lowed by a spontaneous change in its surface properties, could also account for some of the change observed following irradiation of chlorpromazine. The sulfoxide, at these concentration levels, would not be expected to contribute to this change.

Since all of these compounds are metabolites of chlorpromazine (7), it would appear that the accumulation in the skin of not only the parent compound, but, more importantly, of some of its metabolites, may be responsible for the reported phototoxic reactions. It would be interesting to determine whether a relationship exists between the level of chlorpromazine and its *N*-oxide in the skin and the incidence of phototoxicity.

REFERENCES

(1) A. Felmeister and R. Schaubman, *J. Pharm. Sci.*, **58**, 64 (1969).

(2) A. W. Adamson, "Physical Chemistry of Surfaces," 2nd ed., Interscience, New York, N. Y., 1967, p. 26.

(3) C. L. Huang and F. L. Sands, *J. Chromatog.*, **13**, 246(1964).

(4) W. Burckhardt and M. Schwartz-Speck, *Schweiz. Med. Wochschr.*, **87**, 954(1957).

(5) I. Willis and A. M. Kligman, *J. Invest. Dermatol.*, **51**, 378 (1968).

(6) L. C. Harber, H. Harris, and R. L. Baer, *ibid.*, **46**, 303(1966).

(7) H. S. Posner, R. Culpan, and J. Levine, *J. Pharmacol. Exptl. Therap.*, **141**, 377(1963).

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Metabolism of the Plant Growth Regulator 2,3,5-Triiodobenzoic Acid in Soybeans

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Abstract □ This study investigates the residues resulting from the treatment of soybean plants with 1-^{14}C-2,3,5-triiodobenzoic acid. TLC was used to characterize and quantitate the residues present in the various samples. Residues of 1-^{14}C-2,3,5-triiodobenzoic acid, 1-^{14}C-2,5-diiodobenzoic acid, and 1-^{14}C-3,5-diiodobenzoic acid were found in the various plant parts and in the harvested seeds. The seeds also contained a large amount of the ^{14}C activity as a hexane-soluble material which was not identified.

Keyphrases □ 2,3,5-Triiodobenzoic acid metabolism—soybeans □ Soybean plants—1-^{14}C-2,3,5-triiodobenzoic acid residues □ TLC—separation, analysis □ Scintillometry—analysis

Reports of an increased soybean yield following the use of the plant growth regulator, 2,3,5-triiodobenzoic acid (I) (1–3) have led to a proposal for widespread use of the compound on soybeans (4). A more recent publication, dealing with the residue properties of 1-^{14}C-2,3,5-triiodobenzoic acid (II) (5), reported that ^{14}C activity was lost rapidly from the plant but that a residue containing ^{14}C was detected in the harvested seeds. The nature of the ^{14}C residue was investigated.

EXPERIMENTAL

Materials and Methods—The chemicals used in this investigation were reagent grade with the exception of the chromatographic standards.

All samples were counted in a liquid scintillation counter[1] with a gain of 18% and a window setting of 50 to 900 units. The scintillation cocktail used consisted of 15 ml. of a 1:1 mixture of toluene and 2-ethoxyethanol with 3.0 g. of PPO[2]/l. of cocktail. All samples were corrected for quenching by the addition of an internal standard.

The synthesis of the 1-^{14}C-2,3,5-triiodobenzoic acid used in this investigation has been described in a previous publication (5). Samples of leaves and stems from the earlier study were frozen and stored for use in the metabolite analysis. The harvested soybeans from the previous study were stored at room temperature.

TLC was used for the metabolite analysis. The chromatograms were prepared in layers that were 250 μ thick using a 1:2 mixture of an adsorbent containing a fluorescent indicator[3] and plain adsorbent.[3] The chromatograms were activated for 1 hr. at 110° immediately prior to use.

Three solvent systems were used for TLC. Solvent System *A* consisted of a 1:10 mixture of propionic acid and petroleum ether (30–60°), which was freshly prepared and placed in a chamber lined with filter paper. Chromatograms were developed in Solvent System *A* after the solvent had reached the top of the filter paper liner (1.5–2.0 hr.).

Solvent System *B* consisted of a 1:2:10 mixture of propionic acid, methanol, and benzene. Freshly prepared solvent was placed in a chamber lined with filter paper and the chamber was allowed to equilibrate for 24 hr. prior to use.

Solvent System *C* consisted of a 3:7 mixture of pyridine and benzene. Solvent System *C* gave better separation when the chromatograph was placed into the solvent chamber immediately after the addition of the solvent.

After all aromatic solvents had evaporated from the developed chromatograms, the compounds were located by placing the chromatogram under UV light. Table I lists the compounds which were used as chromatographic standards, their source, and their R_f values in the above-mentioned solvent systems.

Preparative thin-layer chromatograms were prepared from a

[1] Packard Tricarb, Packard Instrument Co., Inc.

[2] 2,5-Diphenyloxazole.

[3] Adsorbosil-p-1, and Adsorbosil-1, Applied Science Laboratories, Inc.

Table I—Chemicals Used in an Investigation of the Metabolism of 1-^{14}C-2,3,5-Triiodobenzoic Acid in Soybeans

Symbol	Chemical Name	Source[a]	R_f Value: Solvent System A[b]	Solvent System B[c]	Solvent System C[d]
I	2,3,5-Triiodobenzoic acid	1	0.19	0.50	0–0.13
II	1-^{14}C-2,3,5-Triiodobenzoic acid	2	0.19	0.50	0–0.13
III	2,5-Diiodobenzoic acid	1	0.44	0.62	0.23
IV	3,5-Diiodobenzoic acid	1	0.74	0.72	0.20
V	2,3-Diiodobenzoic acid	1	0.21	0.45	0–0.13
VI	2-Iodobenzoic acid	3	0.48	0.62	0.42
VII	3-Iodobenzoic acid	3	0.78	0.72	0.38
VIII	Benzoic acid	4	0.87	0.73	0.77
IX	2-OH-3,5-Diiodobenzoic acid	1	0.16	0.44	0.06
X	4-OH-3,5-Diiodobenzoic acid	4	0.22–0.53	0.67	0.52
XI	2-OH-3-Iodobenzoic acid	1	0.26	0.46	0–0.08
XII	2-I-3-Hydroxybenzoic acid	1	0.15	0.41	0.08–0.20
XIII	2-OH-5-Iodobenzoic acid	3	0.31	0.50	0.15
XIV	3-OH-5-Iodobenzoic acid	1	0.11	0.55	0.27
XV	1-Glycinyl-2,3,5-triiodobenzoate	1	0.0	0.38	—[e]
XVI	1-Glycinyl-2,5-diiodobenzoate	1	0.0	0.36	—[e]
XVII	1-Glycinylbenzoate (hippuric acid)	3	0.0	0.27	—[e]

[a] Sources of chemicals, 1. Purified chromatographic standard obtained from International Minerals and Chemical Co., Inc. 2. Synthesized by a method previously described (5). 3. Technical grade chemical obtained from commercial sources. 4. Reagent grade chemical obtained from commercial sources. [b] A 1:10 Mixture of propionic acid and petroleum ether (30–60°). [c] A 1:2:10 Mixture of propionic acid, methanol, and benzene. [d] A 3:7 Mixture of pyridine and benzene. [e] This chemical was not tested in this solvent system.

plain adsorbent[4] in layers that were 1.0 mm. thick. The chromatograms were activated for 1 hr. at 110° immediately prior to use. Samples were applied to the chromatogram in streaks that were 2–8 cm. long. The preparative thin-layer chromatograms were developed to a height of 10–12 cm. in Solvent System *A*. Zones of radioactivity were detected by scraping consecutive 1-cm. sections from each streak and counting in a liquid scintillation counter.

Extraction of Radioactive Metabolites—The following samples were chosen to study the metabolites of II present in them: old leaves and stems from plants taken 2, 4, 22, and 60 days after application of II; new leaves were included from plants taken 22 and 60 days after application of II; and seeds which were harvested from the remaining plants, 110 days after application of II.

The plant material was thawed and blotted prior to weighing. After weighing, the plant material was macerated, 10 ml. of *n*-hexane added for each gram of plant material, and the mixture placed on a platform shaker[5] for 24 hr.

The hexane mixture was filtered through a sintered-glass funnel and the plant material washed with three 50-ml. portions of hexane. The ^{14}C content of the hexane was determined by evaporating the hexane extract to a known volume and counting three 1.0-ml. samples in a liquid scintillation counter.

Extraneous material was separated from the radioactive metabolites in the hexane extract of harvested seeds by preparative TLC in Solvent System *A*. The extract separated into three distinct zones, one containing the yellow pigments (R_f 0.08–0.1), another the oils (R_f 0.5–0.75), and another a fluorescent material which traveled above the oil (R_f 0.70–0.85). Liquid scintillation counting of the various zones revealed that ^{14}C was present only in the fluorescent zone. Acetone extracts of the fluorescent zones from several preparative thin-layer chromatograms yielded a very small amount of an oil. When the oil was chromatographed, it gave an R_f of 0.8 in Solvent System *A*, 0.36 in Solvent System *B*, and 0.00–0.45 in Solvent System *C*.

The radioactivity in the hexane extract was soluble in ether at pH 10. Refluxing the hexane extract with 2 *N* HCl for 4 hr. did not alter its ether solubility or chromatographic behavior. Alkaline hydrolysis, by refluxing with 1 *N* NaOH for 1 hr. caused 33% of the ^{14}C to remain in the aqueous phase following ether extraction at pH 10. Since alkaline hydrolysis of II caused decomposition, yielding several colored zones on a thin-layer chromatogram, the alkaline hydrolyzed hexane extract was not chromatographed. Control seeds to which II had been added yielded less than 1.0% of the added ^{14}C in the hexane extract. However, the control seeds did yield a fluorescent material which had chromatographic properties similar to the material found in the treated seeds. No further attempts were made to identify the hexane-soluble fraction.

The plant material remaining after the hexane extraction was next extracted with 10 parts of an ethanolic-formic acid mixture (three parts ethanol, 95%, and one part formic acid, 88%) by shaking for 24 hr. Preliminary studies had shown that ethanolic-formic acid would extract 100% of the ^{14}C residue from treated seeds.

The ethanolic-formic acid extract of the seeds was diluted with two volumes of acetone in order to facilitate filtration through a sintered-glass funnel. The ^{14}C content of the ethanolic-formic acid extract was determined by evaporating the extract to a known volume and counting three 1.0-ml. samples. Table II gives the percent of the ^{14}C residue which was removed from the various samples by the solvent extractions.

Acid Hydrolysis—Acid hydrolysis has been shown to hydrolyze conjugates of both I (2,3,5-triiodobenzoic acid) and its metabolites (6); therefore, one-half of each ethanolic-formic acid extract of the plant parts was hydrolyzed. Each sample to be hydrolyzed was evaporated to an aqueous residue and added to a flask containing 25 ml. of 2 *N* HCl and some glass beads. The flask was attached to a water-cooled condenser and the material refluxed for 4 hr. after which the mixture was neutralized.

Attempts were made to remove extraneous material from the hydrolyzed and nonhydrolyzed ethanolic-formic acid extracts by extracting with ether, first at a pH of 10.0 and then at a pH of 2.0. The radioactivity was distributed in both ether fractions and in the remaining aqueous phase. TLC of the three phases from several samples of plant material indicated further clean-up was necessary before thin-layer chromatographic analysis could be used to identify the ^{14}C residue. Accordingly, each of the solvent fractions

Table II—Percent of the ^{14}C Residue Removed by Solvent Extraction from the Various Plant Parts of Soybean Plants Treated with 1-^{14}C-2,3,5-Triiodobenzoic Acid

Plant Part	Day[a]	Percent[b] Extracted by: Hexane	Ethanolic Formic Acid[c]	Percent[b] Unextracted
Old leaves	2[d]	5.8	122.5	0
	4	5.4	83.5	11.1
	22	2.0	75.2	22.8
	60	1.6	64.4	34.0
Stems	2	1.5	72.3	26.2
	4[d]	2.2	110.4	0
	22	5.0	81.6	13.4
	60	5.4	35.7	58.9
New leaves	22	4.2	80.5	15.3
	60	1.6	56.8	41.6
Harvested seeds	110[d]	48.2	57.5	0
Control seeds + II[e]		0.2	98.4	1.4

[a] Days after application of 1-^{14}C-2,3,5-triiodobenzoic acid to field-grown soybeans. [b] Percent of the ^{14}C residue based on the ^{14}C content determined by prior wet oxidation procedures (5). [c] Refers to a mixture of three parts ethanol 95% and one part formic acid 88%. [d] The excess activity can be attributed to poor mixing of the samples prior to the analysis by wet oxidation. [e] 1-^{14}C-2,3,5-Triiodobenzoic acid was added to the treated seeds; the results are the average of three determinations.

[4] The adsorbent was purified by extracting with ether in a continuous extraction apparatus for 24 hr. prior to use.
[5] Eberbach Corp., Ann Arbor, Mich.

Table III—Percent[i] of the Total ^{14}C Contained by the Old Leaves and Stems of Soybean Plants Treated with 1-^{14}C-2,3,5-Triiodobenzoic Acid, and Found in Four Zones on Preparative Thin-Layer Chromatograms

Day[a]		Percent[b] of the Total ^{14}C in the Old Leaves					Percent[b] of the Total ^{14}C in the Stems				
		Zone 1 (Unidentified)[c]	Zone 2 (II)[d]	Zone 3 (XIX)[e]	Zone 4 (XX)[f]	Total (Unidentified)[g]	Zone 1 Unidentified)[c]	Zone 2 (II)[d]	Zone 3 (XIX)[e]	Zone 4 (XX)[f]	Total (Unidentified)[g]
2	Free[h]	18.2	76.7	2.9	17.0		15.5	42.4	4.4	2.1	
	Conjugated[i]	0.0	0.0	0.0	0.0		0.0	0.0	0.0	2.4	
	Total	18.2	76.7	2.9	17.0	24.0	15.5	42.4	4.4	4.5	43.2
4	Free	11.0	44.0	4.2	15.7		14.7	71.6	9.6	6.8	
	Conjugated	0.0	0.0	0.9	0.8		2.6	4.1	0.0	3.3	
	Total	11.0	44.0	5.1	16.5	27.5	12.1	75.7	9.6	10.1	14.3
22	Free	34.8	7.1	1.6	22.6		20.6	30.5	4.2	13.2	
	Conjugated	20.9	7.6	2.1	7.1		13.1	16.2	0.0	0.2	
	Total	13.9	14.7	3.7	29.6	38.7	7.5	46.7	4.2	13.4	25.9
60	Free	35.2	4.6	0.0	15.5		14.4	8.5	0.0	3.8	
	Conjugated	19.1	4.2	2.0	12.3		1.8	1.4	1.1	0.6	
	Total	16.1	8.8	2.0	27.8	51.7	12.6	9.9	1.1	4.4	76.9

[a] Days after application of 1-^{14}C-2,3,5-triiodobenzoic acid. [b] Calculated from the total radioactivity found in the plant part by wet oxidation techniques (5) and its subsequent distribution into the various zones of the chromatograms. [c] This zone remained at the origin in a solvent system of propionic acid and petroleum ether (1:10) and was not identified. [d] This zone was identified by isotope dilution as II(1-^{14}C-2,3,5-triiodobenzoic acid). [e] This zone was identified by isotope dilution as XIX (1-^{14}C-2,5-diiodobenzoic acid). [f] This zone was identified by isotope dilution as XX (1-^{14}C-3,5-diiodobenzoic acid). [g] Found by summation of the percent remaining at the origin after hydrolysis (Zone 1), the percent in the hexane solvent fraction (Table II), and the percent unextracted (Table II). [h] Refers to material which was not hydrolyzed. [i] Refers to the decrease in Zone 1 after hydrolysis and the increase in the percent of the metabolites identified after hydrolysis.

was subjected to preparative TLC in Solvent System *A*. Four main zones of ^{14}C activity were detected on the preparative thin-layer chromatograms. The percent of the chromatographed radioactivity represented by each of the zones was determined by liquid scintillation counting. By multiplying the radioactivity extracted from each plant part by the fraction found in each zone of the preparative chromatograms the total radioactivity represented by the metabolites present in each zone was calculated. The results for each zone were expressed as a percent of the total radioactivity found in the plant part by wet oxidation techniques (5). The results of these calculations are presented in Table III for the old leaves and stems, and in Table IV for the new leaves and harvested seeds.

After separating the metabolites into four distinct zones, attempts were made to identify as many of the metabolites as possible. Identification was complicated by the small amount of each metabolite contained by and separable from the plant material.

The metabolites were eluted from each chromatograph with three 2.0-ml. portions of acetone. A carrier technique was used to "identify" the metabolites represented by each zone of radioactivity. A portion of the extract from each zone was mixed with each of the suspected metabolites (Table I). The added material was then separated and purified by TLC in two different solvent systems.

Solvent System *A* (propionic acid and petroleum ether, 1:10) was capable of separating most of the compounds from each other. However, Solvent System *C* was used to confirm the results obtained with Solvent System *A* and to separate compounds which had similar R_f values in Solvent System *A*.

The radioactivity contained by Zone 1 (origin) could not be characterized by this method. The radioactivity streaked and overlapped with the added XV (1-glycinyl-2,3,5-triiodobenzoate), XVI (1-glycinyl-2,5-diiodobenzoate), and XVII (1-glycinylbenzoate) when developed in Solvent System *B*.

The described carrier technique was used to characterize the radioactive metabolites in each of the remaining zones. The only radioactive compound found in Zone 2 (R_f 0.14–0.33) was II (1-^{14}C-2,3,5-triiodobenzoic acid). The labeled form of III (2,5-diiodobenzoic acid) was the only radioactive compound found in Zone 3 (R_f 0.4–0.6) and it was referred to as XIX (1-^{14}C-2,5-diiodobenzoic acid). The labeled form of IV (3,5-diiodobenzoic acid) was the only radioactive compound found in Zone 4 (R_f 0.65–0.85), and it was referred to as XX (1-^{14}C-3,5-diiodobenzoic acid.)

The specific activity of XIX and XX was calculated from the specific activity of II (5). The amounts of XIX and XX which were identified in the various plant parts were calculated using the specific activity of each. The results of these calculations are given in Table V. The values for the harvested seeds are given as parts per billion

Table IV—Percent of the Total ^{14}C Contained by the New Leaves and the Harvested Seeds of Soybean Plants Treated with 1-^{14}C-2,3,5-Triiodobenzoic Acid, and Found in Four Zones on Preparative Thin-Layer Chromatograms

Plant Part	Day[b]		Percent[a] of the Total ^{14}C in				
			Zone 1 Unidentified[c]	Zone 2 III[d]	Zone 3 XIX[e]	Zone 4 XX[f]	Total Unidentified[g]
New leaves	22	Free[h]	19.1	26.5	8.8	16.1	
		Conjugated[i]	3.4	6.2	0.0	6.5	
		Total	15.7	32.7	8.8	22.6	35.2
	60	Free	18.3	5.7	1.3	22.6	
		Conjugated	1.0	2.2	0.0	0.0	
		Total	17.3	7.9	1.3	22.6	60.5
Seeds	110	Free	10.0	21.0	11.0	6.5	
		Conjugated	0.0	5.0	0.0[j]	11.0	
		Total	22.0[j]	26.0	11.0	17.5	55.0

[a] Calculated from the total radioactivity found in the plant part by wet oxidation techniques (5) and its subsequent distribution into the various zones of the chromatograms. [b] Days after application of 1-^{14}C-2,3,5-triiodobenzoic acid. [c] This zone remained at the origin in a solvent system of propionic acid and petroleum ether (1:10) and was not identified. [d] This zone was identified by isotope dilution as II(1-^{14}C-2,3,5-triiodobenzoic acid). [e] This zone was identified by isotope dilution as XIX(1-^{14}C-2,5-diiodobenzoic acid). [f] This zone was identified by isotope dilution as XX(1-^{14}C-3,5-diiodobenzoic acid). [g] Found by summation of the percent remaining at the origin after hydrolysis (Zone 1), the percent in the hexane fraction (Table II), and the percent unextracted (Table II). [h] Refers to material which was not hydrolyzed. [i] Refers to the decrease in Zone 1 after hydrolysis and the increase in the percent of the metabolites identified after hydrolysis. [j] The amount remaining at the origin increased after acid hydrolysis while Zone 3 contained no radioactivity after acid hydrolysis.

Table V—The Quantity of Each Metabolite Found in Plant Parts of Soybeans Treated with 1-^{14}C-2,3,5-Triiodobenzoic Acid

Plant Part	Day[a]	Micrograms II[b]	XIX[c]	XX[d]
Old leaves	2	40.2	1.1	6.7
	4	13.9	1.2	3.9
	22	3.2	0.61	4.9
	60	0.81	0.14	1.9
Stems	2	5.6	0.44	0.45
	4	9.3	0.88	0.93
	22	2.8	0.19	0.61
	60	0.35	0.03	0.12
New leaves	22	3.5	0.71	1.8
	60	0.22	0.03	0.48
Seeds[e]	110	43 p.p.b.	14 p.p.b.	22 p.p.b.

[a] Days after application of 1-^{14}C-2,3,5-triiodobenzoic acid. [b] Identified by isotope dilution as 1-^{14}C-2,3,5-triiodobenzoic acid. [c] Identified by isotope dilution as 1-^{14}C-2,5-diiodobenzoic acid. [d] Identified by isotope dilution as 1-^{14}C-3,5-diiodobenzoic acid. [e] Expressed in p.p.b. because the average weight of seeds per plant was not determined.

because the average weight of seeds per plant at harvest was not determined.

DISCUSSION

The major compounds identified as residues from the application of II (1-^{14}C-2,3,5-triiodobenzoic acid) were II, XIX (1-^{14}C-2,5-diiodobenzoic acid), and XX (1-^{14}C-3,5-diiodobenzoic acid).

The percent of the ^{14}C residue in the old leaves which was identified, following hydrolysis, as II decreased rapidly from 75% two days after application to 47% by four days and was down to 8.6% by 60 days, indicating a rapid metabolism of II. The percent of the ^{14}C residue in the old leaves which was identified as XIX increased from two to four days after application indicating some metabolism of II to XIX. The percent of the ^{14}C residue identified as XIX had decreased by 22 days indicating a further metabolism of XIX, or its translocation out of the leaves. The percent of the ^{14}C residue identified as XX did not increase significantly during the first two sampling periods, but it had increased to 30% by 22 days and was the major metabolite of II identified in the old leaves at both 22 and 60 days. Deiodination of II to XIX did not appear to be an important metabolic pathway. However, deiodination of II to XX appears to be an important step in the metabolism of II. The amounts of unidentified and unextractable ^{14}C residue indicate alternate pathways for the metabolism of II. The percent of the residue in the stems identified as II, increased from 2 to 4 days after application of II. Four days after application of II, 75% of the residue in the stems was identified as free II indicating that II was translocated in the free form. The slight increase in the amounts of XIX and XX seems to indicate that these compounds were not translocated. The percent of the residue present in the new leaves at 22 days as II indicates that II was translocated from the old leaves. Some acid hydrolyzable conjugates of II were also found in the new leaves.

The largest identifiable residue in the seeds was II. A small amount of II was present as a conjugate which was hydrolyzable by acid. More than one-half of the XX present in the seeds was in the form of a conjugate hydrolyzable by acid. As in the new leaves and the stems, the amount of XIX identified in the seeds decreased after hydrolysis. Hexane soluble material accounted for 48% of the ^{14}C residue in the harvested seeds *versus* less than 6% for the other plant parts. All of the radioactive residue was extracted from the seeds while a large fraction of the radioactive residue in the other plant parts could not be extracted from the later samples.

The 11% free XIX found in the seeds seems to indicate a selective translocation of XIX to the seeds; no other plant part contained that large a percentage of XIX.

SUMMARY

The use of hexane allowed the extraction of 48% of the ^{14}C-containing metabolites from harvested seeds of soybean plants which had been treated with 1-^{14}C-2,3,5-triiodobenzoic acid. However, hexane extraction of the green plant parts did not remove more than 6% of the ^{14}C-containing metabolites. The hexane-soluble metabolites were characterized as lipid-like materials, but were not identified.

Free 1-^{14}C-2,3,5-triiodobenzoic acid was found to be translocated to sites of new growth within the plant. 1-^{14}C-3,5-triiodobenzoic acid was the major identifiable metabolite found in the old leaves. However, much of the radioactive residue in the old leaves became unextractable in the later time intervals, indicating other metabolic products.

The use of a mixture of three parts ethanol, 95%, and one part formic acid, 88%, allowed extraction of all of the ^{14}C residue in the harvested seeds. The harvested seeds contained 26% of the radioactive residue as 1-^{14}C-2, 3,5-triiodobenzoic acid (43 p.p.b.), 11% as 1-^{14}C-2,5-diiodobenzoic acid (13.7 p.p.b), and 17.5% as 1-^{14}C-3,5-diiodobenzoic acid (21.8 p.p.b.).

REFERENCES

(1) H. A. L. Greer, Ph.D. thesis, Iowa State University, Ames, Iowa (1964).

(2) H. A. L. Greer and I. C. Anderson, *Crop Sci.*, **5**, 229(1965).

(3) I. C. Anderson, *Crops Soils*, **18**, 8(1966).

(4) *Chem. Eng. News*, August 7, 22(1967).

(5) L. A. Spitznagle, J. E. Christian, A. J. Ohlrogge, and C. E. Breckrinridge, Jr., *J. Pharm. Sci.*, **57**, 764(1968).

(6) W. M. Barker, D. J. Thompson, and J. H. Ware, from a paper presented at the 51st meeting of the Federation of American Societies for Experimental Biology, Chicago, 1967.

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