Identification of a Unique Glucosylsulfate Conjugate Metabolite of Profenofos in Cotton

Thomas M. Capps,*,[†] V. Michael Barringer,[†] William J. Eberle,[†] Denee R. Brown,[‡] and
Dale R. Sanson[‡]

Metabolism Department, Ciba Crop Protection, Ciba-Geigy Corporation, P.O. Box 18300,
Greensboro, North Carolina 27419, and Metabolism Programs, ABC Laboratories, P.O. Box 1097,
Columbia, Missouri 65205

The major metabolic pathway for profenofos in cotton involves cleavage of the phosphorothioate ester to yield 4-bromo-2-chlorophenol followed by conjugation with sugars. This investigation found a unique sugar conjugate in cotton stalks and seed. The unique metabolite is identified as a glucosylsulfate conjugate of 4-bromo-2-chlorophenol, and its isolation and structure elucidation are reported.

Keywords: *Organophosphate metabolism; cotton plants; conjugation reactions; O-glucosylsulfate formation*

Profenofos (CGA-15324), the active ingredient in Curacron insecticide−miticide, is currently registered by Ciba Crop Protection for control of insects and mites in cotton. The major pathway of metabolism of profenofos in cotton plants involves cleavage of the phosphorothioate ester to yield 4-bromo-2-chlorophenol followed by conjugation with glucose. In this study, a major aqueous soluble metabolite was detected which was more polar in nature than the glucose conjugate of 4-bromo-2-chlorophenol.

This investigation reports on the isolation and structure elucidation of this polar aqueous soluble metabolite from immature cotton stalks.

MATERIALS AND METHODS

Materials. Profenofos, *O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate, labeled uniformly in the phenyl ring (U-ring-^{14}C), was prepared with a specific activity of 14.5 μCi/mg. Other metabolite standards utilized for cochromatography were also prepared by the Chemical Synthesis Group.

Solvents utilized in various extraction and purification (HPLC, TLC) procedures included methanol, water, hexane, heptane, chloroform, acetonitrile, and isooctane. These solvents were obtained from Burdick and Jackson (Muskegon, MI) and were of pesticide grade.

Scintillation cocktails used were Ready Gel (Beckman, Arlington Heights, IL) and Ultima Gold XR (Packard Instruments Co., Downers Grove, IL).

Test System. Cotton (variety DPL-51) was planted in a sandy loam soil at the Ciba Crop Protection Delta Research Station in Greenville, MS. The cotton received a foliar treatment of [14C]profenofos at the rate of 1.0 lb of ai/acre as a 6E (6 lb of ai/gal) formulation approximately 2 months after the cotton was planted. Five additional applications of [14C]-profenofos were made at this rate on a weekly basis for a total application of 6.0 lb of ai/acre. Plant samples were taken just prior to the fourth application and at maturity (final harvest). The final harvest consisted of both cotton stalks and bolls.

Liquid Scintillation Counting. All samples for liquid scintillation counting were assayed in a TM Analytic Delta

---

* Author to whom correspondence should be addressed.

† Ciba-Geigy Corp.

‡ ABC Laboratories.

Table 1

solvent system		
% A	% B	time (min)
60	40	0
40	60	10
30	70	20
0	100	40
0	100	45
60	40	50

[a] Solvents A and B are defined as 0.1 M ammonium acetate and acetonitrile, respectively.
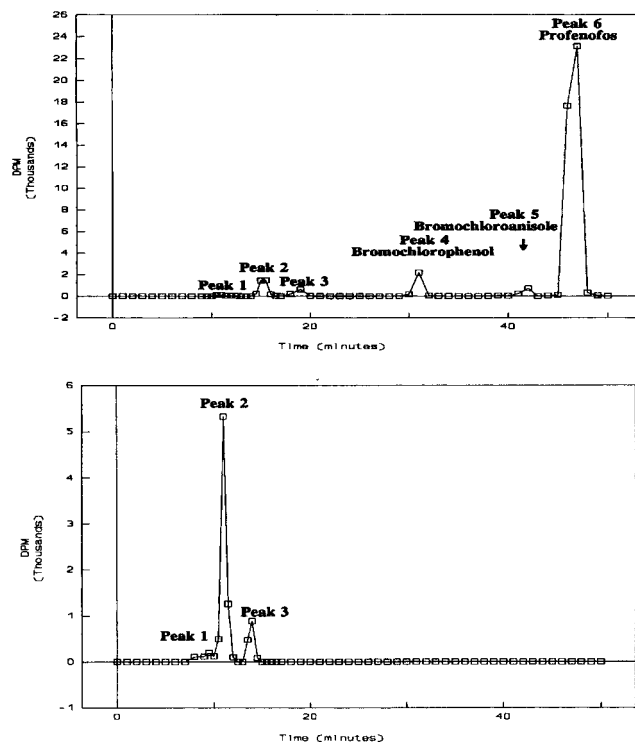
Figure 1. HPLC radiochromatogram of chloroform fraction (top) and aqueous fraction (bottom) of immature cotton stalk.
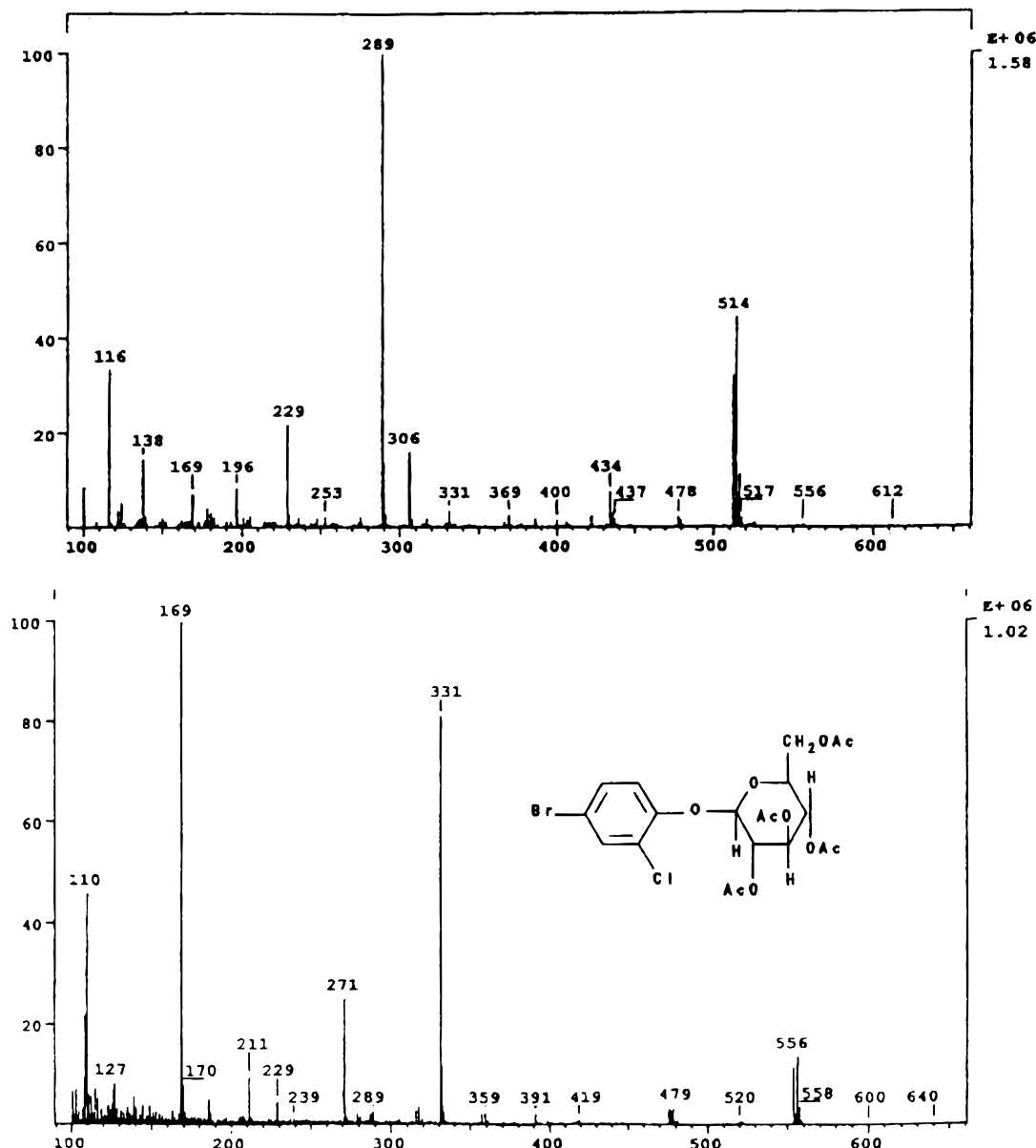
300 scintillation counter. Counting efficiency was determined by external standard calibration.

Tissue Extraction Method. Immature cotton stalks were extracted by blending three times with methanol/water (9:1)

Glucosylsulfate Conjugate Metabolite of Profenofos

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2409**

Figure 2. Mass spectrum of acetylated peak 2 (CI/DIP/MS) (top) and acetylated 4-bromo-2-chlorophenol glucoside (CI/DIP/MS) (bottom).

and vacuum filtered. The methanol was evaporated from the filtrate via a nitrogen stream. The resultant aqueous solution was partitioned with heptane/hexane (1:1 v/v) to remove chlorophylls and other pigments. The remaining aqueous fraction was partitioned with chloroform (v/v, three times). Triplicate aliquots of aqueous and organosoluble fractions were radioassayed. Triplicate aliquots (~0.25 g) of air-dried residue were combusted in a Harvey oxidizer; $^{14}CO_2$ was collected in Ultima Gold XK scintillation cocktail (Packard Instruments) and radioassayed. The ^{14}C distribution among the aqueous and organic soluble fractions and nonextractable residue was then calculated.

HPLC Analysis and Purification of Cotton Extracts.
A dual pump system (Shimadzu LC6A7) with high-pressure-gradient-forming capabilities equipped with a Rheodyne 7125 injector was used for HPLC analysis. Detection of nonradio-labeled column eluates was achieved using a variable-wavelength spectrophotometer at 240 nm (Shimadzu SPD-6A UV−visible). Radiolabeled components were detected using a flow-through radioisotope detector equipped with a 400-μL calcium fluoride solid cell (Raytest Ramona 90). Fractions were also collected and radioassayed by LSC as previously described. All output from the UV−visible and Raytest detectors was captured by a CompuAdd 316s computer.

The HPLC method employed a 250 × 10 mm i.d. semi-preparative YMC-AQ ODS (5 μm, 120 A) column (YMC Inc., Wilmington, NC) with a 2 × 0.2 cm i.d. Upchurch guard column. Elution was achieved with a flow rate of 1.5 mL/min using the gradient system outlined Table 1. One minute functions were collected, and a histogram was generated by assaying aliquots by LSC.

Mass Spectrometry. Identification of the polar isolates from immature stalk was performed using a Finnigan MAT TSQ-700 mass spectrometer in the positive chemical ionization (PCI) mode. The chemical ionization reagent gas used for the CI analysis was 5% NH_3/CH_4 with direct insertion probe analysis (DIP). Data were acquired with scans observed from 100 to 650 m/z. The source temperature was 150 °C. The electron energy used was 70 eV.

A PE Sciex API III triple-quadrupole mass spectrometer was also used for identification of the isolates. It was operated in positive and negative ion modes. The instrument was equipped with a pneumatically assisted electrospray source (ESI) which was interfaced to a Hamilton syringe pump for infusion of the sample in acetonitrile at a flow rate of 10 μL/min. The spray assembly was operated between 4500 and 5500 V with an orifice potential of 60−80 V, which generated the maximum ion intensity for available standards.
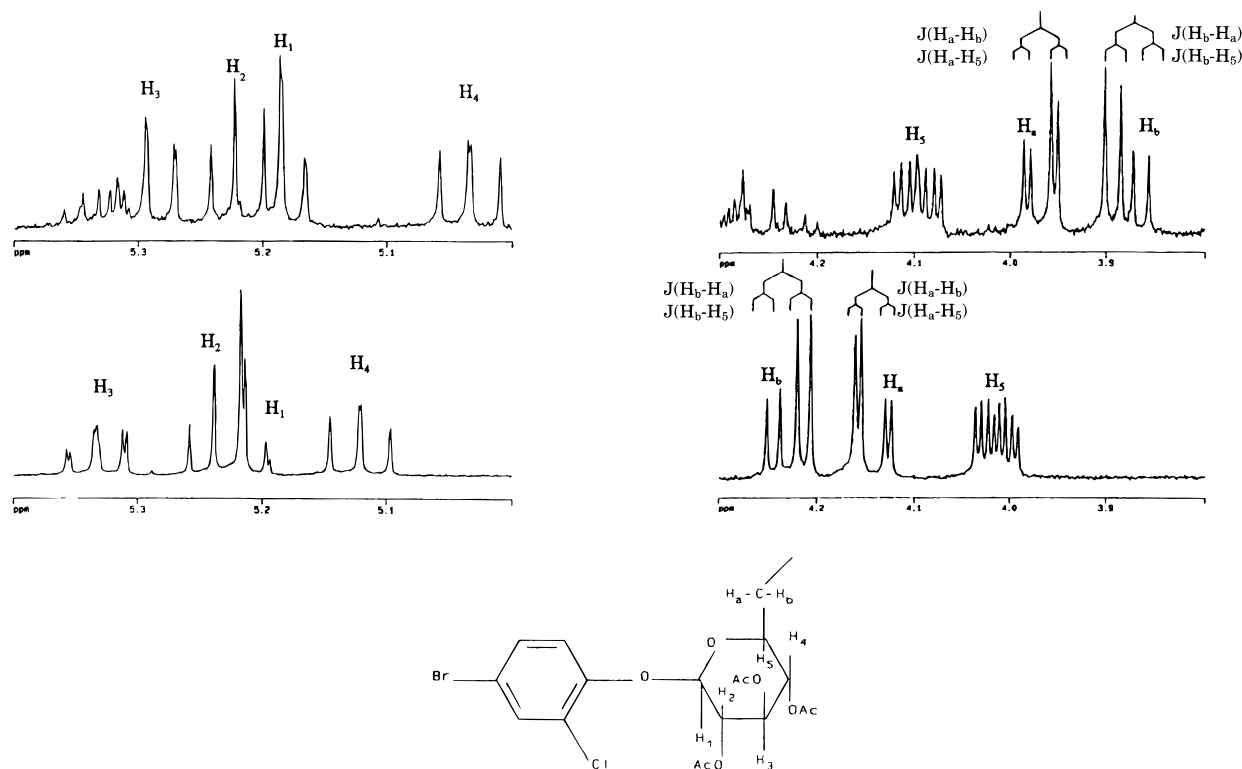
Figure 3. ^{1}H NMR analysis of acetylated peak 2 (top) and acetylated 4-bromo-2-chlorophenol glucoside (bottom).

Confirmation of the identification of the most abundant isolate was done by fast atom bombardment (FAB) at a resolving power of 7500. Accurate mass was obtained using a VG 70-250 SQ instrument. PEG 600 in glycerol was used as the internal reference. Acquisition was done in the negative detection mode. The source temperature for FAB was ambient, and xenon fast atoms were generated at 8 kV and 1 mA.

^{31}P NMR and ^{1}H NMR. NMR analyses were performed on a Bruker AMX-400 NMR spectrometer. Spectral data were obtained in acetonitrile-d_3 (100% d) from Isotec. ^{1}H NMR spectra were obtained using a 5-mm inverse detection probe operating at 400.13 MHz. ^{31}P NMR spectra were obtained using a 5-mm QNP detection probe operating at 161.98 MHz.

Acetylation. Aqueous samples of immature stalk and seed and isolates from the immature stalk were acetylated. Prior to derivatization, aliquots were dried under a nitrogen stream and acetylation was accomplished by adding a 9/1 or 1/9 ratio of pyridine and acetic anhydride to a final volume of 1 mL. The vials were tightly sealed, sonicated briefly, and allowed to sit overnight at room temperature in a hood. The samples were then dried under a nitrogen stream and reconstituted in a minimum volume of methanol or acetone. To ensure solubilization of the sample, each vial was sonicated briefly in a bench-top sonicator and radioassayed.
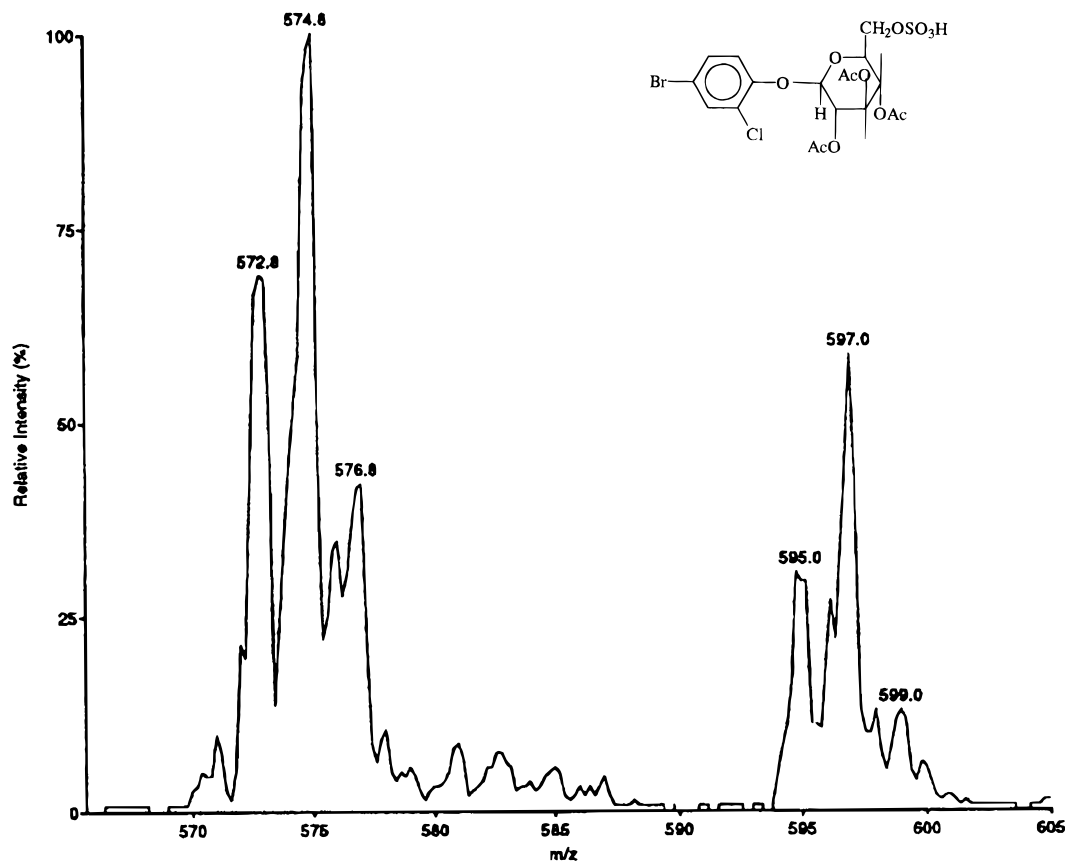
RESULTS AND DISCUSSION

HPLC analysis of the chloroform soluble metabolites (Figure 1) showed six defined radioactive peaks. Metabolites 1−3 were minor in the chloroform fraction but major in the aqueous fractions. The chloroform soluble metabolites were identified as profenofos (peak 6), bromochloroanisole (peak 5), and bromochlorophenol (peak 4) by mass spectroscopy and cochromatography with synthetic standards.

Peak 3 from the aqueous solubles cochromatographed with a standard of the glucose conjugate of bromochlorophenol. Acetylation of peak 3 followed by analysis by mass spectroscopy yielded a spectrum identical to that of the acetylated glucose conjugate.

The major aqueous soluble metabolite was peak 2 (Figure 1). Acetylation of peak 2 with acetic anhydride

and pyridine resulted in a shift of HPLC retention time from 10 to 19 min. During characterization, there was some indication that peak 2 could be converted to bromochlorophenol. After hydrolysis at 60 °C overnight with 3.0 M HCl, peak 2 was completely converted to bromochlorophenol. Peak 2 was further analyzed by ^{31}P NMR, which confirmed that no phosphorus was present in the metabolite.

Analysis of acetylated peak 2 by CI/DIP/MS (Figure 2) yielded an apparent pseudomolecular ion cluster at m/z 512, 514, and 516 typical of the presence of both bromine and chlorine. Major fragment ions were present at m/z 478 [(M + NH$_4$)$^+$ − HCl], 434 [(M + NH$_4$)$^+$ − HBr], 289, and 229. Comparison of these data with the CI/DIP/MS spectrum of acetylated bromochlorophenol (M + NH$_4^+$ = m/z 554, 556, 558) showed a similar fragmentation pattern with the exception that all ions in the MS of peak 2 were 42 amu less than that in the analytical standard. A key ion in the acetylated standard (m/z 331) was the tetraacetate of glucose (C$_{14}$H$_{19}$O$_9$). Peak 2 showed a base peak at m/z 289, 42 amu less than that of the tetraacetate of glucose, indicative of a triacetate of glucose. ^{1}H NMR analysis of acetylated peak 2 showed the presence of the bromochlorophenol and glucose proton resonances in a ratio indicative of a monosaccharide conjugate (i.e., one molecule of bromochlorophenol attached to one molecule of glucose). In addition, ^{1}H NMR showed the presence of the anomeric proton and the three C*H*OAc resonances in the 5.0−5.4 ppm region for both the acetylated metabolite and the acetylated standard (Figure 3). An upfield chemical shift for the C*H*$_2$O protons of the metabolite was observed relative to the standard, indicating the structural differences between the metabolite and the standard occurred at the methylene group of the sugar. Absence of additional couplings to the methylene protons of the metabolite eliminated the possibility of a glycophosphate. On the basis of the above data, it was postulated that this metabolite

Glucosylsulfate Conjugate Metabolite of Profenofos

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2411**

Figure 4. ESI mass spectrum of acetylated peak 2.

contained a nonvolatile blocking group at C_6 of the sugar. Consequently, soft ionization MS techniques were attempted. Analysis of peak 2 by ESI (Figure 4) showed pseudomolecular ion clusters at m/z 573, 575, and 577 $(M - H)^-$ and at m/z 595, 597, 599 $[(M^- + Na^+) - H]^-$. The foregoing data are consistent with a sodium salt of a glucosylsulfate conjugate of bromochlorophenol. High-resolution FAB yielded a molecular formula of $C_{18}H_{19}O_{12}BrClS$. The original DCI data (i.e., pseudomolecular ion at m/z 512, 514, and 516) are explained by loss of SO_3 by pyrolysis from the sulfate conjugate to form the volatile triacetate derivative, which gives the expected CI spectrum $(M - SO_3 + NH_4)^+$.

Although sulfated glucosides are not as well-known in plants as in animals, the presence of sulfated carbohydrates in plant tissues has been documented (Anderson et al., 1969; Kitajima et al., 1990; Paulson, 1976). A review by Lamoureux (1989) reports that phemedipham was metabolized in sugar beet to two glucosylsulfate conjugates. Since the PAPS (3′-phosphoryl-5′-adenosine phosphatosulfate) sulfation pathway is known to exist in plants (Paulson, 1976), the tentative identification of peak 2 as the glucosylsulfate conjugate of bromochlorophenol is reasonable.

LITERATURE CITED

Anderson, N. S., et al. X-ray diffraction studies of polysaccharide sulfates: double helix models for K- and 1-carrageenans. *J. Mol. Biol.* **1969**, *45*, (1), 85−99.

Kitajima, J., et al. Two new triterpenoid sulfates from the leaves of *Schefflera octophylla. Chem. Pharm. Bull.* **1990**, *38*, 714−716.

Lamoureux, G. L. Plant metabolism of herbicides in relation to detoxification, selectivity, anidoting, and synergism. In *Xenobiotic Metabolism and Disposition;* Kato, R., Estabrook, R. W., Cayen, M. N, Eds.; Taylor and Francis: Bristol, PA, 1989; pp 267−274.

Paulson, G. D. Sulfate ester conjugates—their synthesis, purification, hydrolysis, and chemical properties. *Bound and Conjugated Pesticide Residues,* ACS Symposium Series 29; American Chemical Society: Washington, DC, 1976; pp 86−102.