

Pentafluorobenzyl, (Trifluoromethyl)benzyl, and Diazomethane Alkylation of Bentazon for Residue Determination in Soil by Gas Chromatography

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The reaction of bentazon [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] with three alkylating agents was studied. Bentazon was rapidly and quantitatively methylated with diazomethane but interfering coextractives obviated its use for residue analysis in soil. Extractive alkylation of bentazon was quantitative in pentafluorobenzyl bromide or *m*-(trifluoromethyl)benzyl chloride when 0.1 M tetrabutylammonium hydrogen sulfate was used as the counterion in 0.2 M Na₂CO₃. The pentafluorobenzyl derivative showed 14-fold greater sensitivity to an electron capture detector compared to the methyl derivative while [*m*-(trifluoromethyl)benzyl]bentazon was 3 times more sensitive. The response of the (pentafluorobenzyl)bentazon to the electron capture detector was linear from 0.01 to 6 ng. The preferred alkylating agent was pentafluorobenzyl bromide since derivatized bentazon permitted direct analysis of soil extracts by gas chromatography. All three derivatives were stable in hexane for at least 1 week.

Bentazon [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] is a postemergence herbicide used to control broadleaf weeds in most grains and many large-seeded leguminous crops (Weed Science Society of America, 1974). Bentazon is a weakly acidic herbicide ($pK_a = 3.2$) (Abernathy and Wax, 1973) with a secondary nitrogen in the sulfonamide group which is susceptible to alkylation by reagents such as diazomethane (Otto et al., 1979) (Figure 1). The derivatized bentazon can be assayed by gas chromatography with an electron capture or a sulfur-flame photometric detector. The preparation of the methyl derivative of bentazon with diazomethane requires special equipment, e.g., polished joints on glassware, shielded hoods, and care because CH₂N₂ is explosive and carcinogenic (Blau and King, 1978). The hazards of using diazomethane can be reduced with microanalytical techniques (Roper and Ma, 1957), but extensive column cleanup of plant and soil extracts is generally required to remove coextractives which interfere with the response to the electron capture detector. Assay by sulfur-flame photometric detector obviates the need to purify extracts but is less sensitive (Patterson et al., 1978; Cochrane and Greenhalgh, 1976). The response of the detector to sulfur also varies from linear to a square root function, depending upon the design of the detector and concentration and molecular structure of the compound (Hasinski, 1976). Since sulfonamide drugs have been shown to undergo alkylation with a number of electron capture enhancing reagents (Gyllenhaal and Ehrsson, 1975), the purpose of this investigation was to assess the use of alternative less hazardous alkylating agents which would form derivatives of bentazon sensitive to an electron capture detector. The applicability of the alkylating agents for bentazon residue determination in a clay loam soil was assessed.

EXPERIMENTAL SECTION

Methyl Derivative. An aliquot (15 mL) of freshly prepared diazomethane (from Diazald, Aldrich Co. Inc., Milwaukee, WI) (Deboer and Backer, 1954) was reacted with 50 μ g of bentazon in 2 mL of methanol. The reaction was allowed to proceed for 5, 10, 30, 60, 90, 120, or 180 min at room temperature after which the solvents were removed by flash evaporation and the residue was dissolved

in 15 mL of hexane for analysis by gas chromatography. The strength of the ethereal-diazomethane solution was determined after production and before use by reacting a suitable aliquot with an excess of benzoic acid and titrating with 0.01 N NaOH (Roper and Ma, 1957).

Pentafluorobenzyl and *m*-(Trifluoromethyl)benzyl Derivatives. A benzene solution containing 78 μ g of bentazon was reduced to dryness under a stream of air in a 5-mL glass screw-cap vial. One microliter of pentafluorobenzyl bromide (Pierce, Rockford, IL) or *m*-(trifluoromethyl)benzyl chloride (ICN-K and K Laboratories, Cleveland, OH) and 1 mL of an aqueous 0.2 M Na₂CO₃-0.1 M tetrabutylammonium hydrogen sulfate (pH 10) solution were added, and the solution was stirred for 2, 5, 10, 20, 30, or 60 min at 0, 25, 40, or 60 °C. The mixture was then neutralized and the alkylated bentazon extracted 3 times with 4 mL of toluene. The toluene was removed under vacuum and the residue dissolved in 15 mL of hexane for analysis by gas chromatography.

For verification by mass spectrometry, the alkylated products were purified by column chromatography on Florisil (60-100 mesh). Structures were confirmed by a direct inlet probe using a Finnigan 3100 D mass spectrometer interfaced with a Model 6000 data system.

The reaction of pentafluorobenzyl bromide with bentazon yielded a white crystalline powder with a melting point of 101.6-101.8 °C and *m/e* 420. The *m*-(trifluoromethyl)benzyl and methyl derivatives (*m/e* 398 and 254, respectively) were liquids at room temperature.

Thin-Layer Chromatography. The alkylated bentazon derivatives were eluted by ascending chromatography on silica gel plates (Eastman 6060 with a fluorescent indicator) with 10% acetone-hexane. The derivatives were visualized under 254-nm ultraviolet light, and *R_f* values of 0.01, 0.53, 0.32, and 0.24 for bentazon, methylbentazon, (pentafluorobenzyl)bentazon, and [*m*-(trifluoromethyl)benzyl]bentazon determined.

Soil Extraction. A Brookston clay loam soil (3.6% organic matter) was fortified at 1 and 0.1 ppm of bentazon. Fortified and untreated soil (50 g) was shaken for 1 h on a mechanical shaker with 100 mL of methanol and suction filtered, and the residue leached with an additional 80-100 mL of extractant. The combined filtrate was reduced to dryness at 40 °C on a rotary evaporator. The residue was transferred with benzene to Reacti vials, and the final volume adjusted to ~1 mL under a gentle stream of air. The samples were prepared as above for the pentafluoro-

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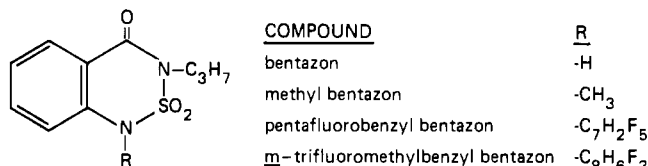


Figure 1. Structure of bentazon and derivatives.

benzyl derivative and reacted 30 min at 60 °C. The methylated samples were reacted for 10 min directly in round-bottom flasks and carried through the above procedure after filtrate evaporation.

Gas Chromatograph Conditions. A Varian 3700 gas chromatograph equipped with a ⁶³Ni electron capture detector was used to analyze for the alkylated derivatives. Samples were injected with a Varian 8000 auto injector and output was processed on a Varian CDS 111 data system. The 1.4 m × 3 mm i.d. glass column was packed with 5% OV-17 on 60–80-mesh Gas-Chrom Q. Inlet, column, and detector temperatures were set at 250, 230, and 300 °C, respectively, and the analyte was eluted with an argon-methane (95%) carrier gas (60 mL/min).

RESULTS AND DISCUSSION

Methylation of bentazon was complete after reaction for 5 min at room temperature with a 50-fold excess of diazomethane. Reaction times up to 180 min did not change the yield of methylbentazon or increase the interference of the chromatograms from polymerized diazomethane. A freshly prepared solution of diazomethane should be used to ensure complete methylation of bentazon, since it was found that 50% of the diazomethane was lost within 5 days of preparation when stored at -5 °C. Also, as the diazomethane solution was removed from the storage bottle, the gas was lost more rapidly because of the increased volume of air.

The alkyl derivatives of bentazon were easily produced with good yields as determined by the absence of unreacted bentazon on thin-layer chromatography plates and only one reaction product for each of the three derivatives. It was possible to detect 0.1 µg of bentazon on the thin-layer chromatography plates which in the absence of a bentazon spot would indicate that less than 0.2% of the bentazon remained unreacted. Alkylation of bentazon was judged to be complete when no further increase in peak area as determined by gas chromatography was observed and by the absence of unreacted bentazon on thin-layer chromatography plates.

The relative response of the alkylated products was investigated with the ⁶³Ni detector. The peak area response of the electron capture detector increased with the degree of halogenation or electron capturing groups on the compound as would be expected (Gyllenhaal and Ehrsson, 1975). (Pentafluorobenzyl)bentazon generated a peak area response 4.6 times greater than that of the *m*-(trifluoromethyl)benzyl derivative which in turn gave a response 3.1 times greater than that of the methyl derivative. No comparison in sensitivity could be made with the parent herbicide, bentazon, because it is strongly adsorbed to the column packing. The peak area response of the ⁶³Ni detector to bentazon as the pentafluorobenzyl derivative was linear from 0.014 to 3.333 ng (Figure 2). A detection limit of ~6 pg of bentazon could be achieved. Typical chromatograms for the three alkylated products and relative peak area sensitivities are shown in Figure 3.

Since (pentafluorobenzyl)bentazon gave a greater response on the electron capture detector than the *m*-(trifluoromethyl)benzyl derivative, more definitive studies were conducted with the pentafluorobenzyl reagent to

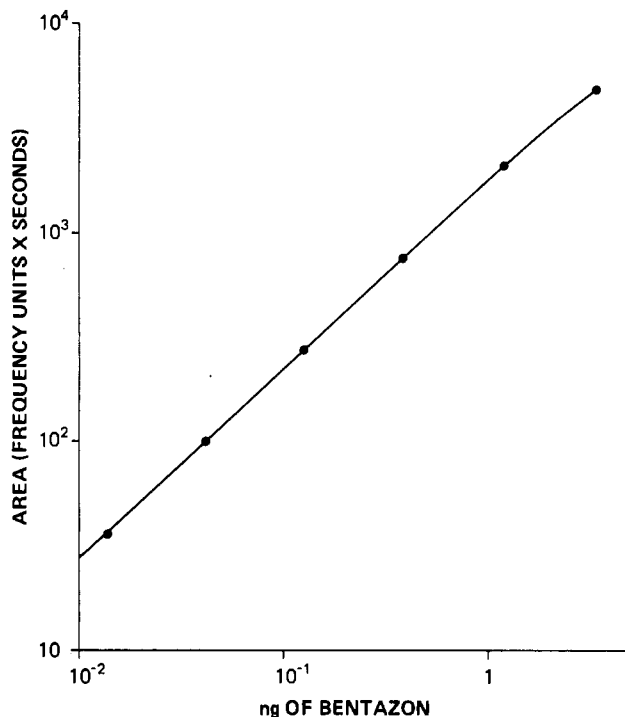


Figure 2. Response of the ⁶³Ni detector to (pentafluorobenzyl)bentazon. The abscissa is normalized as nanograms of bentazon.

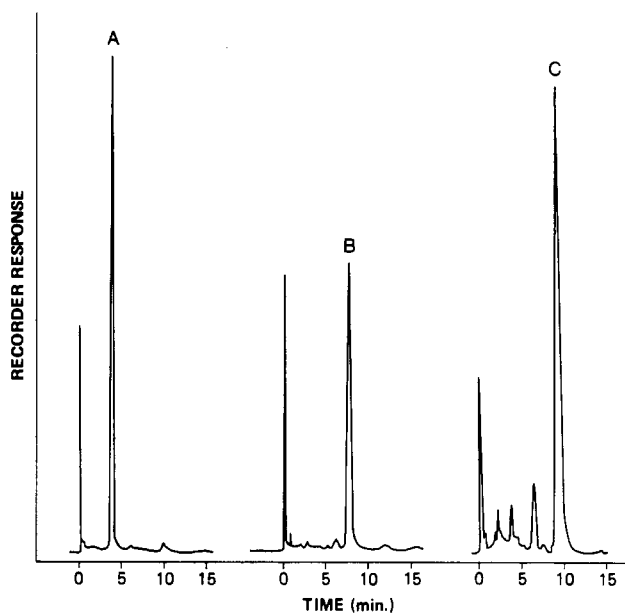


Figure 3. Gas chromatograms of (A) 4.9 ng of methylbentazon, attenuation 640, (B) 1.9 ng of (pentafluorobenzyl)bentazon, attenuation 2560, and (C) 4.9 ng of [*m*-(trifluoromethyl)benzyl]-bentazon, attenuation 640.

determine the optimum conditions for alkylation. Derivatization of bentazon 20 min at 60 °C produced a high but inconsistent yield of product (coefficient of variation = 21%) which indicated either incomplete extraction or slow breakdown of the derivative in the alkaline medium. Neutralizing the reaction medium before extraction reduced the coefficient of variation to 3%. The effect of pH on the stability of the (pentafluorobenzyl)bentazon was further investigated in aqueous Na₂CO₃ solutions adjusted from pH 2 to pH 11 with 0.4 M H₂SO₄. After equilibrating for 20 min at room temperature in the above solution, the samples were dried on a rotary evaporator and the (pen-

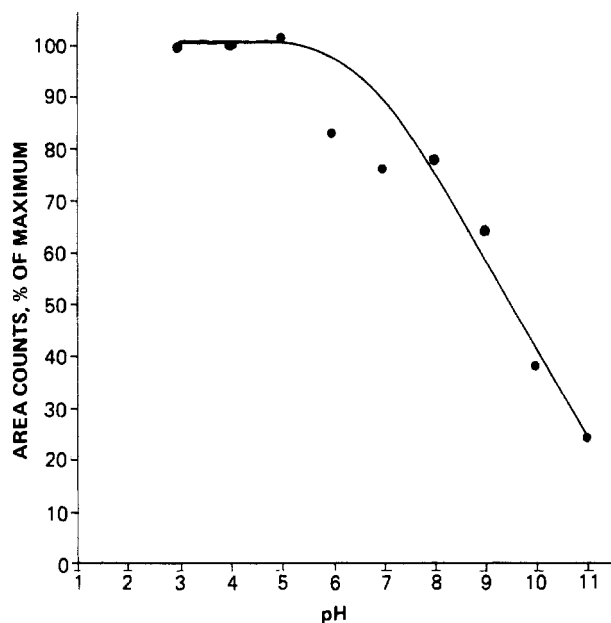


Figure 4. Degradation of (pentafluorobenzyl) bentazon reacted 30 min at 60 °C in 2 mL of aqueous 0.2 M Na₂CO₃ adjusted to various pH levels with 0.4 M H₂SO₄ or 0.1 N NaOH.

Table I. Effect of Temperature on Pentafluorobenzylation^a of Bentazon

temp, °C	yield, %	coeff of variation, %
0	37a ^b	72
25	71b	21
40	98b	12
60	100b	3

^a 78 µg of bentazon reacted 20 min with pentafluorobenzyl bromide. ^b Means followed by similar letters are not significantly different at the 5% level (Student's *Q* test).

Table II. Pentafluorobenzylation^a of Bentazon at Selected Reaction Times

time reacted, min	2	5	10	20	30	60
yield, %	44a ^b	57b	85c	100d	101d	99d

^a 78 µg of bentazon reacted at 60 °C with pentafluorobenzyl bromide. ^b Means followed by similar letters are not significantly different at the 5% level (Student's *Q* test).

tafluorobenzyl) bentazon was extracted from the residue with hexane for analysis by gas chromatography. The recovery of the product increased as pH decreased (Figure 4), suggesting the product was hydrolyzed in base.

The optimum temperature for alkylation was determined for a 20-min reaction time. The yield of (pentafluorobenzyl) bentazon reached a maximum at temperatures above 25 °C (Table I), but the yield was spurious as reflected by the high coefficient of variation (21% vs. 12% at 40 °C). At 60 °C the yield was more consistent with a coefficient of variation of 3.0%. The yield of the pentafluorobenzyl derivative reached a maximum after a 20-min reaction at 60 °C (Table II).

The utility of pentafluorobenzylation as a residue method for analysis of bentazon in soil was investigated with a Brookston clay loam soil. Methylation of a methanol soil extract produced an interference peak at the retention time for methyl bentazon which could not be resolved by different column packing materials or removed by Florisil cleanup. The interference was highest in soil samples collected in April but stabilized to a corresponding ben-

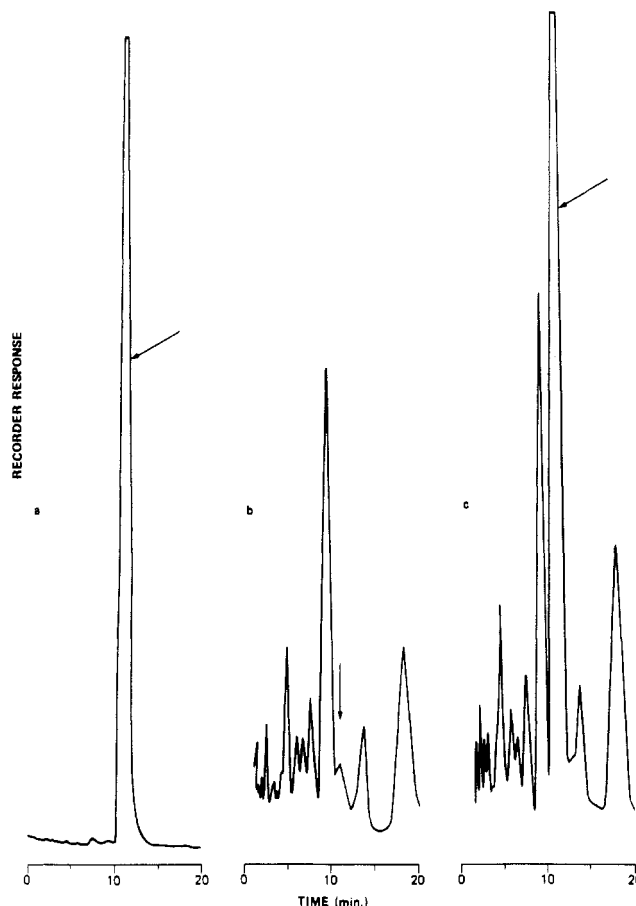


Figure 5. Gas chromatograms of (a) the (pentafluorobenzyl) bentazon standard, (b) pentafluorobenzylated check soil, and (c) fortified (0.1 ppm) pentafluorobenzylated soil. Attenuation 512; injector temperature, 240 °C; column temperature, 220 °C; detector temperature, 300 °C. Other conditions are as described in the text.

tazon residue of 0.1 kg/ha in August. Bentazon is applied at 0.75–1.0 kg of a.i./ha for weed control in soybean (*Glycine max.*) (Singh-Verma and Luib, 1974). The interference was not attributed to bentazon contamination since no response was noted with a flame photometric detector operated in the sulfur mode.

Methanol extracts of soil reacted with pentafluorobenzyl bromide recovered 62 ± 1% (1 ppm) and 73 ± 8% (0.1 ppm) of the bentazon. Minor interference peaks in the (pentafluorobenzyl) bentazon region (Figure 5) of the chromatograms were noted, but the greater response of the electron capture detector to the (pentafluorobenzyl) bentazon permitted operation of the gas chromatograph at a higher attenuation where background interference was minimized. Bentazon residues in soil to 10 g/ha could be detected with the pentafluorobenzyl derivative.

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Analysis of Carbofuran and Atrazine in Soil Samples

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A very rapid procedure for extracting carbofuran and atrazine from soil is described. Both pesticides were extracted from soil with ethyl acetate following soil-moisture adjustment. Minimal or no effects were caused by soil type, pH, and pesticide concentration. When compared with other carbofuran and atrazine extraction techniques, the ethyl acetate technique was equally effective, less time consuming, and permitted extraction of larger soil samples. Extraction of whole samples was preferred over sub-sampling for carbofuran analyses, because of very uneven distribution of that chemical in soil samples. Carbofuran and atrazine in extracts were measured without extract concentration or cleanup, by a gas-liquid chromatograph (GLC) with a thermionic specific detector. This technique was corroborated by high-pressure liquid chromatography. Atrazine, carbofuran, and several of their metabolites were separated by GLC with an Apiezon N liquid phase. It was the only one of nine liquid phases tested that adequately separated those compounds.

Two pesticides commonly used on corn in the Midwest are atrazine, for control of annual broad-leaved weeds and some grasses, and carbofuran, for control of northern, *Diabrotica longicornis* (Say), and western, *D. virgifera* LeConte, corn rootworm larvae. Frequently, these two chemicals are applied to the same fields, so it would be useful to have procedures for analyzing both compounds simultaneously.

There are numerous extraction procedures that have been used successfully for each of these pesticides. Many of the extraction procedures are similar, so it is likely that a single procedure would effectively extract both pesticides. We hoped to develop an extraction procedure for both pesticides that would work efficiently on the large numbers of samples and large quantities of soil that must be extracted for pesticide residue work. We hoped to eliminate time-consuming steps such as refluxing, filtration, centrifugation, solvent partitioning, solvent evaporation, and extract cleanup. One technique that had the potential of meeting these criteria was shaking the soil with ethyl acetate. Kadoum and Mock (1978) showed that ethyl acetate extracted both carbofuran and atrazine from freshly treated samples, but their technique included two solvent evaporation steps and an extract cleanup step. Gorder et al. (1980) described two techniques for extracting carbofuran from soil. One of those techniques utilized ethyl acetate and had no time-consuming evaporation or cleanup steps prior to measurement of carbofuran by gas-liquid chromatography (GLC) with a thermionic specific detector (TSD).

This paper describes the extension of that methodology to soils containing both atrazine and carbofuran. The

procedure required for analysis of both pesticides is described in detail and corroborated with a second analytical procedure. The extraction procedure was tested for the influences of a variety of factors, and work with freshly treated laboratory soils (Kadoum and Mock, 1978; Gorder et al., 1980) was extended to aged residues and field residues. Procedures widely accepted for extraction of carbofuran (Cook, 1973) and atrazine (Tweedy and Kahrs, 1978) were used for comparative extraction of aged and field residues.

MATERIALS AND METHODS

Analytical Chemicals. Analytical-grade 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine (atrazine), 2-chloro-4-amino-6-(isopropylamino)-s-triazine (deethylated atrazine), and 2-chloro-4-amino-6-(ethylamino)-s-triazine (deisopropylated atrazine) were received gratis from CIBA-GEIGY Corp., Greensboro, NC. Commercial-grade atrazine (AAtrex 4L) was purchased. Analytical, technical, and commercial (Furadan 10G) grades of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (carbofuran) and analytical-grade 2,3-dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl methylcarbamate (3-hydroxy-carbofuran) and 2,3-dihydro-2,2-dimethyl-2-oxo-7-benzofuranyl methylcarbamate (3-oxocarbofuran) were received gratis from FMC Corp., Middleport, NY.

Soils. Soils used in these experiments were classified as Canisteo (formerly a high pH variant of Webster) and a mixture of Kenyon and Floyd. Composites of three, 10.5 cm diameter by 7.5 cm deep soil cores were tested for pH (glass electrode; soil:water ratio of 2:1), particle size (Bouyoucos, 1936) after partially destroying the organic matter with H₂O₂, and organic carbon (Mebius, 1960). The percentage of organic matter was determined by multiplying the percentage of organic carbon by 1.724 (Allison, 1965). The Canisteo soil was pH 7.8, 20% sand, 59% silt, and 21% clay and had 5.2% organic matter. The mixed Kenyon and Floyd soil was pH 6.1, 26% sand, 51% silt,

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