Post-mortem examination revealed marked glandular hyperplasia of the major intrahepatic bile ducts of the liver in the cow and the gall bladder of the sheep. This unusual condition is characteristic of hyperkeratosis, the only known cause of which in farm animals is ingestion of chlorinated naphthalenes (Clarke and Clarke, 1970). Symptoms of hyperkeratosis in cattle foraging on PBB contaminated feed were also observed by Jackson and Halbert (1974).

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A Specific Gas–Liquid Chromatographic Method for Analysis of Some Amine Salts of 2,4-Dichlorophenoxyacetic Acid

The methyl-, dimethyl-, *n*-butyl-, and *n*-dodecylamine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) were analyzed by conversion to the corresponding amide and subsequent gas–liquid chromatographic analysis. As the amide function retains the identity of the original acid and amine, the original amine salt can be identified.

The herbicidal halophenoxyacetic acids are usually formulated as a mixture of esters, or of amine salts. It is often desirable to know which derivative was originally present.

The amine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) are too involatile for direct gas–liquid chromatography. Thus, these compounds need to be derivatized to more volatile forms to take advantage of the sensitivity of this method of analysis. Various derivatives of 2,4-D can be quantitated individually by such methods as alkaline hydrolysis with subsequent free acid isolation and then esterification (Que Hee et al., 1975; Henshaw et al., 1975), or by total acid equivalent methods (Horwitz, 1970). Unfortunately, the quantitative nature of these methods is at the expense of the specificity of the derivative originally present, although the type of halophenoxyacetic acid present can be found (Henshaw et al., 1975). In the case of the amine salts, previous work (Que Hee and Sutherland, 1974) has shown that the corresponding amides are produced after pyrolysis. These pyrolysis products retain the identity of both acid and amine, and so the original amine salt can be identified. This paper presents a specific analytical technique for some amine salts of 2,4-dichlorophenoxyacetic acid (2,4-D) based upon this fact.

EXPERIMENTAL SECTION

Reagents. Commercial *n*-dodecyl- and *n*-butylamines (Aldrich) were purified by vacuum distillation. Commercial 2,4-D (Aldrich) was recrystallized from benzene until a constant melting point of (140–143 ± 0.5°) was attained. The purity of these chemicals was confirmed by NMR and mass spectroscopy. Reagent grade dimethylamine (Eastman) and a 20% aqueous solution of methylamine were used without further purification.

Salts (1:1) of 2,4-D were made by adding stoichiometric amounts of the amines dissolved in benzene–acetone solutions to solid 2,4-D at 10°. The solutions were shaken until all the 2,4-D had disappeared. The solvent was then removed under vacuum at room temperature. The resultant salts were recrystallized from ether–acetone–hexane (1:1:1) by volume to constant melting points.

Pyrolysis Experiments. Known masses of solid salts (ca. 200 mg) were pyrolyzed directly in sealed Pyrex tubes covered with aluminum foil for 1 hr at 160 and 190°. The tubes were then cooled. Experiments were done in triplicate.

Quantitation and Analysis. The pyrolyzed salts and equivalent amounts of corresponding nonpyrolyzed salts were dissolved in known volumes of methanol and also of acetonitrile. In the case of the methylamine salt, enough methanol was added to the acetonitrile solution to dissolve the salt.

Known aliquots (ca. 25 μl) of pyrolyzed salts were injected onto a 6-ft long × 3.5 mm i.d. copper column packed with 10% SE-30 on 60–80 mesh Chromosorb W (DMCS-AW). The injector, column, and thermal conductivity detector were maintained at temperatures of 200, 170, and 210°, respectively. The flow of helium carrier was 25 ± 1 ml/min. The filament current was 200 mA. The separated amides were collected manually in tared glass tubing, cooled externally. The weight of collected amide was compared with the amount of amide expected if pyrolysis was 100% efficient, and the purified amides then used to quantitate the amide produced in the original pyrolysis. This was done by injecting known masses of purified amide and constructing calibration curves using a 6 ft × 3.5 mm i.d. Pyrex U-tube column packed with 10% SE-30 impregnated

60–80 mesh Chromosorb W (AW-DMCS) at injector, column, and flame ionization detector temperatures of 200, 170, and 210°, respectively. The helium carrier was regulated at 25 ± 1 ml/min. Hydrogen and compressed air flows were 37 and 181 ml/min, respectively. Known aliquots of the pyrolyzed and unpyrolyzed salts were then injected, and the amide yields found.

The procedure of prior purification using the copper column before quantitation utilizing the Pyrex column worked only for the short-chain amides, i.e. the methyl-, dimethyl-, and *n*-butylamides, since the long-chain amides could not be collected. However, the long-chain amides were detected upon injection onto the Pyrex U-tube column at temperatures above 230°. The dodecylamide was purified by vacuum distillation, and was then quantitated at 250° on the Pyrex U-tube column.

The pyrolysis products were identified by GC–mass spectroscopy done with a flame ionization/gas–liquid chromatograph and an MS-12 mass spectrometer. The GC column used was a 6 ft × 3.5 mm i.d. stainless steel tube, packed with 10% SE-30 impregnated 60–80 mesh Chromosorb W (AW-DMCS), injector and detector temperatures being 232 and 350°, respectively. The temperature program involved holding the column at 70° for 6 min after injection and then heating the column to 200° at 4°/min; the column was held at 200° until all short-chain amides eluted. The flow rates of compressed air, hydrogen, and helium carrier were 500, 35, and 20 ml/min, respectively. The amide, 2,4-dichlorophenol, and 2,4-D methyl ester were identified from their retention times and mass spectra with authentic standards.

The best conditions for isothermal separation of a mixture of the short-chain amides on the Pyrex U-tube column were found by varying the temperature between 165 and 200°, while monitoring the resolution of the peaks. All other conditions were as described above.

RESULTS AND DISCUSSION

Pyrolysis of the methyl-, *n*-butyl-, *n*-dodecyl-, and dimethylamine salts of 2,4-D at 160° caused formation of the corresponding amides in yields of 75 ± 3, 77 ± 4, 40 ± 2, and 46 ± 3%, respectively; 2,4-dichlorophenol was also produced in 2 ± 0.2% yield. The remainder was undecomposed salt. Pyrolysis at 190° increased the yields to 77 ± 4, 82 ± 4, 80 ± 3, and 82 ± 4%, respectively, and the yield of 2,4-dichlorophenol increased to 5.0 ± 0.6% for all salts. Only a trace of unreacted salt survived pyrolysis at 190°. The balance of the pyrolysis products at 190° were nonvolatile under the GLC conditions used.

The GC yields above were computed using acetonitrile as solvent and the Pyrex U-tube column. An additional GC peak appeared when methanol was the solvent; this was the methyl ester of 2,4-D. The yields of ester from the pyrolyzed salts were higher than those from the unpyrolyzed compounds, implying the amides solvolyzed to some extent. Only a trace of the corresponding amide was found on direct GC of the unpyrolyzed lower chain amine salts.

The major advantage of this analytical method is that the amide function retains the identity of both acid and amine, and so the original amine salt can be identified. The retention of the nitrogen in the amide group also allows use of a nitrogen detector.

A mixture of methyl-, dimethyl-, and *n*-butylamides of 2,4-D is resolved isothermally between 165 and 185°. However, the long-chain amides require temperatures above 230°. Pyrex columns must be used to quantitate the amides since the yields obtained using the copper column were lower than those obtained on the Pyrex columns.

A minor disadvantage of the present method is that conversion to the amide is not completely quantitative necessitating the use of calibration curves.

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Synthesis and Herbicidal Activity of *N,N*-Diethyl-2-(1-naphthyloxy)propionamide and Its Optical Isomers

Extensive field tests have shown *N,N*-diethyl-2-(1-naphthyloxy)propionamide or Devrinol to be a highly active preemergence herbicide. The two optically active stereoisomers were synthesized separately and their herbicidal activities were evaluated. The D(−) isomer was found to be about eightfold more active than the L(+) isomer.

Considerable interest has been generated on the relationship between the steric configuration of aryloxypropionic acids and the growth regulating effects in plants (Aberg, 1953, 1965; Matell, 1953; Sjoberg, 1960). Our interest in this area is to study the herbicidal activity of the derivatives of naphthyloxyalkanoic acids. Among those derivatives we have prepared, *N,N*-diethyl-2-(1-naphthyloxy)propionamide or Devrinol (1) was found, after extensive evaluation and field trial studies, to have high preemergence herbicidal activity. The synthesis of 1 was reported by Tseng et al. (1973). We wish to report in this paper the synthesis of the optical isomers of 1 and their comparative herbicidal activities.

CHEMICAL METHOD

The NMR spectra were obtained on a Varian HA-60-IL spectrometer in deuteriochloroform solution with tetramethylsilane as an internal reference. The mass spectra