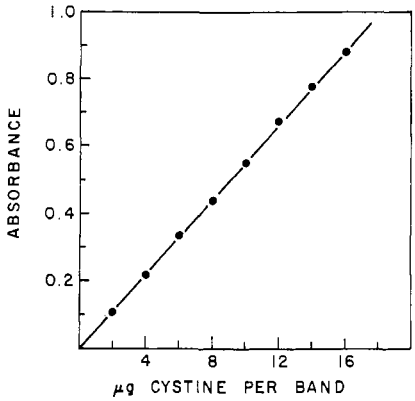

Figure 1. Absorbance of color produced by various amounts of cystine in 5 ml. of methanol as measured in 16-mm. cuvettes at 505 mμ

Table IV. Cystine in Various Foods (Gram %)

Food	Found	Lit. (10)
Casein	0.31	0.33
Promine[a]	1.09	0.97[a]
Whole egg (dry)	1.20	1.10
Yeast	0.56	0.55
Milk solids (fat-free)	0.26	0.32
Fish flour (alcohol extraction)	0.83	0.95
Fish flour (ethylene dichloride extraction)	0.61	...
Rice	0.18	0.10
Wheat	0.34	0.32

[a] Information on Promine obtained from Central Soya Lab. (2).

and the line did not always go through the origin, so that it was necessary to repeat the standard curve at four levels with each series of assays.

The reproducibility of the method was tested by assaying on 7 different days a hydrolyzate of oxidized casein with and without added cysteic acid in an amount equivalent to half that present in the sample. Table II shows that the method was reproducible and that recoveries were complete.

Rice was added to small portions of fish flour and dry milk and assayed (Table III). Taking the value for rice alone as 100%, the expected value due to addition of previously assayed fish flour and milk was calculated. Results were close to 100% of the expected value and the standard deviation was 3%. These data furnish additional proof of the reproducibility of the method.

Cystine contents of various types of foods are reported in Table IV and compared with data of Orr and Watt (*10*). Most values agree fairly well with those from the literature, but significantly higher values were obtained for rice; these are probably due to a difference in the cystine content of the samples of rice.

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LABELED HERBICIDES

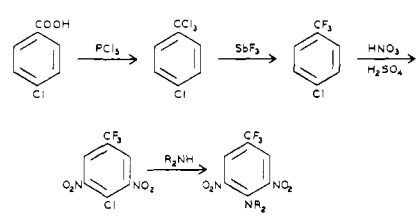
Preparation and Stability of C^{14}-Labeled Trifluralin and Related Compounds

F. J. MARSHALL, R. E. McMAHON, and R. G. JONES

Lilly Research Laboratories, Indianapolis, Ind.

Trifluralin-ring-(Universally Labeled)-C^{14} was prepared by a five-step synthesis from 4-chlorobenzoic-ring-(U.L.)-C^{14} acid with an over-all radiocarbon yield of 31%. Trifluralin-$C^{14}F_3$ and trifluralin-propyl-1-C^{14} were prepared also. The methods developed for these syntheses were utilized also for the preparation of radiocarbon labeled benefin and of several *N*-monoalkyl analogs. Samples of C^{14}-labeled trifluralin and benefin were found to be radiochemically stable when stored in the dark as evaporated films. When in dilute heptane solution, exposure to ultraviolet light produced extensive photodecomposition.

TRIFLURALIN (Treflan, Elanco Products Co., *N*,*N*-dipropyl-2,6-dinitro-4-trifluoromethylaniline), is a very effective pre-emergence herbicide (*1*) for the control of both grasses and weeds. The increasing use of trifluralin has led to the need for material labeled with carbon-14 for use in metabolism (*3*), transport, and stability studies. To have the C^{14}-label in various positions in the molecule was desirable so that all possible routes of metabolism could be followed. Thus, trifluralin-trifluoromethyl-C^{14}, trifluralin-propyl-1-C^{14}, and trifluralin-ring-(U.L.)-C^{14} were prepared. The general reaction procedure was as follows:



Benefin (Balan, Elanco Products Co.), *N*-butyl-*N*-ethyl-2,6-dinitro-4-trifluoromethylaniline, is an analog of trifluralin which is also a valuable herbicide. As a part of this study, benefin-butyl-1-C^{14} and benefin-ring-(U.L.)-C^{14} were prepared by the same general procedure outlined above.

A number of related *N*-alkyl-2,6-dinitro-4-trifluoromethylanilines, *N*-methyl-C^{14}, *N*-ethyl-1-C^{14}, *N*-propyl-1-C^{14}, and *N*-butyl-1-C^{14}, were also prepared. In addition to these, the *N*-ethyl-, *N*-

propyl-, and *N*-butyl-2,6-dinitro-4-trifluoromethylaniline-ring-(U.L.)-C^{14} derivatives were obtained as by-products of the preparation of trifluralin-ring-(U.L.)-C^{14} and benefin-ring-(U.L.)-C^{14}, evidently resulting from the primary amines present as impurities in the secondary amines used in the preparations. With the exception of the *N*-methyl-C^{14} compound, all of these *N*-monoalkyl compounds are possible metabolites which could result from the enzymatic dealkylation of trifluralin or benefin.

Trifluralin-C^{14} and benefin-C^{14} were found to be radiochemically stable for at least 2 years when stored in the dark as evaporated films. Solutions in *n*-heptane were stable at least 3 months when kept in the dark. The solutions were also stable to oxygen. When, however, dilute heptane solutions in borosilicate glass or quartz vials were exposed to ultraviolet light, extensive photodecomposition occurred. These results are not surprising since the photochemical transformations of substituted aromatic nitro compounds is well known (*6*). Of interest in the present connection is the report of Wright and Warren (*7*) who have reported that a film of trifluralin on glass undergoes photochemical decomposition in the presence of ultraviolet light. The as yet unidentified photodecomposition products of trifluralin do not appear to have herbicidal properties (*7*). Recently (*5*), the product isolated from *N*-propyl-2,6-dinitro-4-trifluoromethylaniline has been shown to be 2-nitro-6-nitroso-4-trifluoromethylaniline. Studies on the trifluralin products are being continued.

Table I. Labeled Herbicides and Related Compounds

Compound	Method	Thin-Layer System	Approx. R_f A	Approx. R_f B	Radioyield μc.	Radioyield %	Specific Activity, μc./mg.
Trifluralin			0.69	0.32			
Propyl-1-C^{14}	I[a]	A			950	47	3.08
$C^{14}F_3$	II[b]	A			5750	29	9.00
Ring-(U.L.)-C^{14}	II[c]	A			2810	31	13.60
Benefin			0.67	0.30			
Butyl-1-C^{14}	I[a]	B			1700	57	7.50
Ring-(U.L.)-C^{14}	II[c]	A			1418	31	14.04
N-Monoalkyl							
N-$C^{14}H_3$	I[a]	A	0.22	0.03	238	48	3.98
N-Ethyl			0.43	0.09			
1-C^{14}	I[a]	B, A			364	73	4.87
Ring-(U.L.)-C^{14}	[d]	A			17		
N-Propyl			0.51	0.13			
1-C_{14}	I[a]	A			265	53	3.10
Ring-(U.L.)-C^{14}	[e]	A			31		
N-Butyl			0.56	0.15			
1-C^{14}	I[a]	B, A			292	58	3.06
Ring-(U.L.)-C^{14}	[d]	A			47		

[a] Starting with the 1-C^{14}-amine hydrochloride and cold 2,6-dinitro-4-trifluoromethylchlorobenzene. The yield is thus for a one-step synthesis.
[b] Starting with 4-chlorobenzoic-7-C^{14} acid. The final step involved the reaction between 2,6-dinitro-4-trifluoromethyl-C^{14}-chlorobenzene and dipropylamine. The per cent radioyield is based on the starting acid.
[c] Starting with 4-chlorobenzoic-ring-(U.L.)-C^{14} acid. The final step involved the reaction between 2,6-dinitro-4-trifluoromethylchlorobenzene-ring-(U.L.)-C^{14} and cold amine. The per cent radioyield is based on the starting acid.
[d] Isolated from the thin-layer chromatogram of benefin-ring-(U.L.)-C^{14}. Estimated specific activity at 14 μc./mg.
[e] Isolated from the thin-layer chromatogram of trifluralin-ring-(U.L.)-C^{14}. Estimated specific activity at 14 μc./mg.

Experimental

The data on the labeled compounds prepared in this study are summarized in Table I. The procedures described below are general for all of the compounds. All the labeled starting materials were obtained commercially: 4-chlorobenzoic-7-C^{14} acid, dipropyl-1-C^{14}-amine hydrochloride, and ethylbutyl-1-C^{14}-amine hydrochloride from New England Nuclear Corp., Boston, Mass.; methyl-C^{14}-, ethyl-1-C^{14}-, propyl-1-C^{14}-, and butyl-1-C^{14}-amine hydrochloride, and 4-chlorobenzoic-ring-(U.L.)-C^{14} acid were supplied by Nuclear Research Chemicals, Inc., Orlando, Fla. All were used as obtained from the suppliers.

Thin-Layer Chromatography. Because of the small scale of the reactions, the isolation of pure products from the crude reaction mixtures could be done easily only by use of preparative thin-layer chromatography. The separations were carried out on plates which were coated with 0.5-mm. layers of silica gel GF-254 (E. Merck) and which had been dried at 60° C. The solvent systems used were A (1:1 benzene–methylcyclohexane) and B (carbon tetrachloride). A single 200 × 200 mm. plate served to separate 50 to 60 mg. of the *N*,*N*-dialkyl products, but the monoalkyl derivatives were usually run at that level and then were rechromatographed at about 20 mg. per plate. The products were isolated by scraping the band from the plate and eluting it with ether in a small column. These operations were carried out as rapidly as possible, and care was taken not to expose either the plates or the solutions to sunlight. All the compounds prepared were stored as evaporated films in borosilicate glass vials and were kept in the dark.

4 - Trichloromethylchlorobenzene. The procedure used was a modification of that described by Limpricht (*4*) for the preparation of benzotrichloride from benzoic acid. A longer period of heating at a higher temperature gave much higher yields.

In a small dry Carius tube were placed 940 mg. (6 mmoles) of dry 4-chlorobenzoic acid and 2.5 grams (12 mmoles) of phosphorus pentachloride. The tube was sealed, placed in a steel pressure reactor, and then, was heated at 200° C. for 120 hours. After cooling, the tube was opened and a magnetic stirring bar was introduced. Excess phosphorus pentachloride and the phosphorus oxychloride formed during the reaction were decomposed by the cautious dropwise addition of 5*N* sodium hydroxide solution to the stirred mixture. Then there was added 5 ml. of methylene chloride. The aqueous layer was separated by use of a dropper, and the organic layer was washed thoroughly by stirring overnight with 5 ml. of fresh 1*N* sodium hydroxide solution. The organic layer was separated and partially dried by passage over a small silica gel column. The dry product, obtained after removal of the solvent and a small added portion of dry benzene in a microdistillation apparatus, weighed 1.1 grams (80%).

4 - Trifluoromethylchlorobenzene. The procedure was based on the method of Booth, Elsey, and Burchfield (*2*). To 1.38 grams (6 mmoles) of 4-trichloromethylchlorobenzene was added 1.18 grams (6.6 mmoles) of fresh commercial antimony trifluoride, and the flask immersed in an oil bath at 155° C. This reacted in about 2 minutes, and the mixture turned black. The bath temperature was then increased to 220° C. The product, which distilled during this time, boiled at 135° to 140° C. and weighed 760 mg. (70%).

2, 6 - Dinitro - 4 - trifluoromethylchlorobenzene. To a mixture of 1 ml. of sulfuric acid and 0.4 ml. of fuming nitric acid was added 670 mg. (3.7 mmoles) of 4-trifluoromethylchlorobenzene. The reaction mixture was stirred for 1 hour at 40° C. and then was cooled to room temperature. The upper organic layer was removed with a small pipet, the transfer was completed with a little petroleum ether, and added to a mixture of 2.8 ml. of fuming sulfuric acid and 0.8 ml. of fuming nitric acid. Then the mixture was stirred for 2 hours

at 100° C. and, after cooling, was added cautiously to 25 grams of ice. The product was isolated by ether extraction and removal of the solvent to give 830 mg. of crude 2,6-dinitro-4-trifluoromethylchlorobenzene. This was purified by preparative thin-layer chromatography (system A) and was recovered by ether elution in a small column. The oil crystallizes on standing.

***N*-Alkyl- and *N,N*-Dialkyl-2,6-dinitro-4-trifluoromethylanilines.** METHOD I. Equimolar amounts of 2,6-dinitro-4-trifluoromethylchlorobenzene and the amine hydrochloride in dry triethylamine (2 ml. for 0.5-mmole run) were heated overnight at 80° C. After cooling, ether was added, and the mixture was washed with 1*N* HCl, water, 1*N* NaOH, and water. Purification was accomplished by thin-layer chromatography.

METHOD II. To 1 mmole of 2,6-dinitro-4-trifluoromethylchlorobenzene in 25 ml. of ether was added 4.25 mmoles of the *N,N*-dialkylamine, and the mixture was allowed to stand overnight. Six milliliters of 1*N* HCl was added, and then, the ethereal solution was washed with water. The ether was removed under reduced pressure, and the product was purified by thin-layer chromatography.

Acknowledgment

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2,4-D METABOLISM

Metabolism of C^{14}-Labeled 2,4-Dichlorophenoxyacetic Acid in Rats

SUCHITRA KHANNA and S. C. FANG

Department of Agricultural Chemistry, Oregon State University, Corvallis, Ore.

C^{14}-labeled 2,4-dichlorophenoxyacetic acid (2,4-D-1-C^{14} or -2-C^{14}) was fed to adult rats at a dose of 1 to 100 mg. per rat, and the expired air, urine, feces, internal organs, and tissues were analyzed for radioactivity. No C^{14} was found in the expired air during a three-day period following dosing. The rate of 2,4-D elimination was dosage dependent. Radioactivity was found in all organs and tissues examined. The maximum radioactivity in all tissues was generally reached at 6 to 8 hours after dosing, and started to decrease immediately for 1-mg. 2,4-D dosage. At 100-mg. dosage, the peak concentration persisted until about 17 hours. The urine and the extracts of several tissues contained mainly unchanged 2,4-D residue. A study of the intracellular distribution of 2,4-D in six organs has shown that the soluble fraction of the cells contained the major portion of radioactivity, followed by the nuclear fraction, and the mitochondrial and microsomal fractions.

FOR A NUMBER of years, 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely used as a selective herbicide. In 1947, Levey *et al.* (*4*) reported a study of the metabolism of phenoxyacetic acid and monochloro- derivatives in rabbits. They found that when phenoxyacetic acid was given orally, 96% of the administered dose was excreted in the urine in 24 hours. In the case of *o*- or *p*-monochlorophenoxyacetic acid, the recovery was 70 to 90% in 24 hours. There was a difference in the rate of excretion between these two isomers. Clark *et al.* (*2*) reported on the metabolism of 2,4-D-1-C^{14} in sheep. They found that approximately 96% of a dose of 2,4-D was excreted unchanged in the urine and 1.4% in the feces in 72 hours. Very little residual radioactivity was found in sheep tissues after 72 hours. The purpose of the present investigation was to study the metabolic fate, tissue accumulation, cellular incorporation, and excretory pattern in adult rats receiving varying amounts of C^{14}-labeled 2,4-D.

Materials and Methods

Adult rats of the Wistar strain, approximately 4 to 6 months old and weighing 350 to 400 grams for males, and 225 to 275 grams for females, were used in this study. Aqueous solution of 2,4-D-1-C^{14}, 3.03 mc. per mmole, or 2,4-D-2-C^{14}, 1 mc. per mmole, at a concentration ranging from 1 to 50 mg. per ml. was prepared by dissolving it in water containing equal molar amounts of tribasic potassium phosphate. Neither labeled 2,4-D contains any isotopic impurity as revealed by paper chromatography. The same amount of 1 mg. of labeled 2,4-D was used at all dose levels with the balance being made up of nonlabeled 2,4-D acid in the case where doses were greater than 1 mg. The herbicide was administered to the rat by stomach tube at different dose levels, ranging from 1 to 100 mg. per animal—approximately 3 to 300 mg. per kg. of body weight. The oral LD_{50} for 2,4-D in rats has been found to be 300 to 1000 mg. per kg. of body weight (*3*). After dosing, the animals were placed individually in metabolism cages, and the urine and feces were collected periodically. The expired CO_2 was trapped in a dilute sodium hydroxide solution, which was changed at hourly intervals and checked for radioactivity for three days following 2,4-D administration. During this period, no radioactivity was found in the expired air from rats fed 2,4-D labeled either at the 1-C or 2-C position. The radioactivity in all urine samples was measured in a Packard Tricarb liquid scintillation spectrometer Model 314S. Aliquots of urine