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Keyphrases

Thalidomide, D and L—synthesis
TLC separation
Optical rotation—identity
IR spectrophotometry—structure

Study of Absorption, Translocation, and Residue Properties of 2,3,5-Triiodobenzoic Acid in Field-Grown Soybeans

By L. A. SPITZNAGLE, J. E. CHRISTIAN, A. J. OHLROGGE, and C. E. BRECKINRIDGE, JR.*

The synthesis of carboxyl-^{14}C-2,3,5-triiodobenzoic acid is described. The translocation, biological half-life, and residues remaining in the plant after application to field-grown soybeans are discussed.

INCREASED KNOWLEDGE of plant metabolism has led to a search for chemicals which stimulate or inhibit the growth of plants. One such chemical is 2,3,5-triiodobenzoic acid, commonly referred to as TIBA. TIBA has been shown by Galston (1) to decrease apical dominance and to affect the flowering of soybean plants, causing an increased number of flowers. Anderson (2) reported that the result of several combined effects is an increased yield of soybeans per acre, making TIBA a useful agricultural chemical. However, since soybean products are used in animal feeds and for human consumption, the residue and metabolism properties of TIBA must be determined prior to general usage.

Wheeler and Johns (3) reported the first synthesis of TIBA by reacting anthranilic acid with iodine monochloride. The product was diazotized, and allowed to react with potassium iodide yielding TIBA. Olivier and Combe (4) were able to obtain 95% of the theoretical yield of TIBA by making minor changes in the method of Wheeler and Johns (3). Munakata and Nakai (5), and Ice, Breckinridge, and Christian (6) described the synthesis of 2(^{131}I),3,5-triiodobenzoic acid. They followed closely the procedure of Olivier and Combe (4) substituting sodium iodide-131 for potassium iodide. Jarboe (7) synthesized 2,3(^{131}I),5(^{131}I)-triiodobenzoic acid by substituting iodine-131 monochloride for iodine monochloride.

EXPERIMENTAL

Synthesis—To facilitate tracing the TIBA molecule through the entire growth period of the soybean plant from the flowering to the bean stage, radioactive TIBA was synthesized containing carbon-14 in the carboxyl position. One hundred millicuries (2.584 Gm.) of anthranilic acid[1] ($C_6H_4{}^{14}CO_2H$-2-NH_2), was suspended in 60 ml. of

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[1] Volk Radiochemical Co., Inc., New York, N. Y.

0.7 *N* hydrochloric acid and cooled to 0°. Eleven grams (3.5 ml.) of iodine monochloride[2] and 30 ml. of 0.7 *N* hydrochloric acid were added to the cold suspension with stirring. To allow for completion of the reaction, stirring was continued for 48 hr. at room temperature after which excess iodine was reacted with sodium bisulfite. The solution was cooled to 0°, the precipitated reaction product (3,5-diiodoanthranilic* acid)[3] was collected by filtration. The reaction product was partially purified by acid-base reaction, ammonium hydroxide solution was added to give a pH of 9.5. The alkaline solution was filtered through a sintered-glass funnel. Glacial acetic acid was added to give a pH of 3. The solution was cooled to 0° and the resulting crystals of 3,5-diiodoanthranilic* acid collected on a sintered-glass funnel.

The 3,5-diiodoanthranilic* acid was diazotized by dissolving it in 40 ml. of concentrated sulfuric acid and cooling to 0°, followed by slowly adding 4 Gm. of sodium nitrite with stirring. After 2 hr. of cooling and stirring, the diazotized product was poured on ice. Nitrogen dioxide was removed from the solution by bubbling air through the solution for 30 min.

To the cold solution, 12 Gm. of potassium iodide in 12 ml. of water were added slowly with stirring. Nitrogen was removed from the solution by heating, and excess iodine was reacted with a sodium bisulfite solution.

After the solution was cooled to 0°, crude TIBA* was collected on a sintered glass-funnel. Fifteen milliliters of ether and enough hot ethanol to effect solution was added to the crude TIBA* and the solution was filtered. Ten milliliters of water was added, and the solution cooled to 0°. The crystals of TIBA* which formed were dried and weighed, the compound melted at 223–225.5°. The reported melting point for TIBA is 223–226° (3, 8). Approximately 55% of the theoretical yield (5.15 Gm.) of TIBA* (specific activity 16.240 dpm/mcg.[4]) was obtained.

The radiochemical purity of the compound was studied using thin-layer chromatography and autoradiography. Adsorbosil-1 (Applied Science Laboratories, Inc.), 250-μ thick, and a solvent system of petroleum ether (30–60° fraction)–propionic acid (10:1) were used. Nonradioactive compounds were chromatographed simultaneously and located by spraying with a 0.1% methanol solution of bromocresol green. Radioactive spots on the thin-layer plate were scraped from the plate and analyzed by internal liquid scintillation counting techniques to obtain a quantitative evaluation of the components.

The following compounds in addition to the TIBA* were separated: 3,5-diiodobenzoic* acid (1.36% of the radioactivity spotted), 2,5-diiodobenzoic* acid (1.16% of the radioactivity spotted), and an unidentified compound remaining at the origin (3.88% of the radioactivity spotted). Repeated recrystallization failed to improve the radiochemical purity of the compound. Work by another investigator (9) indicates that photochemical degradation occurs during chemical manipulation, resulting in the presence of impurities.

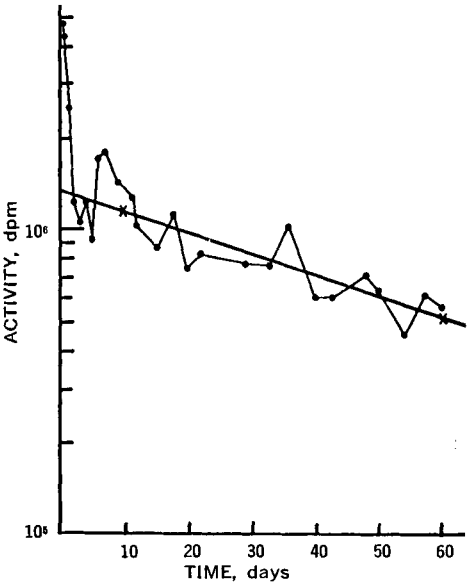

Fig. 1—Carbon-14 activity in soybean plant.

Plant Studies—A soybean plot, which at the beginning of the flowering stage contained approximately 600 plants averaging in the ninth trifoliate stage and about 35.5 cm. (14 in.) in height, was sprayed with a solution of TIBA* in water (650 mg. of TIBA* dissolved in ethanol 95% and brought up to a volume of 3 L. with distilled water). The spray was applied to the soybean plants with a hand sprayer to provide even application at the level of about 1 mg. of TIBA* per plant.

Three plants were taken from the plot at various time intervals (see Fig. 1) up to 60 days after application of the TIBA*, and the remaining plants were harvested at 110 days. Each sample was separated into the following parts: roots, stems, old leaves and old petioles, new leaves and new petioles (those which formed after the ninth trifoliate stage), pods, and seeds. After weighing, the samples were frozen in liquid nitrogen and ground in a blender to reduce the particle size and to provide a uniform mixture.

Five- to ten-gram samples of the plant parts were quantitatively converted to carbon dioxide using a modification of the Van Slyke-Folch wet oxidation procedure described by Jeffay and Alvarez (10). Each sample plus 7 Gm. of potassium iodate and 25 Gm. of chromium trioxide was placed in a suction flask connected to the fritted-glass dispersion tube of a gas-washing tower. Two hundred fifty milliliters of acid mixture[5] was added slowly to the suction flask from a separator. The mixture was boiled for 10 min. and the emitted carbon dioxide collected in 200 ml. of a mixture of ethanolamine and 2-ethoxyethanol (1:2). Heating was then discontinued and the system flushed with dry, carbon dioxide-free air. Controls were run using a known

[2] Eastman Organic Chemicals, Rochester, N. Y.
[3]* Denotes carboxyl-^{14}C.
[4] Determined by dissolving 10 mg. of TIBA* in 100.0 ml. of ethanol and counting 0.100-ml. samples and correcting for quenching by the addition of an internal standard.
[5] Prepared by mixing one part 85% phosphoric acid with two parts fuming sulfuric acid (20% SO_3) and allowing the mixture to cool before use.

TABLE I—CARBON-14 ACTIVITY FOUND IN THE WHOLE PLANT AND IN THE OLD LEAVES OF SOYBEAN PLANTS AFTER TREATMENT WITH CARBOXYL-^{14}C-2,3,5-TRIIODOBENZOIC ACID

Days After Application	Whole Plant		Old Leaves	
	dpm/Gm.[a]	dpm Total[b]	dpm/Gm.	dpm Total[b]
0	53,240	4,767,000	115,600	4,215,000
0.5	44,800	4,307,000	87,500	3,713,000
1	36,630	2,516,000	74,600	2,105,000
2	14,190	1,200,000	26,630	852,000
3	14,470	1,062,000	24,330	570,000
4	15,600	1,230,000	24,500	512,000
5	9,800	930,000	16,900	506,000
6	12,100	1,718,000	19,400	1,009,000
7	18,800	1,782,000	39,400	2,280,000
9	12,360	1,423,000	19,200	714,000
11	11,350	1,284,000	18,900	594,000
12	9,910	1,029,000	16,000	448,000
15	7,600	867,000	12,300	400,000
18	8,300	1,119,000	13,900	525,000
20	6,500	749,000	7,700	222,000
22	5,430	834,000	10,500	358,000
29	4,820	772,000	8,000	226,000
33	4,690	754,000	10,200	305,000
36	5,340	1,021,000	16,600	502,000
40	3,570	593,000	11,600	264,000
43	3,760	600,000	3,800	70,000
48	2,970	704,000	8,200	200,000
50	4,100	633,000	10,100	206,000
54	1,780	454,000	4,600	119,000
57	2,900	601,000	10,000	217,000
60	2,340	551,000	8,500	151,000

[a] Disintegrations/min./Gm. of plant material. [b] Calculated by multiplying the weight in grams of plant material by the dpm/Gm.

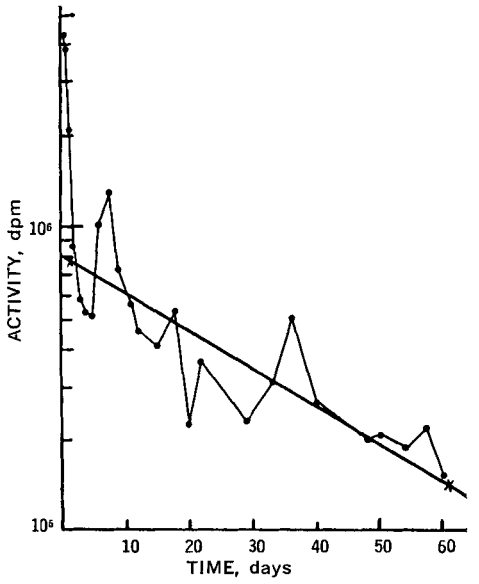

Fig. 2—Carbon-14 activity in old soybean leaves.

amount of TIBA* and 10 Gm. of plant material.

The liquid scintillation solution consisted of toluene and 2-ethoxyethanol (1:1), PPO[6] (4 Gm./L.) and dimethyl-POPOP[7] (100 mg./L.). All samples (3 ml. of the ethanolamine solution) were counted in a Tricarb liquid scintillation counter[8] and the results corrected for background and quenching following the method suggested by Schrodt, Gibbs, and Cavanaugh (11).

Results and Discussion—The radioactivity data for the whole soybean plant and the old leaves, Table I, indicated a rapid initial loss of radioactivity. Figures 1 and 2 are semi-log plots of the radioactivity against time for the soybean plant and the old leaves, respectively. At application the total

TABLE II—% CARBON-14 FOUND IN THE VARIOUS PLANT PARTS OF SOYBEAN PLANTS AFTER TREATMENT WITH CARBOXYL-^{14}C-2,3,5-TRIIODOBENZOIC ACID

Days After Application	%[a] in New Leaves	% in New Petioles	%[b] in Pods	%[b] in Seeds
6	5.0	2.2	...	...
7	6.4	1.3	...	...
9	10.4	3.6	...	...
11	21.3	4.7	...	...
12	21.1	5.4	...	...
15	21.1	5.5	...	...
18	21.0	2.7	...	...
20	27.6	5.5	2.4	...
22	21.0	7.7	2.2	...
29	25.8	6.4	8.7	0.2
33	14.5	2.5	5.6	3.5
36	11.9	3.1	8.0	2.2
40	11.0	1.2	6.5	4.9
43	21.1	3.4	6.8	21.5
48	14.3	3.3	12.0	12.3
50	15.0	3.0	11.4	14.4
54	13.4	3.0	9.8	13.2
60	8.3	3.7	5.4	31.1

[a] Calculated by dividing the total carbon-14 radioactivity in the plant by the amount in each of the plant parts and multiplying by 100. [b] TIBA* was applied at the flowering stage; consequently pods and seeds were not sufficiently formed for sampling and analysis until 20 and 29 days after application, respectively.

[6] 2,5-Diphenyloxazole.
[7] Dimethyl-1,4-bis-2-(5-phenyloxazolyl)-benzene.
[8] Model 3003, Packard Instrument Co., La Grange, Ill.

activity of a plant was 4,767,000 dpm; by 2 days after application the activity found on a plant was 1,200,000 dpm. Since absorption was being studied, the first 2 days were not included in the calculations of the linear regression for the soybean plant and the old leaves. From the linear regression lines shown on the graphs, the biological half-lives were calculated to be 42.5 and 24 days from the soybean plant and the old leaves, respectively. The difference between the half-lives indicated a translocation to other parts of the plant.

To better indicate this translocation, the percent of the carbon-14 in the various plant parts at the various sampling times was determined (Table II) and plotted against time. Figures 3–6 show the

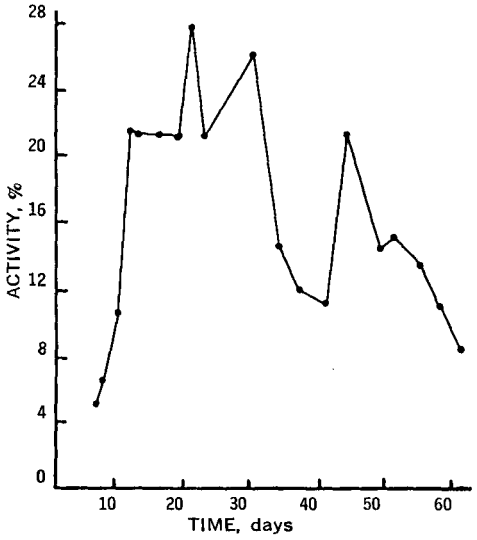

Fig. 3—Percent carbon-14 activity of the soybean plant in new leaves.

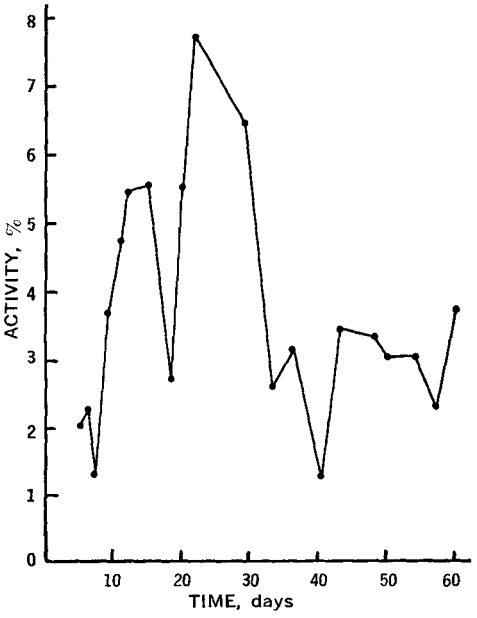

Fig. 4—Percent carbon-14 activity of the soybean plant in new petioles.

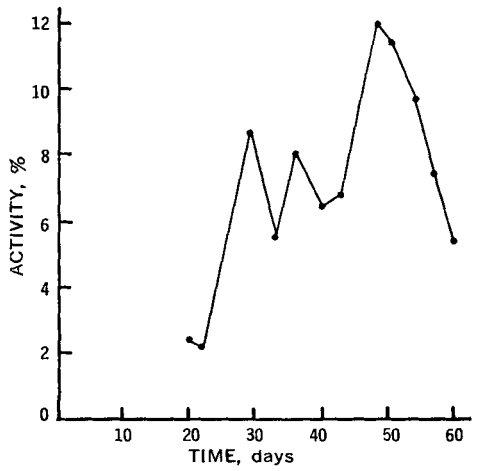

Fig. 5—Percent carbon-14 activity of the soybean plant in pods.

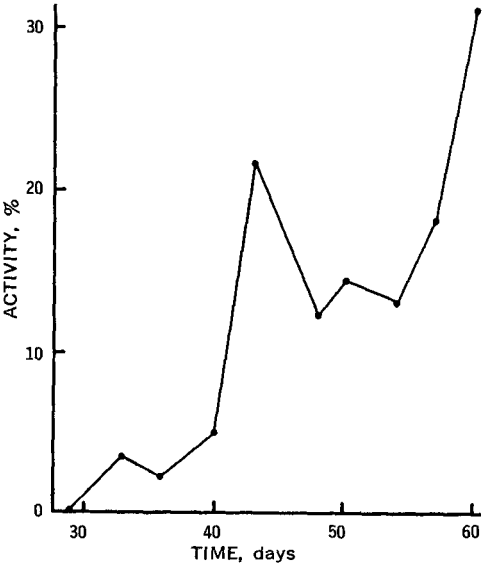

Fig. 6—Percent carbon-14 activity of the soybean plant in seeds.

percent of the carbon-14 activity in the soybean plant found in the new leaves, new petiole, pods, and immature seeds, respectively. From these figures it can be seen that carbon-14 was translocated to sites of new growth within the plant, and that as these parts aged, there was a translocation to newer parts of the plant. Although the seeds accounted for an increasing percentage of the radioactivity in the plant, the actual dpm/Gm. of soybeans did not increase after day 50.

Eleven samples of harvested soybeans were analyzed and the 95 percentile confidence level for the average radioactivity per Gm. of seeds was calculated. The 95 percentile confidence limits equaled 2709 dpm ± 950 dpm/Gm. of seeds, equivalent to 167 ± 59 ng. of TIBA* and/or metabolites per Gm. of seeds.[9]

[9] The 95% confidence interval = mean ± (1.96) (standard error of the mean).

SUMMARY

The plant growth regulator 2,3,5-triiodobenzoic acid containing carbon-14 in the carboxyl position was synthesized and applied to field-grown soybeans. A biological half-life of 42.5 days was found for the radioactivity in the soybean plant. Radioactivity was translocated to sites of new growth within the plant. At harvest the seeds contained a residue equivalent to 167 ng. of TIBA* and/or its metabolites per Gm. Before an assessment of the health hazard associated with the use of this chemical on soybeans can be made, there must be further study as to the nature of the residue.

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Keyphrases

Carboxyl-^{14}C-2,3,5-triiodobenzoic acid—synthesis
Absorption, translocation, residue—soybean plants
TLC—analysis
Autoradiography—analysis
Liquid scintillation counting—^{14}C, soybean plants

Mechanism of Action of Phenolic Disinfectants VIII

Association of Phenolic Disinfectants With Proteins

By JON E. STARR and JOSEPH JUDIS

Association of phenol-^{14}C (P-C-14), *p-tert*-amylphenol-^{14}C (PTAP-C-14), and 2, 4-dichlorophenol-^{14}C (DCP-C-14) with human serum and bacterial (*Micrococcus lysodeikticus*) proteins was investigated by means of density gradient ultracentrifugation and Sephadex gel filtration. Definite association between the phenols, PTAP-C-14 and DCP-C-14, and human serum proteins could be demonstrated by sucrose density gradient ultracentrifugation and with Sephadex gel filtration. The major protein in human serum involved in this association appeared to be albumin. Sucrose density gradient ultracentrifugation provided data indicating association of bacterial proteins with the three phenolic compounds, but most clear-cut in the case of PTAP-C-14. Protein binding could explain interference of serum with germicidal effects of phenolic disinfectants and enzyme inhibition and structural damage may account for bactericidal action.

TWO MAJOR CONSIDERATIONS motivated the investigation of the possible association of proteins and phenolic disinfectants. The interference of organic matter with the action of many disinfectants including phenol derivatives is a well recognized, troublesome phenomenon (1) and the authors desired to gain direct evidence for the basis of this interference, namely, whether binding of disinfectant by protein occurs. Secondly, an understanding of the mechanism by which phenolic disinfectants are lethal to bacteria would be much more complete if it could be shown that certain microbial cell components are specifically attacked by these disinfectants. One could presume that if a chemical component of the microbial cell bound phenolic disinfectants, this component could be, hypothetically, the one damaged by these germicides. Proteins, of course, play a major structural and functional role in all living cells and the general toxicity of phenolic disinfectants could be best explained on the basis of affinity for a generally distributed cell component, such as proteins.

The proteins selected for the preliminary studies were human serum, human serum albumin,

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