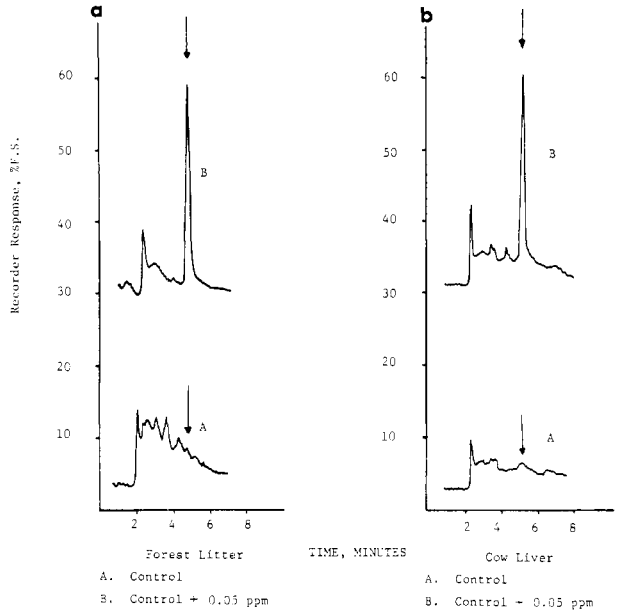

Figure 3. (a) Forest litter: (A) control, (B) control + 0.05 ppm; (b) cow liver: (A) control, (B) control + 0.05 ppm.

and soil samples utilizing the analytical procedures described here. The results are shown in Table I. Typical chromatograms representative of those normally encountered during the course of routine analyses for controls and fortified samples of water, soil, fish, forest litter, and cow liver, as well as a diflubenzuron standard, are presented in Figures 1–3.

Pesticides having a tolerance established in water, animal tissues, and cotton seed, as well as pesticides registered for use on forest insects, have been studied for interference when diflubenzuron is analyzed by HPLC. None of the compounds were found to have a retention time within 10% of diflubenzuron.

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Separation of Amino Acid Conjugates of 2,4-Dichlorophenoxyacetic Acid by High-Pressure Liquid Chromatography Employing Ion-Pair Techniques

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A high-pressure liquid chromatographic procedure was developed for the analysis of 15 amino acid conjugates of 2,4-dichlorophenoxyacetic acid. The procedure employs a μBondapak C_{18} column with a methanol–water solvent and the use of ion-paired chromatography. The method is sensitive (<50 ng) and quantitative over a wide range (50 ng to 100 μg), and conjugates are easily recoverable.

Amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) have been shown to be important metabolites of 2,4-D in plants and plant tissue cultures (Andrea and Good, 1957; Klämbt, 1961; Feung et al., 1971, 1972, 1973b, 1975). Other investigations have demonstrated the herbicidal properties of these conjugates (Krewson et al., 1956; Wood and Fontaine, 1952; and Feung et al., 1974). Analytical methods for the analysis of the amino acid conjugates are needed so that we can better understand their significance in our environment. Recently, Arjmand and Mumma (1976a,b) reported the gas chromatographic separation of the methyl esters and of the trimethylsilyl ethers of the amino acid conjugates of 2,4-D. These techniques gave good resolution, but sensitivity was relatively low and recovery of parent conjugates could be difficult. We now report the development of a high-pressure liquid chromatographic (LC) technique for the analysis of 15 amino acid conjugates of 2,4-D employing ion-pair methods (Kissinger, 1977; Walters Associates Inc., 1975). The procedure is more sensitive than existing methods, yields quantitative results over a wide range, and allows for ease of recovery.

Pesticide Research Laboratory and Graduate Study Center and the Department of Entomology (M.A., R.O.M.) and the Department of Biology, (R.H.H.) The Pennsylvania State University, University Park, Pennsylvania 16802.

EXPERIMENTAL PROCEDURE

Reagents and Materials. All solvents were distilled in glass (Burdick and Jackson) and filtered through a 0.45-μm Millipore filter before use. Water was doubly distilled and also filtered just prior to use. The amino acid conjugates of 2,4-D were synthesized as previously reported (Feung et al., 1973a). The conjugates used were 2,4-D-Ala, 2,4-D-Arg, 2,4-D-Asp, 2,4-D-Cys, 2,4-D-Glu, 2,4-D-Gly, 2,4-D-Ile, 2,4-D-Leu, 2,4-D-Met, 2,4-D-Pro, 2,4-D-Phe, 2,4-D-Ser, 2,4-D-Thr, 2,4-D-Trp, and 2,4-D-Val. The ion-pair chromatographic agent was PIC Reagent A (tetrabutylammonium phosphate, Waters Associates, Inc., Milford, Mass.).

Stock solutions of each conjugate and of 2,4-D (4 mg/mL) were prepared in methanol. A small amount of ammonia was added to the vials containing 2,4-D-Gly and

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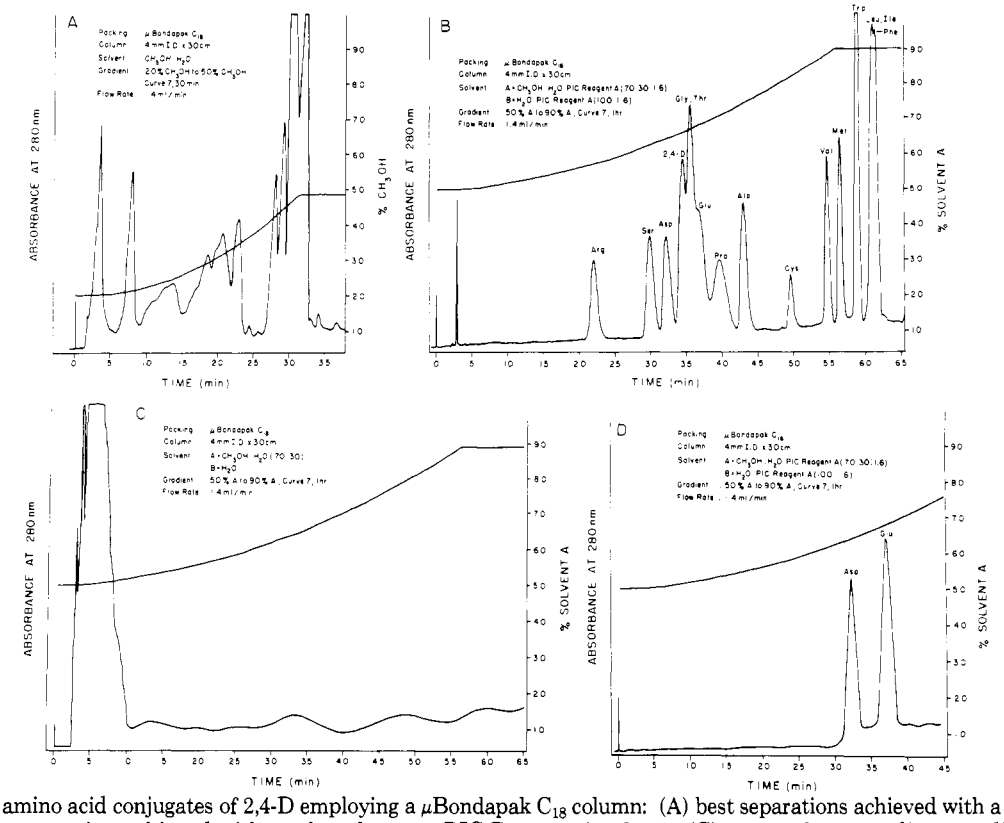

Figure 1. LC of amino acid conjugates of 2,4-D employing a μBondapak C_{18} column: (A) best separations achieved with a methanol–water solvent; (B) best separation achieved with methanol–water–PIC Reagent A solvent; (C) same solvent gradient conditions as used in B except PIC Reagent A was absent; (D) the separation of synthetic 2,4-D-Glu and 2,4-D-Asp under the conditions of B.

2,4-D-Cys to increase their solubility.

Instruments. A Model ALC/GPC 244 high-pressure liquid chromatograph equipped with a 6000A pump, U6K injector, 440 UV detector, and a 660 solvent programmer (Waters Associates, Inc., Milford, Mass.) was used. The eluting compounds were recorded at 280 nm. Three types of columns were examined (Waters Associates, Inc.): (1) a 30 cm × 4 mm i.d. μPorasil column using solvents chloroform–hexane, hexane–diethyl ether, and diethyl ether–petroleum ether, (2) a 30 cm × 4 mm i.d. μBondapak C_{18} column using solvents acetonitrile–0.1 M sodium acetate, ethanol–water (pH 4), methanol–water (pH 4), tetrahydrofuran–methanol, tetrahydrofuran–water, and methanol–water–PIC Reagent A, and (3) a 61 cm × 7 mm i.d. preparative μBondapak C_{18} column. The relative composition of the solvents was varied in all cases and evaluated under isocratic and gradient conditions. Pressures ranged from 500 to 3500 psi and chromatography was conducted at room temperature.

RESULTS AND DISCUSSION

Separations of the 2,4-D conjugates by LC were evaluated with a μPorasil column and with reverse-phase conditions with a μBondapak C_{18} column. Various solvents (chloroform, hexane, diethyl ether, and petroleum ether) and combinations of these solvents were tried under isocratic and gradient conditions, but no good separations were achieved with the μPorasil column. The μBondapak C_{18} column was evaluated using various solvents (methanol, ethanol, acetonitrile, tetrahydrofuran, water, and 0.1 M sodium acetate) under similar conditions as above. The best separation of the 15 conjugates and 2,4-D achieved with a methanol–water solvent is shown in Figure 1A. When PIC Reagent A was used with the μBondapak C_{18} column along with methanol–water as the solvent, conditions were achieved to effect a better separation (Figure 1B). Of the 15 conjugates used in these studies, nine conjugates were separated. The unresolved compounds were: (1) 2,4-D-Gly, 2,4-D-Thr, and 2,4-D-Glu and (2) 2,4-D-Leu, 2,4-D-Ile, and 2,4-D-Phe. As would be expected on reverse-phase chromatography, the more polar the amino acid conjugate, the earlier it emerges from the column. The importance of the PIC Reagent A can be easily shown by the poor separation achieved under identical conditions when PIC Reagent A was omitted (Figure 1C).

With LC the eluting compounds are easily collected. This ease of recovery permits this technique to be particularly useful for metabolic studies and for purification of the synthetic metabolites. For example, the two major 2,4-D conjugates in many plant tissues, 2,4-D-Glu and 2,4-D-Asp, are very difficult to separate on thin-layer chromatography (TLC) (Feung et al., 1973a). Therefore, it is difficult to quantitate these metabolites from plant sources. As a practical example Figure 1D shows a separation of synthetic 2,4-D-Glu and 2,4-D-Asp which has been spiked with ^{14}C-labeled 2,4-D-Glu and 2,4-D-Asp derived from a single spot of a TLC of the [^{14}C]2,4-D metabolites of soybean callus tissue. These two conjugate peaks were collected and quantified by radiochemical procedures. The results indicated the radiochemical mixture was 89% 2,4-D-Glu and 11% 2,4-D-Asp. In this way LC can assist difficult problems of metabolism studies. By eluting the 2,4-D-Glu and 2,4-D-Asp spots from a TLC plate, other possible interfering conjugates and 2,4-D were eliminated, and thus good separations were achieved.

Another practical application of this technique is its use in the purification of synthetic amino acid conjugates. The crude solid product obtained from the reaction of the acid chloride of 2,4-D with the amino acid (Feung et al. 1973a) contains only the amino acid conjugate and 2,4-D. When only two compounds are present, conditions can be achieved in the absence of PIC Reagent A, to effect a good purification of amino acid conjugates. Figure 2 shows the

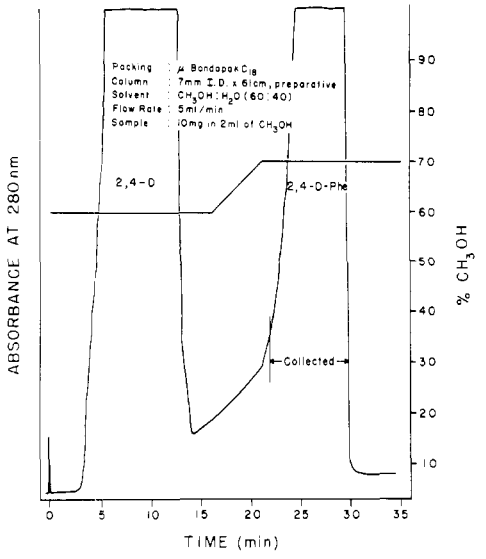

Figure 2. The purification of 2,4-D-Phe by LC employing a preparative μBondapak C_{18} column.

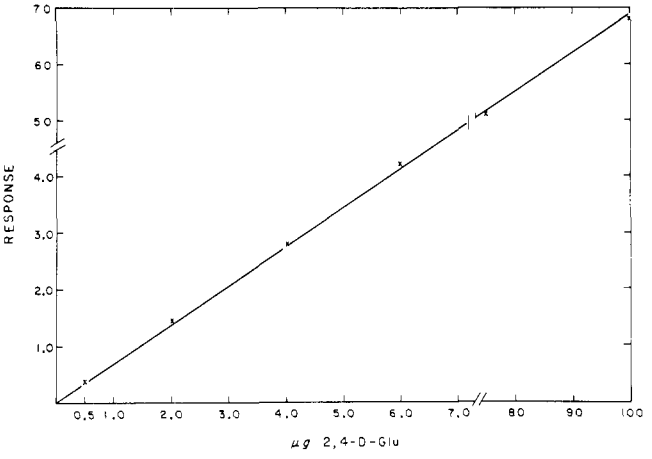

Figure 3. Response of the UV detector to 2,4-D-Glu.

separation achieved to purify 100-mg quantities of 2,4-D-Phe using a preparative μBondapak C_{18} column.

The UV detector gave a linear response over the entire range analyzed from 100 ng to 100 μg (Figure 3). This is a much wider linear response than that achieved with gas chromatographic procedures (Arjmand and Mumma, 1976a,b). The lower sensitivity limit for the conjugates was ca. 50 ng which is nearly 20 times better than what was achieved by gas chromatography methods employing flame detection (Arjmand and Mumma, 1976b).

LC offers a good alternative method of analysis for the amino acid conjugates of 2,4-D. This technique is more sensitive than reported procedures; it provides good separations, allows for ease of collection of samples, can be used in the purification of synthetic conjugates, and can assist in the quantification of metabolites. The importance of the use of ion-pair chromatography must be emphasized for good resolution of the mixture of the conjugates. These procedures have potential applicability, not only to amino acid conjugates of 2,4-D, but to amino acid conjugates of other xenobiotics and perhaps to other ionic metabolites.

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COMMUNICATIONS

A Simple and Rapid Quantitative Method for Total Phenols

A simple and rapid quantitative procedure for determining total phenols is described based on the colored complex formed with titanium. Determination of the chlorogenic acid content of defatted sunflower meal by the titanium reagent demonstrates the applicability of this method to agricultural products.

Phenolic compounds are widely distributed in plants contributing to flavor and color problems associated with flours, grains, and oilseeds (Cater et al., 1972; Sabir et al., 1974; Maga and Lorenz, 1973). A number of methods are

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