since preparations vary in response to added Polyclar AT, depending on the plant species. Comparison of aldrin epoxidase activity of various plants used in this study can be best made by considering the preparations without added Polyclar AT, since optimization for all plants was not conducted. Addition of calcium chloride and bovine serum albumin to incubations, along with optimum levels of Polyclar AT, did not enhance the epoxidase activity above that brought about by use of Polyclar AT alone.

The Broad bean root extracts, which were very inefficient in aldrin epoxidation and turn dark upon incubation or standing, were tested for inhibitory activity. Since darkening of plant preparations is thought to be an indication of intense polyphenol oxidase activity (Jones *et al.*, 1965), it was possible that the low aldrin epoxidase activity was caused by the presence of quinolic products resulting from polyphenol oxidase activity. A very effective inhibitory activity was located in the soluble fraction of the Broad bean root extracts. This activity was not associated with the protein precipitates. Characterization and identification of this inhibitor could provide a useful tool for the elucidation of the fate of aldrin and other pesticides in plants.

Use of higher substrate levels was beneficial in this study and permitted detection of aldrin epoxidase activity in corn roots previously reported to be devoid of this activity (Yu *et al.*, 1971). Incubations for longer than 2 hr also proved advantageous in obtaining higher levels of dieldrin. However, further research will be required for the optimization of the total incubation parameters and for the most efficient procedures for extracting active enzymes from plant material.

LITERATURE CITED

Bendall, D. S., Gregory, R. P. F., "Enzyme Chemistry of Phenolic Compounds," Pergamon Press, London, 1963, p 7.
Frear, D. S., Swanson, H. R., Tanaka, F. S., *Phytochemistry* **8,** 2137 (1969).
Gustavson, K. H., *J. Soc. Leather Trades' Chem.* **50,** 144 (1966).
Hulme, A. C., Jones, J. D., Wooltorton, L. S. C., *Nature (London)* **201,** 795 (1964).
Jones, J. D., Hulme, A. C., *Nature (London)* **191,** 370 (1961).
Jones, J. D., Hulme, A. C., Wooltorton, L. S. C., *Phytochemistry* **4,** 659 (1965).
Kleinhofs, A., Haskins, E. A., Gorz, H. J., *Phytochemistry* **6,** 1313 (1967).
Lichtenstein, E. P., Corbett, J. R., J. AGR. FOOD CHEM. **17,** 589 (1969).
Oloffs, P. C., *Pest. Sci.* **1,** 228 (1970).
Yu, S. J., Kiigemagi, U., Terriere, L. C., J. AGR. FOOD CHEM. **19,** 5 (1971).

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Metabolism of 4-Chloro-2-butynyl-3-chlorocarbanilate by Soybean Plants

Gerald G. Still* and Eugene R. Mansager

Root-treated soybean plants absorb, translocate, and metabolize 4-chloro-2-butynyl-3-chlorocarbanilate-^{14}C (barban-^{14}C). Water-soluble products and insoluble residues are rapidly formed in the roots. Only water-soluble residues are found in the shoots after 3 days posttreatment. Time-course experiments indicated a possible precursor product relationship between barban, soluble products, and insoluble residues. Water-soluble metabolites are not translocated once they are formed in the shoot tissues. The radiocarbon distribution in soybean plants root-treated with phenyl-^{14}C, carbonyl-^{14}C, and butynyl(C-1)-^{14}C-labeled barban were identical in all comparative experiments. These studies indicate that the barban molecule was not cleaved in soybean. Barban-phenyl-^{14}C was root fed to soybean plants for 4 days and the polar metabolites were isolated. Caustic hydrolysis of polar metabolites from either root or shoot yielded 3-chloroaniline-^{14}C. The recovery of 3-chloroaniline-^{14}C indicated that barban-^{14}C metabolites are formed by the alteration of the 4-chloro-2-butynyl side chain and not by hydroxylation of the aromatic nucleus, as was found for isopropyl-3-chlorocarbanilate in soybean.

One of the most effective herbicides used in the control of wild oat (*Avena fatua*) has been 4-chloro-2-butynyl-3-chlorocarbanilate (barban). Though this herbicide has been widely used in agriculture, its metabolism by plants is not well understood. Foliar absorption of barban in the various plant species has been reported by Foy (1961), Riden and Hopkins (1962), and Crafts (1964). Foliar translocation both acropetally and basipetally has been confused by conflicting reports. It appears that one could expect only a small percentage of barban to translocate after leaf treatment. Root absorption of barban has been reported (Crafts, 1964; Jacobsohn, 1970) in several plant species. These studies have demonstrated limited acropetal translocation of barban.

Riden and Hopkins (1961, 1962) demonstrated the conversion of barban to a polar metabolite (Compound X), which was shown to be a nonproteinaceous, dialyzable material which yielded 3-chloroaniline upon caustic hydrolysis. The conversion of barban to aniline-containing polar metabolites occurred in 13 different plant species, including grasses and broad leaf plants. The appearance and disappearance of the polar metabolite (Compound X) was a function of time. Jacobsohn (1970) also demonstrated the conversion of leaf-treated barban to polar metabolites and extended the investigation to root-treated wild oat and barley plants. These plants also converted barban to 3-chloroaniline containing polar metabolites. Riden and Hopkins (1962) showed that there was no free 3-chloroaniline in their barban leaf-treated plants. Further, they were able to isolate

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2-chloro-4-aminophenol from barban-treated plant tissue. Riden and Hopkins concluded that in leaf-treated tissues the barban-^{14}C concentration decreased with time and that the 3-chloroaniline moiety of barban was complexed as polar metabolites.

Still and Mansager (1971a,b) have studied the metabolism of isopropyl-3-chlorocarbanilate (chlorpropham or CIPC) in soybeans. Preliminary studies indicated that Riden and Hopkins' polar barban metabolite behaved in a manner similar to the polar metabolites from chlorpropham. Soybean plants root-treated with chlorpropham converted the herbicide to isopropyl-2-hydroxy-5-chlorocarbanilate glucoside in root tissue, while shoot tissue yielded the same metabolite along with a second metabolite whose identification has not been established. Caustic hydrolysis of the polar chlorpropham metabolites yielded 2-hydroxy-5-chloroaniline and isopropyl alcohol. The report by Riden and Hopkins (1962) on the isolation of 2-chloro-4-aminophenol from barban-treated plants suggested that an aryl hydroxylation similar to the one observed in chlorpropham soybean metabolism may be responsible for the formation of the polar metabolite from barban metabolism. This paper describes our studies with soybean barban metabolism.

EXPERIMENTAL

Plant Material and Treatment. Soybean [*Glycine max* (L.) Merr. variety Hawkeye] seeds were germinated between moist paper towels at 25°C in the dark. The 4-day-old seedlings were transferred to pint jars (two seedlings/jar) containing Hoagland's solution and grown in an environmental chamber (Still, 1968). A 14-hr day at 27°C and a 10-hr night at 23°C was used with a relative humidity of approximately 45% and a light intensity of 1.72×10^4 lux at the leaf surface. When the third trifoliate was approximately half expanded the time-course experiments were initiated. Both the time-course experiments and the pulse-time course experiments were conducted as described by Still and Mansager (1971a). At the termination of the pulse-time course experiment, the plants were sectioned to yield root, primary leaf, stem sections 1 and 2, trifoliate groups 1 and 2, pods, and the seeds. Stem section 1 and trifoliate group 1 are defined as those tissues present during the barban-^{14}C treatment, while stem section 2 and trifoliate group 2 were those tissues that emerged after removal of the ^{14}C substrates. In all experiments the plant sections were freeze-dried and stored dry at freezer temperatures until analysis. In each experiment at least four plants (two jars) were sampled at each harvest. The radiocarbon content of each plant section was assayed.

For large scale barban-phenyl-^{14}C root treatment, the soybean plants were germinated as described above and reared in stainless steel trays. Each tray held 4 l. of half-strength Hoagland's solution. The soybean plants (24 per tray) were suspended over the nutrient solution through holes in the stainless steel cover. When the third trifoliate leaves were expanded, the Hoagland's solution was replaced with half-strength Hoagland's solution, which contained 1×10^{-5} *M* barban-phenyl-^{14}C. Preparation and quantitation of these solutions are described by Still and Mansager (1971a). At the end of 4 days the experiment was terminated by sectioning the soybean stems, at the cotyledon, to yield root and shoot fractions. The roots were blotted to remove excess treating solution and the root or shoot fractions were extracted.

Extraction of Plant Material. The extraction procedure used for the time-course experiments and the isolation of the polar metabolites from the mass culture were modifications of the techniques described by Bligh and Dyer (1959). The modified extraction procedure, as described by Still and Mansager (1971a), yielded a chloroform layer and a polar (methanol–water) layer which were assayed directly for radiocarbon. After extraction, the insoluble plant residue material was dried and combusted by the oxygen flask technique for radiocarbon assay.

Tissues from the large-scale barban-phenyl-^{14}C treatment (9700 g of shoot tissue and 4000 g of root tissue, fresh weight) were subjected to the modified Bligh–Dyer extraction, as described by Still and Mansager (1971b). The chloroform extract from both roots and shoots contained only unchanged barban-^{14}C and was discarded. The freeze-dried polar extract was solvated in water at 4°C and centrifuged at $1 \times 10^5 \times g$ for 2 hr. The precipitate was free of radiocarbon and discarded. The aqueous supernatant was adjusted to 0.01 *N* with HCl and extracted five times with 1-butanol. The aqueous acid solution was discarded and the 1-butanol was removed as a 1-butanol–water azeotrope *in vacuo*, leaving an aqueous solution. The resulting water solution was centrifuged again and the radiocarbon free precipitate discarded. The aqueous supernatant was adjusted again to 0.01 *N* with HCl, and extracted five times with 1-butanol and the aqueous acid solution discarded. The 1-butanol was then extracted five times with 1.0 *N* NaOH, in the cold, and the basic extract neutralized immediately after the extraction. The polar metabolites in the roots partitioned with twice as much label in the 1-butanol as in the basic solution, while the polar metabolites in the shoots partitioned approximately equally between the aqueous base and the 1-butanol. In either the root or the shoot, all of the radiolabel could be accounted for in both of the two fractions. The neutralized aqueous solution was freeze-dried and stored at 0°C for further analysis. The 1-butanol solution was extracted in the ternary mixture of hexane–butanol–water (10:1:1), with the aqueous phase extracting all of the radiocarbon. The organic phase was discarded and the aqueous phase freeze-dried and stored at 0°C for further analysis. At this point in the procedure, the recovery of the polar metabolites was nearly quantitative.

Either the base-soluble or the 1-butanol-soluble components of root or shoot were further purified by DE-52-acetate column chromatography on 50-mm × 77-cm columns. Stepwise elution from 0 to 0.5 *N* acetate was used, yielding broad, poorly defined peaks. The radio-labeled eluates were concentrated by freeze-drying. In all cases, substantial losses of radiocarbon were observed during the freeze-dry process.

Radiochemical Assays. Quantitative measurements of radiocarbon were made on a liquid scintillation spectrometer. All samples were counted as homogeneous solutions in either toluene or alcohol–toluene scintillation cocktails. Each sample was corrected for quenching by external standardization. Radiochemical assay of barban-^{14}C treating solution was accomplished by transferring the treating solution into a 500-ml liquid–liquid extractor and extracting for 48 hr with ethyl ether. The ether extract was assayed by liquid scintillation spectrometry.

All particulate samples, such as the solid residues, were dried and combusted using the oxygen combustion flask described by Kelly *et al.* (1961), as modified by Oliverio *et al.* (1962). The $^{14}CO_2$ from these combustions was assayed by liquid scintillation spectrometry.

Purification of Barban Radiochemical Substrate. Barban-phenyl-^{14}C, barban-carbonyl-^{14}C, and barban-butynyl(C-1)-

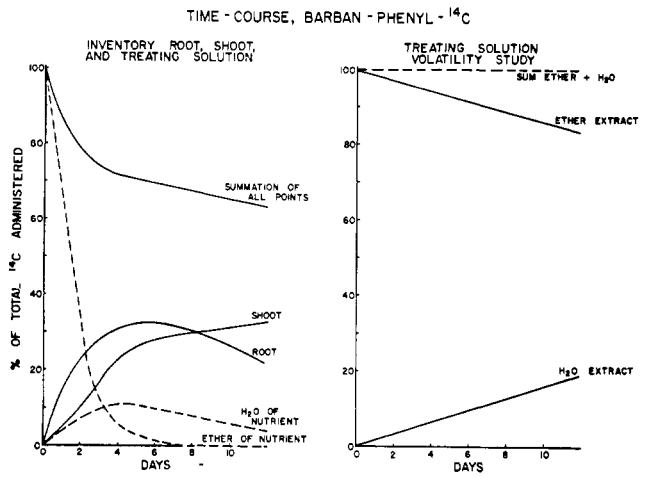

Figure 1. Volatility and time-course experiments of barban-phenyl-^{14}C in root-treated soybean plants

Table I. Pulse Time-Course Experiment. Percent ^{14}C Administered in 3-Day Incorporation of Barban-^{14}C

	Days posttreatment			
	0	3	9	23
Barban-phenyl-^{14}C				
Root and shoot	55	50	41	44
Treating solution	19			
Total	74			
Barban-carbonyl-^{14}C				
Root and shoot	47	42	38	29
Treating solution	17			
Total	64			
Barban-butynyl-^{14}C				
Root and shoot	58	44	49	37
Treating solution	18			
Total	76			
Average				
Root and shoot	53	45	43	37
Treating solution	18			
Total	71			

^{14}C were purified by preparative thin-layer chromatography. The impure substrate was streaked on 200 μ Silica gel HF thin-layer plates and developed to 15 cm with benzene:chloroform:carbon tetrachloride (6:9:5). The barban-^{14}C and other impurities were visualized in bands under ultraviolet light and ^{14}C bands were located with a windowless Geiger counter. The barban-^{14}C band was scraped from the plates, eluted from the silica gel with solvent, and concentrated. Substrate purity was confirmed with two-dimensional thin-layer chromatography on silica gel. The first solvent system was benzene:chloroform:carbon tetrachloride (6:9:5) and the second was benzene:acetone (2:1). In all cases, a single spot was observed after autoradiography before the substrate was considered chemically pure.

The radiochemical purity was assayed by caustic hydrolysis, steam distillation, and assay of the products; 3-chloroaniline-^{14}C was the only ^{14}C label found in the case of barban-phenyl-^{14}C and $^{14}CO_2$ from barban-carbonyl-^{14}C. Caustic hydrolysis of barban-butynyl(C-1)-^{14}C yielded no steam distillable radiocarbon. The 4-chloro-2-butynyl alcohol-1-^{14}C was therefore considered radiochemically pure. The preparation of all substrates for root feeding was as described by Still and Mansager (1971a).

Instrumentation. Gas–liquid chromatography was conducted using a glass inlet and glass columns of 1.8 m × 0.6 cm packed with various supports and phases. Helium was used as the carrier gas with a flow rate of 60 cm^3/min; the inlet temperature and the column oven temperature programs were as demanded by the column used. The gas–liquid chromatography of 3-chloroaniline-^{14}C was as described by Bombaugh (1965) using siponate DS-10 on acid-washed Chromosorb W precoated with 2% sodium hydroxide. The column effluent was split with a 10:1 stream splitter with the smallest portion passed to a flame detector. The components trapped from the glc column were assayed for radiocarbon by liquid scintillation counting or were analyzed directly in the mass spectrometer by the use of a solid sample probe. The mass spectra were measured with a Varian M-66 mass spectrometer equipped with a D-5500 console. The spectra were obtained at 70 eV at a source temperature of 180°C and a probe temperature of 25–50°C.

Miscellaneous Experimental Techniques. All β-glucosidase (Worthington Biochemical Corp.) reactions were run at pH 4.8 sodium acetate buffer at 37°C for 4 hr. The procedure described by Paulson and Portnoy (1970) was used for all the acetylation reactions. The procedure of Schlenk and Gellerman (1960) was used for the methoxylation reactions.

RESULTS AND DISCUSSION

Time-Course Experiments. Results from time-course experiments of barban-phenyl-^{14}C root-treated soybean plants and simultaneous volatility studies are reported in Figure 1. In the volatility studies, no plants were placed in the aerated nutrient solution. At the termination of the experiment the entire nutrient solution, either from the volatility study or from the plant feeding experiments, was extracted with ether. In the absence of plants, there was a decrease in ether-soluble material and an increase of water-soluble material with time. The sum of the ether and water extract was at all times equal to 100% of the radio-labeled material placed in the system.

In the presence of plant tissue, the concentration of barban-^{14}C in the nutrient solution fell to zero at approximately 7 days and the water solubles increased to 4 days and then decreased, approaching zero after 10 days of treatment. The radiocarbon concentration in root tissue increased steadily for 5 days and fell thereafter, while the radiocarbon content of shoot tissue increased continually throughout the experiment. The summation of the ^{14}C in root, shoot, or nutrient solution (water and ether extract) showed a loss of ^{14}C with time. At 10 days, greater than 30% of the radiocarbon placed in this system was not recovered. As seen from the volatility study, this loss could not be attributed to the loss of barban-^{14}C in the treating solution, but must be a result of the plant and its metabolism. These time-course experiments clearly demonstrated that root-treated soybean plants are able to absorb barban-^{14}C through the root and translocate it to the foliar tissues.

Pulse time-course experiments were conducted to determine the metabolic fate of barban-^{14}C labeled either in the phenyl nucleus, the carbonyl carbon, or the C-1 of the butynyl moiety. Table I reports the results of these experiments. Soybean plants were root-treated for 3 days, after which time the ^{14}C treating solutions were removed (0 day posttreatment) and samples taken for analysis. Harvesting was repeated at 3 days, 9 days, and 23 days posttreatment. At 0 days the dried plant tissue accounted for 55% of the administered barban-phenyl-^{14}C, while analysis of the treating solution resulted in

Table II. Percent Distribution of Radiocarbon in Soybean Plant Segments after 3-Day Treatment with Barban-^{14}C

	Barban-phenyl-^{14}C days posttreatment				Barban-carbonyl-^{14}C days posttreatment				Barban-butynyl-^{14}C days posttreatment			
Plant segment	0	3	9	23	0	3	9	23	0	3	9	23
Root	37	24	20	21	27	22	18	11	43	26	28	15
Stem 1	6	7	4	5	7	5	5	3	4	5	4	4
Stem 2			T[a]	T			T	T			T	T
Primary leaf	3	4	3	2	3	4	3	4	3	3	3	3
Trifoliate 1	9	14	12	14	9	11	12	11	9	11	12	13
Trifoliate 2			1	1			T	1			1	1
Pod				T				T				T
Seed				0				0				0
Total	55	49	40	43	46	42	38	30	59	45	48	36

[a] T, trace, less than 0.5%.

Table III. Percent Distribution of Radiocarbon in the Bligh–Dyer Extraction of Soybean Plant Segments, Pretreated for 3 days with Barban-^{14}C. Data Normalized to 100%[b]

	Chloroform extract days posttreatment				Polar extract days posttreatment				Solid residue days posttreatment			
Plant segment	0	3	9	23	0	3	9	23	0	3	9	23
Root	3.0	1.3	0.7	0.0	9.6	7.6	7.2	3.7	3.9	4.4	5.0	6.7
Stem 1	0.6	0.3	0.0	0.0	1.8	1.9	1.5	1.2	0.4	0.9	1.3	1.6
Stem 2			N[a]	N			N	N			N	N
Primary leaf	0.2	0.0	0.0	0.0	1.2	2.0	1.9	2.0	0.0	0.0	0.0	0.0
Trifoliate 1	0.5	0.1	0.0	0.0	3.8	6.6	7.1	8.6	0.0	0.0	0.0	0.0
Trifoliate 2			N	N			N	N			N	N
Pod				N				N				N
Fruit				0.0				0.0				0.0

[a] N, the tissue samples did not contain enough radiocarbon to carry out the Bligh–Dyer extraction and assay. [b] The data are an average of experiments in which the barban-phenyl-^{14}C, barban-butynyl-^{14}C, or barban-carbonyl-^{14}C were used as specifically labeled treatments.

19% of the ^{14}C, accounting for 74% of the total. Similar results were observed for the carbonyl and butynyl-^{14}C-labeled barban. After 3 days of root treatment (0 day posttreatment) an average of 53% of the total radiocarbon in the system was incorporated into the roots and shoots, and 18% remained in the treating solution. Approximately 30% of the radiocarbon administered to the system was not accounted for at 0 day posttreatment and was lost by some route other than volatility of parent barban-^{14}C from the nutrient solution. In all cases, the ^{14}C concentration in the root and shoot tissues decreased with time (0 day through 23 days posttreatment). The close correlation between the three specifically ^{14}C-labeled barban substrates indicates that the molecule is not cleaved and that the phenyl ring, carbamate carbonyl carbon, and C-1 of the butynyl alcohol are intact.

Table II reports data from pulse time-course experiments where the plant segments have been separately analyzed: root, stem section 1 (that section of stem that was present during the radiocarbon-labeled treatment), stem section 2 (that section that emerged after removal of radiocarbon from the nutrient solution), primary leaf, trifoliate group 1 (that group of leaves that were present during the ^{14}C treatment), and trifoliate group 2 (those leaves that emerged after treatment), pod, and seed. Data are reported for all three specifically ^{14}C-labeled barbans. In all cases there was little difference in the distribution of radiocarbon in the various plant segments due to specific labeling of the barban-^{14}C substrates. These results also support the thesis that the barban molecule is not cleaved. In all cases, there was a decrease in the ^{14}C in the root tissue with a concomitant increase in radiocarbon label in the trifoliate tissues. No ^{14}C was found in the seeds of these plants; however, a trace of ^{14}C was observed in the pod tissues. As in the other studies the total recovery of ^{14}C at each harvest reflects the loss of radiocarbon with time.

Tissues from all three ^{14}C specifically labeled barban experiments were extracted, and the ^{14}C distribution was found to be identical. A summation of these data is presented in Table III. The chloroform extract contained unaltered barban-^{14}C. As was observed with chlorpropham metabolism (Still and Mansager, 1971a), these data also showed a precursor product relationship between the parent barban, polar metabolites, and the solid residues. The photosynthetic tissues did not form insoluble residues. After 9 days posttreatment, most of the radio-labeled materials are either water-soluble metabolites or solid residues. At 23 days, the highest ^{14}C concentration was in the trifoliate group as polar metabolites. There was no movement of these polar metabolites from the site of origin into the newly emerging tissues, as is evidenced by the absence of appreciable amounts of ^{14}C label in the newly emerging trifoliate tissues or in the pods or seed tissues of these plants. This is contrary to the report by Jacobsohn (1970) from his studies with barley and wild oat biotypes.

These time-course studies have demonstrated the movement of barban-^{14}C from the treating solutions through the roots and into the shoots, primary and trifoliate leaves (Table III). The roots and stems metabolize barban to polar metabolites and solid residues. The polar metabolites are terminal metabolites in the leaf tissues. An inventory of the ^{14}C label shows a loss of 29% of label at 0 days posttreatment. Of the ^{14}C label incorporated into the plant at 0 days posttreatment (53%), 16% is lost by 23 days posttreatment. Therefore, 45% of the administered ^{14}C is not accounted for at the termination of the experiment (Table I). Riden and Hopkins (1961) reported a 40% loss 3 weeks after foliar treat-

ment with barban-butynyl-^{14}C from various crop plants. Both our studies and the work of Jacobsohn (1970) have shown that barban is not volatile. These losses of ^{14}C label may be a result of plant metabolism to yield a volatile metabolite.

At 23 days posttreatment, the soybean plants had 47% of the ^{14}C label in the roots, 17% as polar metabolites, and 30% as solid residues, while the stems, primary and trifoliate leaves contained 53% of the ^{14}C, all as polar metabolites. No parent barban remained unchanged, and 70% of the total ^{14}C label in both root and shoot was present as polar metabolites. Therefore, the chemical character of these polar metabolites was important.

Characterization of the Barban Polar Metabolites. To characterize the polar metabolites, barban-phenyl-^{14}C was incorporated *via* root-treatment into large quantities of soybean tissue. The root and shoot tissues were extracted using the Bligh–Dyer extraction technique (Still and Mansager, 1971b) and the polar metabolites were purified. The purification procedures were similar to those described for the isolation of the chlorpropham polar metabolites (Still and Mansager, 1971b). At the beginning of the purification procedure, the barban-^{14}C polar metabolites behaved similarly as the chlorpropham metabolites, and nearly quantitative recovery of radiocarbon from both root and shoot was observed to the point of DE-52-acetate column chromatography. The barban-^{14}C polar metabolites eluted from these columns as poorly defined peaks requiring high concentration acetate ion (0.5 *N*) to remove the material from the anion exchange column. Freeze-drying procedures were used to concentrate the column eluates and in all fractions from either root or shoot tissue, major losses of radiocarbon were experienced with each freeze-drying procedure (greater than 60%). The column purified materials were subjected to β-glucosidase incubation followed by partition and acetylation, as well as to direct acetylation of the partially purified polar metabolites. In either case, no conclusive results were obtained. Also, treatment of the column purified polar metabolites with diazomethane failed to yield any alteration of the materials. A solubility study of the polar metabolites showed them to be insoluble in nonpolar solvents but highly soluble in methanol or water. A number of gas–liquid chromatographic columns and temperature parameters were used in an effort to purify the components of the barban-^{14}C polar metabolites, but in no case were discrete radiocarbon-labeled peaks eluted from the gas–liquid chromatograph. In all cases gas–liquid chromatography yielded exceedingly low recovery of radiocarbon, indicating that the polar metabolites were either thermally and/or chemically unstable.

At this point in the investigation it became clear that further purification of the barban-^{14}C polar metabolites would not be practical. Therefore, the two samples from either root or shoot were hydrolyzed in 10% NaOH and steam distilled to yield quantitative recovery of radiocarbon in the steam distillate. The ^{14}C distillate was shown to contain only one ^{14}C-labeled component. This component was shown to be 3-chloroaniline by siponate DS-10 gas–liquid chromatography (Bombaugh, 1965) and by mass spectral analysis.

From these data we conclude that in root-treated soybean plants, barban is altered in such a fashion that its polar metabolites are rendered highly reactive and volatile when they are separated from the normal plant constituents. In all cases, the aromatic nucleus of barban is not altered, which is in agreement with Riden and Hopkins (1962) as they observed with their "compound X".

Frear and Swanson (1970) reported a fast *in vitro* reaction of barban with reduced glutathione. Lamoureux *et al.* (1971) have examined barban metabolism in excised leaves of grasses and found that the barban yielded a metabolite which had a thin-layer chromatographic R_f value comparable to a glutathione conjugate of barban synthesized *in vitro*. These workers were unsuccessful in purifying the barban metabolites. In our studies we compared the purified soybean polar metabolite with the barban-conjugate standard prepared by Lamoureux and co-workers and showed them to be dissimilar by thin-layer chromatography. It is our conclusion that the 4-chloro-2-butynyl alcohol moiety of barban is the first reactive site of the molecule in soybean. The polar metabolites found in root and shoot are products of this metabolism and perhaps the precursors to the solid residues found in the root and stem tissues. The absence of aryl hydroxylation of barban may be the result of the reactive 4-chloro-2-butynyl alcohol moiety and subsequent polar products which are not capable of phenyl hydroxylation.

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LITERATURE CITED

Bligh, E. G., Dyer, W. J., *Can. J. Biochem. Physiol.* **37,** 911 (1959).
Bombaugh, K. J., *Anal. Chem.* **37,** 72 (1965).
Crafts, A. S., in "The Physiology and Biochemistry of Herbicides," Audus, L. J., Ed., Academic Press, New York, N.Y., 1964, p 75–110.
Foy, C. L., *Western Weed Control Conf. Red. Prog. Rep.* 96–97 (1961).
Frear, D. S., Swanson, H. R., *Phytochemistry* **9,** 2123 (1970).
Jacobsohn, R., Ph.D. Thesis, The University of Minnesota, St. Paul, Minn., 1970.
Kelly, R. G., Peets, E. A., Gordon, S., Buyski, D. A., *Anal. Biochem.* **2,** 267 (1961).
Lamoureux, G. L., Stafford, L. E., Tanaka, F. S., J. AGR. FOOD CHEM. **19,** 346 (1971).
Oliverio, V. T., Denham, C., Davidson, J. D., *Anal. Biochem.* **4,** 188 (1962).
Paulson, G. D., Portnoy, C. E., J. AGR. FOOD CHEM. **18,** 180 (1970).
Riden, J. R., Hopkins, T. R., J. AGR. FOOD CHEM. **9,** 47 (1961).
Riden, J. R., Hopkins, T. R., J. AGR. FOOD CHEM. **10,** 455 (1962).
Schlenk, H., Gellerman, J. L., *Anal. Chem.* **32,** 1412 (1960).
Still, G. G., *Plant Physiol.* **43,** 543 (1968).
Still, G. G., Mansager, E. R., J. AGR. FOOD CHEM. **19,** 879 (1971a).
Still, G. G., Mansager, E. R., *Phytochemistry* in press (1971b).

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