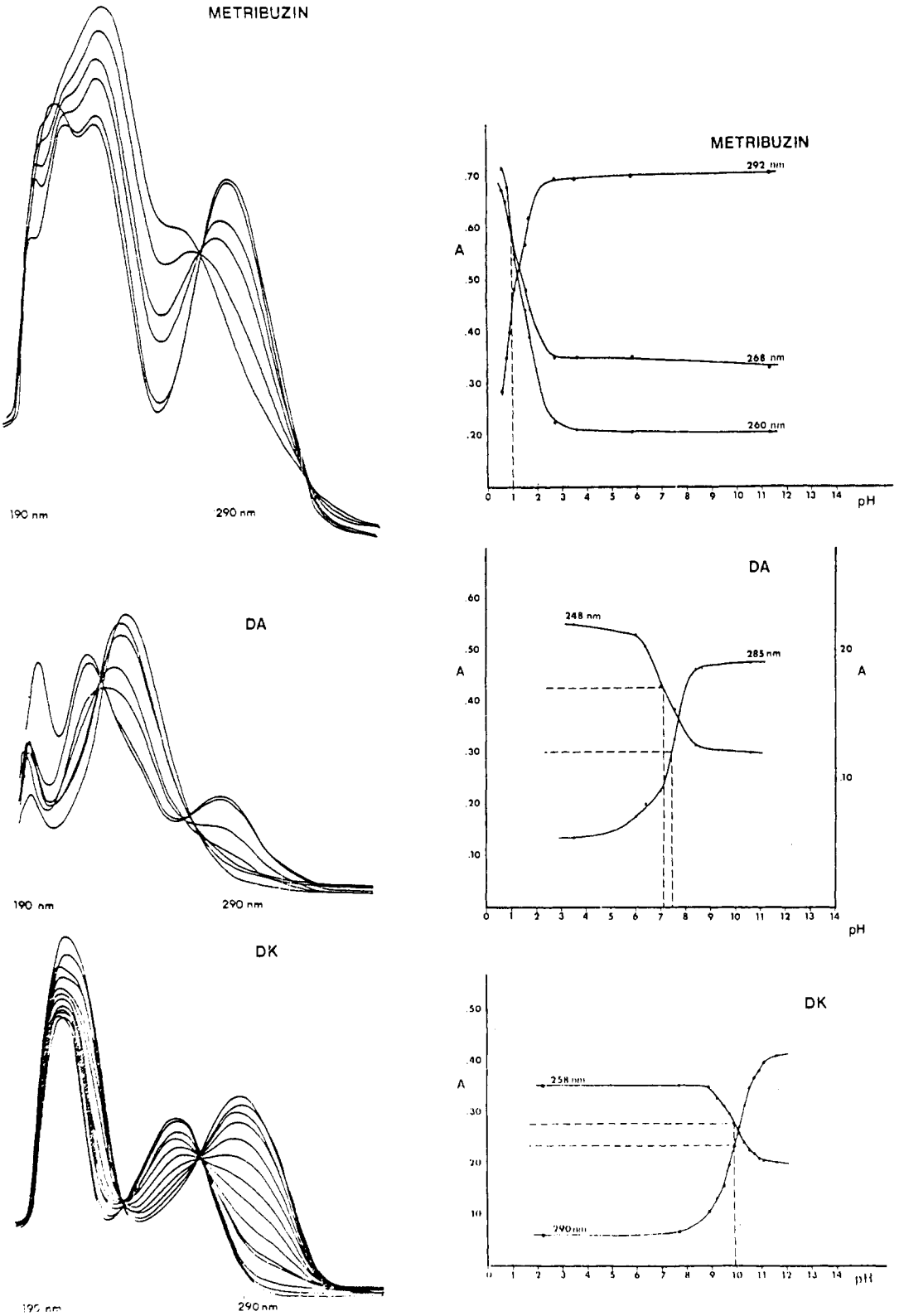

Figure 5. UV spectra at various pHs and plots of UV absorbance vs. pH at selected wavelengths for metribuzin, DA, and DK.

A formol titration of DK, which had been shown potentiometrically to have a pK_a near 10.0 associated with titration of the amino group, still showed an apparent spectral pK_a near 10.0. The reaction of DK with formaldehyde was confirmed by a change in the extinction coefficient for the complex as discussed above. The lack of effect on the λ_{max} ($\Delta\lambda_{max}$ ±1–2 nm) indicates that the reaction does not generate a new chromophore. This may be because the double bond of the –N=CH2, the –NH–CH_2OH, or the –N–$(CH_2OH)_2$ group formed (Levy and Silverman, 1937) is not conjugated to the carbonyl groups. Thus, the chromophore being altered as the pH changes is the ring system, possiby with accompanying tautomerism. A tautomeric shift generating a hydroxyl group followed by ionization of the hydroxyl would be reasonable at pH 10.

Comparison of Results. For the three known metabolites of metribuzin, DA, DK, and DADK, pK_a values obtained by spectrophotometric and potentiometric titration were in good agreement (coincidentally in the case of DK). Deconvolution of the DADK spectrum was required, however. For metribuzin itself, the spectropho-

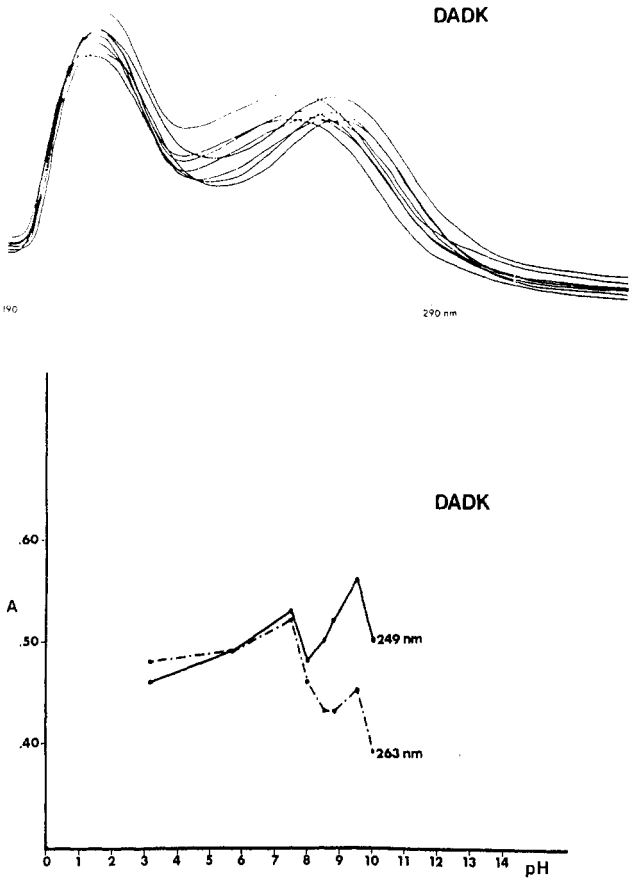

Figure 6. UV spectra at various pHs and plots of UV absorbance vs. pH at selected wavelengths for DADK.

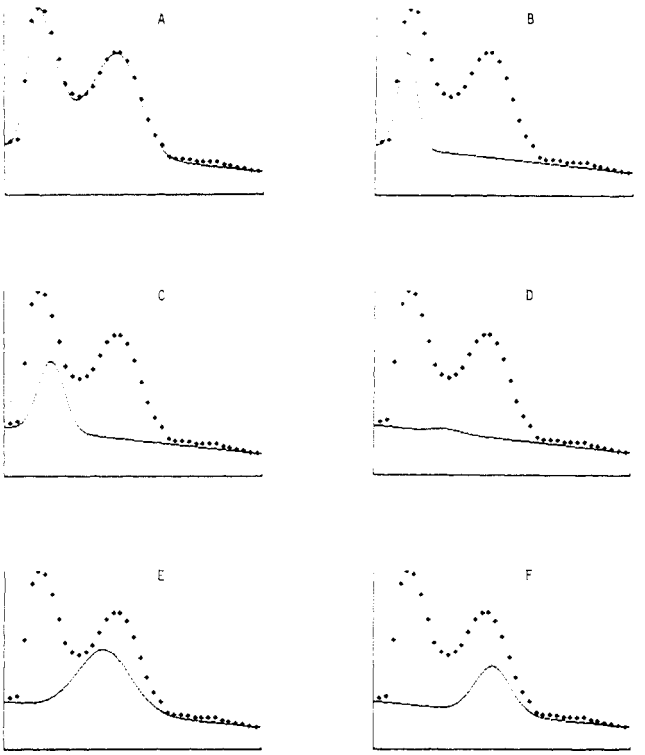

Figure 7. Deconvolution of the DADK UV spectrum at pH 3.2, showing a composite spectrum (A) and the five-component Gaussians (B–F).

tometric pK_a was beyond the pH range examined potentiometrically. In order to determine the cause of the change in the absorbance spectrum that occurs around pH 1.0, 5 mg of metribuzin was dissolved in 1 mL of methanol

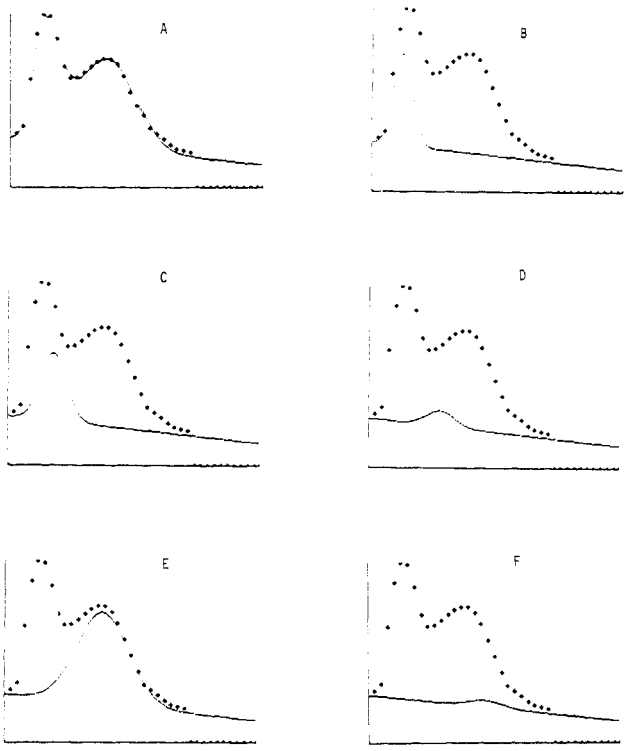

Figure 8. Deconvolution of the DADK UV spectrum at pH 9.5, showing a composite spectrum (A) and the five-component Gaussians (B–F).

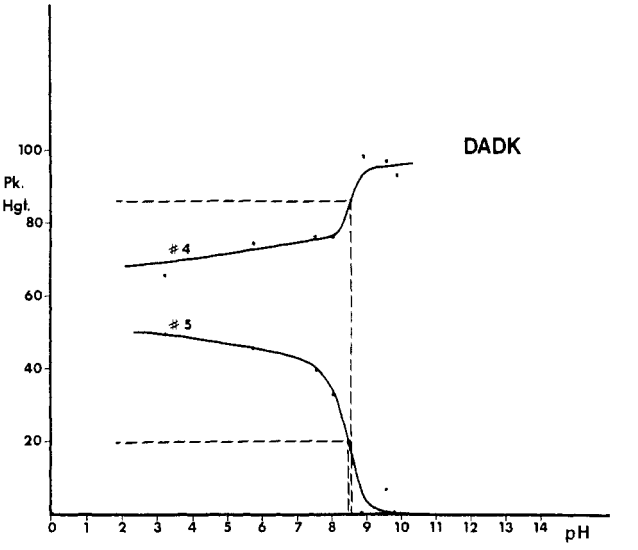

Figure 9. Plot of peak heights of component Gaussians 4 and 5 of DADK vs. pH.

and 10 mL of 2 N HCl was added (pH approximately –0.3). The solution was stirred at room temperature for 1 h, during which a strong mercaptan or sulfide odor was detected. The solution was then treated with 2.5 mL of 5 N NaOH (giving a pH of approximately 13.3), and the products were extracted with ether. Two fractions could easily be separated: metribuzin itself, which is soluble in methanol, and another product (or mixture of products), which was insoluble in methanol but soluble in methylene chloride. The two fractions were approximately equal in mass.

High-performance liquid chromatographic analysis of a 5×10^{-5} M solution of metribuzin before and after treatment with acid at pH –0.3 showed an approximately 50% reduction in the metribuzin peak height but did not indicate formation of any of the known metabolites. A

solvent consisting of 60:40 (v/v) methanol–0.05 N HOAc and a 25-cm Du Pont Zorbax ODS column were used for the analysis (Parker et al., 1983). Both of these experiments provide evidence for the decomposition of metribuzin at low pH. Identification of the decomposition product(s) is presently under study.

CONCLUSIONS

A comparison of spectrophotometric and potentiometric titrations for determining the pK_as of metribuzin's three known metabolites indicates that without computer manipulation of the data, only DA and DK could be successfully titrated spectrophotometrically and the pK_a value obtained for DK may represent only one of the two sites titrating in the pH 9–11 range. With deconvolution, the pK_a of DADK could be correctly determined spectrophotometrically. Spectrophotometric titration could not detect the structural alteration occurring at approximately pH 7.1 in a solution of metribuzin. The possibility also exists that the pK_a value of 1.0 determined spectrophotometrically may involve decomposition of metribuzin rather than just protonation. Formol titrations done to determine the functional group being protonated in DK confirmed the occurrence of two sites that could deprotonate near pH 10 under these conditions and suggested that one of these sites was the amine group.

In conclusion, while the spectrophotometric method has the advantage of requiring a much smaller sample size than does potentiometric titration, errors can result from assuming that all spectral shifts are due to simple ionization of the compound, since such shifts can result from processes other than protonation. In addition, true pK_as can be missed when protonation does not cause a significant change in the absorbance spectrum. Both cases were observed in this study. The spectrophotometric method can, on the other hand, be used to detect processes such as the effect on pH on tautomerization, which cannot be determined potentiometrically. In this way, the two methods can be used to complement one another in the study of the effects of pH on organic compounds.

While the titrimetric procedures employed in the present study can provide quantitative information on the protonation/deprotonation of these compounds, they are not capable of completely defining the structures of the protonated species. A spectral study involving proton NMR, infrared, UV, and mass spectrometry is in progress to hopefully elucidate these uncertainties. Until such structural information is available, it is pointless to speculate on the pH-dependent biophysical properties of these compounds.

Registry No. Metribuzin, 21087-64-9; DA, 35045-02-4; DK, 56507-37-0; DADK, 52236-30-3.

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