METABOLISM OF RICININE IN THE CASTOR PLANT, *RICINUS COMMUNIS* L.

By

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PLANT, RICINUS COMMUNIS L.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Very little is known about the biodegradation of alkaloids in plants. During the early period of the twentieth century, it was generally thought that alkaloids were by-products of a number of irreversible and physiologically useless reactions of nitrogen metabolism (1-3). This idea was gradually discarded as an increasing number of experiments showed that alkaloids are metabolically active.

Indirect evidence for the decomposition of ricinine in the living plant was obtained by several investigators in non-isotopic experiments. In 1933, Weevers (4) showed that ricinine content decreased with increasing age of castor plants grown on nitrogen depleted soil. Bogdashevskaya (5) showed that the content of ricinine was reduced in leaves which were shaded from the light, while the upper unshaded leaves of such plants produced supernormal levels of ricinine. Waller and Nakazawa (6) reported that ricinine was rapidly utilized by the cotyledon in the dark. However, the amount of ricinine did not decrease when nicotinic acid was present in the medium.

Tso and Jeffrey (7) were the first to use isotopic tracers to demonstrate that alkaloids are metabolically active. They fed nitrogen-15 labeled nicotine, nornicotine and anabasine to tobacco plants and found that a major portion of the alkaloids supplied was broken down. Griffith et al. (8) indicated later that nicotinic acid was a metabolite

of nicotine. The catabolism of nicotine was also studied by other investigators (9).

The experiments described here were performed: (a) to determine whether ricinine can be metabolized in the castor plant, (b) to observe the turnover of ricinine in different organs, and (c) to investigate whether ricinine can be converted to nicotinic acid or other compounds containing the pyridine ring.

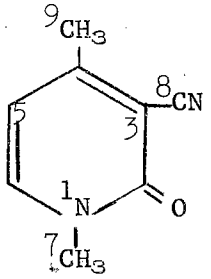
CHAPTER II

LITERATURE SURVEY

A. Properties of Ricinine

Ricinine, $C_8H_8O_2N_2$ is an alkaloid occurring in the castor plant. It was first discovered by Tuson (10) in 1864 in castor beans and was examined by Soave (11), Schulze (12) and Evans (13). Ricinine is a neutral compound and, unlike most alkaloids, it neither forms salts with acids nor reacts with the usual alkaloidal reagents (14). However, it does give some characteristic color reactions such as the Weidel (15) and Dragendorff reactions (14).

Ricinine crystallizes in prisms, melts at 200-201.5° and sublimes at 170-180° under 20 mm pressure. It is optically inactive, and sparingly soluble in water, alcohol, chloroform and ether. Its absorption maxima are 320 mμ in chloroform, 315 mμ in 95 per cent ethanol and 307 mμ in water (16). The structure of ricinine was demonstrated by Spath and Koller (17, 18) to be 1,2-dihydro-4-methoxy-1-methyl-2-oxonicotino-nitrile.



B. Occurrence of Ricinine

Ricinine appears to be distributed throughout all parts of the

castor plant. Young castor plants which had been grown in the dark under laboratory conditions contained about three per cent ricinine in the leaves and about one per cent ricinine in the roots and stems, while the whole plant contained approximately 1.6 per cent of the alkaloid. However, roots from a flowering plant at the 11th nodal stage of development which had been grown in the field contained only 0.13 per cent ricinine, which represented 0.72 per cent of the total alkaloid on a dry weight basis (19). According to Bottcher (20) castor seeds contain about 0.15 per cent ricinine. However, the great bulk of ricinine is located in the seed coat, with only about 0.03 per cent in the kernel. Bogdashevskaya (21) showed that the absolute amount of the alkaloid constantly increased with plant growth, but on a percentage basis rose only up to the age of 20 days, after which it declined rapidly only to show a rise just before bud opening. The rate of formation of the alkaloid was three times greater in 20-day-old plants than in mature plants. Thus the rate of formation of ricinine declined with increasing age of the plants. In addition, Schulze and Winterstein (22) indicated that etiolated castor seedlings produced more ricinine than normal green ones.

C. Biosynthesis of Ricinine

On the basis of the chemical degradation of ricinine formed from various labeled compounds, the following conclusions were reached. The methyl group of ricinine can be formed from methionine (23). The pyridine ring of nicotinic acid and nicotinamide becomes the α-pyridone ring of ricinine and all of the activity of nicotinic acid-7-^{14}C administered to castor plants is located in the nitrile carbon atom of ricinine (24, 25). One of the carboxyl groups of four carbon dicarboxylic

acids of the citric acid cycle provides the carbon for the cyano group, the other carboxyl group presumably being lost by decarboxylation (25). Carbons 2 and 3 of these dicarboxylic acids are incorporated into the pyridone ring at the 2 and 3 positions of ricinine (26). Succinate and glycerol are presumed to be the primary building units of ricinine, but other compounds related to succinic acid through the citric acid cycle are also precursors of ricinine (26).

The biosynthesis of ricinine in vitro was studied by Hadwiger (27), who found that trigonelline accumulated when nicotinic acid was added to homogenates of castor seedling cotyledons. Therefore, experiments were carried out to determine whether trigonelline became labeled in castor plants after injecting ricinine-8-^{14}C.

D. The Function of Ricinine

Although there is no doubt that alkaloids must play an important physiological role in plants, there is as yet little direct experimental evidence on this subject. Some of the functions which have been postulated for alkaloids are: (a) they may serve as a protection against parasites (28) or animals (29), (b) they may be reserves of nitrogen (30), (c) certain alkaloids may function in transmethylation (9), and (d) alkaloids, especially in the root tip, may have antiauxin activity (31).

Nuccorini and Bagnis (32) reported a possible relationship between ricinine and glutamine, since the etiolated castor plant when subjected to pathological changes contained more ricinine and less glutamine than normal plants. Padoa and Spada (33) observed that ricinine could depress lipase function.

A possible role of alkaloids in plants was suggested by the work

of Dawson et al. (34) and Solt et al. (35). They found in their sterile tobacco root culture that nicotine and anabasine production is independent of the quantity of precursor in the tissues. Hadwiger and Waller (36) also found that ricinine biosynthesis in roots of Ricinus communis was not significantly affected by increasing the quantity of precursors. These results indicate that the alkaloid may serve as a regulatory agent in the plant.

From the observations mentioned above, it appears probable that ricinine is not a waste product of nitrogen metabolism in the castor plant.

CHAPTER III

EXPERIMENTAL

A. Conditions for Growing Castor Plants

The plants used in this study were of the Cimarron variety, grown from seeds which had been harvested in 1958. The first group of plants was grown on Port clay loam soil at the Agronomy Farm of Oklahoma State University in Stillwater. The planting dates were May 31 and July 10, 1962. These plants were used for the determination of ricinine, free nicotinic acid and total nicotinic acid.

A second group of castor plants was grown on Port clay loam soil at Perkins, Oklahoma on the Agronomy Farm. The planting date was April 24, 1963. These plants were used for the isolation and determination of radioactive ricinine.

A third group of plants was grown indoors in sand in this laboratory during the summer of 1963. The room temperature was kept at approximately 25° C, and tap water was supplied daily. These plants were used for observing the biodegradation of ricinine.

B. Injection Procedure

The first group of plants was injected in the second node with a 50 µl syringe. Because this node of the plant could not hold 200 µl solution at one time, 50 µl injections were made at intervals of ten minutes until all of the compound had been introduced into the same nodal space.

The method of injection used for the second group of plants was developed by Waller (19). A 22 gauge hypodermic needle was inserted at the top of the second internode of a castor plant to serve as a vent. An aqueous solution of the labeled compound was injected at the bottom of the internode. The solution was completely absorbed within a few minutes after injection. The third group of plants was injected by the same method as the second group, but since these plants were much smaller than those in the second group four hours were required for the complete absorption of the 25 μl volume of solution injected.

The details of the experimental procedures are summarized in Table I.

C. Preparation of Labeled Compounds

1. Tritium labeled ricinine

This compound was prepared at Oklahoma State University in September, 1960, by the Wilzbach method (37) which involves exposing several hundred milligrams of the compound to pure tritium gas for about two weeks. The ricinine-^{3}H was washed with water to remove exchangeable tritium, diluted by adding cold ricinine, and then purified by recrystallization from water until the specific activity reached a constant value. Descending paper chromatography was used to check the radiochemical purity of ricinine-^{3}H. A solution of the ricinine-^{3}H was spotted on Whatman No. 1 paper, and the paper developed in tert-butyl alcohol-water-acetic acid, 4:2:1, and 85 per cent isopropanol. The R_f value of ricinine was 0.75 in both solvent systems. After developing and drying, the paper strip was cut into one inch x two cm pieces, and each piece was placed in a Tri-Carb vial with 10 ml of scintillation solvent for counting.

2. Carbon-14 labeled ricinine

Ricinine-8-^{14}C was prepared biosynthetically by isolating it from

TABLE I

A SUMMARY OF EXPERIMENTAL PROCEDURES

Exp. No.	Location	Planting Date	Injection Date	Age of Plant (Days)	Physiological Stage of Development	Isotope	Conc. of Ricinine (μg/ml)	Amount of Injected Solution (μl)	Specific Activity of Ricinine (μc/mmole)	Total Activity Injected Into One Plant (mμc)
1	Perkins, Oklahoma	April 24, 1963	June 12, 1963	49	10~11 nodules flowering	Ricinine-^{3}H	6.0	500	48.50	890
2	Stillwater, Oklahoma	May 31, 1962	July 5, 1962	36	6~8 nodules	Ricinine-^{3}H	8.025	200	48.70	475
3	Stillwater, Oklahoma	July 10, 1962	August 21, 1962	41	6~9 nodules	Ricinine-^{3}H	8.0	200	21.55	210
4	Laboratory	July 2, 1963	July 14, 1963	12	6 inches	Ricinine-8-^{14}C	6.0	25	4.27	3.90
5	Laboratory	July 18, 1963	July 30, 1963	12	6 inches	Ricinine-8-^{14}C	6.0	---	2.73	---

Note: Exp. 5 see Table V

young castor plants to which nicotinic acid-7-^{14}C or nicotinamide-7-^{14}C had been administered. Both of these compounds gave rise to ricinine labeled solely in the nitrile carbon. Its purity was checked in the same manner as was outlined for tritium labeled ricinine. The results are shown in Table II.

D. Measurements of Radioactivity

In all of these experiments, radioactivity measurements were made using a Liquid Scintillation Spectrometer.[1] The scintillation solvent was composed of 58.75 per cent toluene, 39.25 per cent absolute ethanol and 2 per cent water. This solution contained 0.5 per cent 2,5-diphenyl oxazole and 0.02 per cent p-bis-2-(5-phenyl oxazolyl) benzene (PPO) as phosphors. This system had an efficiency of approximately 8.0 per cent for tritium and 38 per cent for carbon-14 pyridinium compounds. The scintillation solvent used for measuring the $^{14}CO_2$ was made of three ml of ethanolamine-methylcellosolve, 1:2 which was used for CO_2 absorption, and 15 ml of methylcellosolve-toluene (sulfur free), 1:2 which contained 5.5 g of PPO per liter. Ethanolamine was used because it was less quenching than sodium hydroxide solution.

E. Storage of Plant Materials

Whole castor plants were harvested, placed in polyethylene bags and stored at -18° C until used.

F. Isolation of Ricinine

Castor plants were cut into pieces approximately one inch in length. Chloroform in an amount four times the weight of the fresh

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[1]Model 314 E Tri-Carb, Packard Instrument Company, Inc., LaGrange, Illinois

TABLE II

PREPARATION OF RICININE-8-^{14}C

Compounds	Physiological Stage	Volume of Injected Solution	Total Activity Injected Into One Plant	Ricinine Isolated		Duration
				Amount	Specific Activity	
Nicotinic amide-7-^{14}C	8 nodes	100 µl	5,350 mµc	850 mg	2.73 mµc/mmole	96 hours
Nicotinic acid-7-^{14}C	6 inches	10 µl	1,000 mµc	1.6 mg	6.27 mµc/mmole	96 hours

sample was added and the mixture macerated for 15 minutes in a Waring blendor. The mixture was filtered by vacuum through a coarse sintered glass funnel. The residue was returned to the blendor and one volume of chloroform was added and the mixture was reextracted for 15 minutes and again filtered using the same sintered glass funnel. To this combined extract was added 100 ml of NH_4OH:H_2O, 1:1. The mixture was shaken vigorously for one minute and the layers were allowed to separate. The chloroform layer which contained the ricinine was then transferred into a clean beaker and evaporated to dryness on a steam hot plate. The dried residue was transferred to a 40 ml round bottom centrifuge tube with ether and a glass stirring rod (with no rubber policeman since this contaminated the ricinine), and centrifuged for 15 minutes at 850 x g. The ether layer was decanted and discarded. The residue was repeatedly washed with ether until the washings were colorless. Most of the lipids and pigments were removed by this treatment. The residue was dissolved in hot distilled water and filtered through a medium porosity fritted glass funnel (micro). The solution was concentrated to a volume of about three ml and transferred to a sublimation vessel, dried and sublimed for two hours or until sublimation was complete (24).

About 1-3 mg of this purified ricinine was used for determining the specific radioactivity.

G. Estimation of the Amount of Nicotinic Acid in Castor Plants

1. Extraction of free nicotinic acid (8)

The plant was extracted with 80 per cent ethyl alcohol, using a weight ratio of 1:6, in a Waring blendor or Servall homogenizer. The macerated plant tissue was filtered in the same manner as was previously

described for ricinine. The extraction process was repeated and the combined filtrates were evaporated on the steam hot plate until most of the ethanol had been removed. The dark green water insoluble material which precipitated as the ethanol was evaporated was removed by centrifugation at 850 x g. The solution was diluted to 100 ml for liquid-liquid extraction and microbiological assay.

2. Extraction of total nicotinic acid

The castor plants were dried to a constant weight at 70°-80° C, which usually required 48-72 hours. The samples were pulverized, using a motar and pestle. This powder was hydrolyzed by autoclaving for one hour at 120° C and 15 psi in 1.0 N HCl using five ml of HCl per one g of dry sample. This treatment liberates nicotinic acid from the pyridine nucleotides. After filtration through Whatman No. 42 filter paper, the solution was made up to a volume of 100 ml for paper chromatography and microbiological assay.

3. Microbiological assay

A modification of the method of Snell and Wright (38) with Lactobacillus arabinosus ATCC 8014 was used for nicotinic acid analysis. The method employed the titration of the lactic acid produced by the organism after 72 hours with 0.1 N KOH. Aliquots of one, two or three ml of the solutions which were prepared for free or total nicotinic acid analysis according to parts 1 and 2 were neutralized with 6.0 N KOH and diluted to 100 ml. Suitable aliquots were taken so that at least three values agreed within ± five per cent of 0.15 μg/ml.

H. Measurement of Radioactivity of Nicotinic Acid

1. By liquid-liquid extraction (8)

Fifty ml of solution prepared in part G-1 was evaporated to about

twenty ml, and the solution was adjusted to pH 3.3 with 6.0 N HCl since nicotinic acid is soluble in ether at this pH. The solution was extracted with chemically pure anhydrous ether in a liquid-liquid continuous extractor for 48 hours. The extract was evaporated to dryness on the steam hot plate. The dry residue was taken up in acetone, and the acetone solution was saturated with HCl (gas) to precipitate nicotinic acid-HCl. If the nicotinic acid-HCl did not precipitate immediately, the HCl saturated solution was allowed to set overnight in the refrigerator. The precipitate was removed after centrifugation at 850 x g. The nicotinic acid-HCl was purified by sublimation at 110°-120° at a pressure of one mm Hg. The absorption maxima of the nicotinic acid isolated in this manner was 261 mμ. The specific activity of the nicotinic acid was determined using the liquid scintillation spectrometer.

2. Paper chromatography

The labeled castor plants were extracted twice with four volumes of 95 per cent ethanol. The solution was evaporated under vacuum to two ml. At this stage, most of the chlorophyll was removed. One hundred μl of solution was spotted on Whatman No. 1 paper, and descending paper chromatography was carried out. The strip was counted as before (see part C-1). The radioactivity in the total nicotinic acid was determined by applying the same method to the hydrolyzed sample.

3. Ion exchange column chromatography (39)

Twenty-five young plants grown in sand in the laboratory were extracted twice with 80 per cent ethanol and water (solvent:sample, 4:1). The extracts were evaporated under vacuum to ten ml, centrifuged, and the 80 per cent alcohol and water extracts were combined. Ten mg each of unlabeled nicotinic acid and trigonelline were added as carrier.

The solution was passed over a 20 x 3.2 cm^2 column of Dowex-1-formate, eight per cent cross-linked. The column was eluted with increasing concentrations of formic acid as follows:

(a) 150 ml of deionized distilled water

(b) 150 ml of 0.05 N HCOOH

(c) 150 ml of 0.10 N HCOOH

(d) 300 ml of 0.25 N HCOOH

(e) 150 ml of 1.0 N HCOOH

(f) 150 ml of 2.0 N HCOOH

(g) 300 ml of 4.0 N HCOOH

(h) 300 ml of 4.0 N ammonium formate

The flow rate was about one ml/minute. Aliquots of each ten ml fraction were assayed for absorbancy at 260 mμ. A 0.5 ml aliquot of each fraction was withdrawn for radioactivity determination. The fractions making up each ultraviolet absorption peak were combined and concentrated to 2-3 ml, then 1/5 to 1/2 of the total solution was spotted on Whatman No. 1 paper. After developing in 85 per cent isopropanol, the chromatogram was cut into one inch x two cm strips and counted in a Tri-Carb liquid scintillation spectrometer.

I. Measurement of Radioactivity in Respiratory CO_2 From Castor Plant

Carbon-14 labeled ricinine was injected into a castor plant which was immediately placed in a dark, closed system in order to prevent photosynthesis and promote catabolism. Carbon dioxide free air was passed through the system and the respiratory CO_2 was trapped in ethanolamine-methylcellosolve for 28 hours.

CHAPTER IV

RESULTS AND DISCUSSION

A. Turnover of Ricinine in the Castor Plant

The biodegradation of ricinine-^{3}H in the intact castor plant was the subject of a preliminary investigation by Scotts, Goldberg and Waller (40). They demonstrated that all of the radioactivity in ricinine formed from nicotinic acid-7-^{14}C or of ricinine-^{3}H administered in a manner similar to that employed in the experiment reported here, disappeared or reached a negligible level in 70-90 days. It was desirable to confirm and to extend these findings.

Plants from Experiment 1 (Table I) were used in this study. Ricinine samples were isolated from the whole plant, from zero time to one week later, and their specific activities were determined. At this stage a significant amount of flowers and immature seed were beginning to appear and therefore plants were divided into three parts for ricinine analysis, i.e. leaf-stem, flower and seed. Plants obtained from the second week to the thirteenth week were handled in a similar manner so that the radioactivity present in the ricinine of these fractions could be compared directly. The root was not included in this experiment due to the fact that the ricinine content is negligibly small, i.e. about 0.72 per cent of the alkaloid (19). Furthermore, when the plant has grown larger than the ten nodal stage, the root becomes too hard to be handled practically.

Two methods were used on the zero time samples (Table III) to measure the recovery of ricinine-^{3}H. Ricinine-^{3}H was added to the sample after maceration in the blendor for ten minutes. Eighty-five per cent of the added activity was recovered in the ricinine fraction. The 15 per cent loss seemed to be due to technique and the extraction procedure in which the ricinine was slightly soluble in the ether layer and ammonium hydroxide aqueous solutions that had been discarded. Sample-600 was frozen in dry ice immediately after injecting the ricinine-^{3}H. Only 75 per cent of the radioactivity could be recovered. The additional ten per cent loss may have been due to the macerating machine efficiency, but the possibility that some ricinine was conjugated with protein in the plants so that it could not be extracted with chloroform can not be eliminated.

Table III shows the recovery of ricinine-^{3}H from castor plants the first week following administration of the isotope. The total loss of radioactivity during this period was 20 per cent of that added. There was at least a two-fold decrease in the specific activity of the ricinine, indicating that alkaloid synthesis was continually occurring.

In Figure 1 it can be seen that the total activity of ricinine increased in the seed but decreased in the leaf-stem and flower fractions. This indicated that ricinine can be transported from the other tissues to the seed. On the other hand, after nine weeks, both the specific activity and quantity of ricinine in the seed was greater than that of the other parts (Figures 2 and 3). This might indicate that ricinine was metabolically inactive and almost unexchangeable in the seed. This metabolic inactivity of ricinine in the seed is one reason why the rate of ricinine metabolism in the whole plant was faster in the earlier

TABLE III

RECOVERY OF RICININE-^{3}H FROM WHOLE CASTOR PLANTS

Plant No.	Experiment Duration (hours)	Dry Weight (gram)	Ricinine (mmole)		Ricinine/Dry Weight (mmole/gram)		Specific Activity (mμc/mmole)		Total Activity (mμc)		Recovery Per Cent	
			Part	Total	Part	Total	Part	Average	Part	Total	Part	Total
CI-601A	0	15.30	---	0.404	---	0.0264	---	1928	---	778	---	87.5
CI-601B	0	15.00	---	0.298	---	0.0199	---	2470	---	734	---	82.5
CI-600A	0	13.24	---	0.428	---	0.0323	---	1600	---	685	---	77.0
CI-600B	0	24.33	---	0.815	---	0.0335	---	796	---	650	---	73.0
CI-606	8	21.00	---	0.670	---	0.0319	---	878	---	589	---	66.2
CI-608	26	26.00	---	0.900	---	0.0346	---	613	---	552	---	62.0
CI-609	48	21.20	---	0.700	---	0.0329	---	732	---	512	---	57.5
CI-611	168 (one week)	42.20	---	1.158	---	0.0274	---	429	---	497	---	55.8

TABLE III (CONTINUED)

Plant No.	Experiment Duration (week)	Dry Weight (gram)	Ricinine (mmole) Part	Ricinine (mmole) Total	Ricinine/Dry Weight (mmole/gram) Part	Ricinine/Dry Weight (mmole/gram) Total	Specific Activity (mμc/mmole) Part	Specific Activity (mμc/mmole) Average	Total Activity (mμc) Part	Total Activity (mμc) Total	Recovery Per Cent Part	Recovery Per Cent Total
CI-612	2			1.978		0.1135		228.2		452.4		50.84
leaf-stem		72.9	1.680		0.0231		240.0		404.0		45.40	
flower		2.3	0.122		0.0530		239.0		29.2		3.28	
seed		4.7	0.176		0.0374		109.5		19.2		2.16	
CI-613	3			1.807		0.1217		193.0		346.2		38.90
leaf-stem		50.6	1.080		0.0214		219.0		237.0		26.60	
flower		13.3	0.204		0.0616		130.0		26.6		3.00	
seed		13.5	0.523		0.0387		158.0		82.6		9.30	
CI-614	4			1.340		0.0874		236.0		316.2		35.58
leaf-stem		42.2	0.704		0.0167		282.5		199.0		22.40	
flower		1.8	0.061		0.0339		216.0		13.2		1.48	
seed		15.6	0.575		0.0368		181.5		104.0		11.70	
CI-615	5			2.560		0.0892		112.4		287.9		34.80
leaf-stem		79.4	1.166		0.0148		126.8		147.5		16.60	
flower		5.3	0.206		0.0390		116.0		2.4		2.70	
seed		33.6	1.188		0.0354		116.0		138.0		15.50	
CI-617	7			3.194		0.0737		55.8		178.00		19.98
leaf-stem		88.7	1.129		0.0127		41.60		47.00		5.28	
flower		3.7	0.095		0.0256		65.50		6.20		0.70	
seed		55.8	1.970		0.0354		63.30		124.80		14.00	
CI-619	9			2.972		0.0866		57.0		168.95		18.99
leaf-stem		137.5	1.186		0.0087		32.80		39.00		4.38	
flower		2.7	0.101		0.0375		19.23		1.95		0.22	
seed		41.7	1.685		0.0404		75.88		128.00		14.40	
CI-621	11			4.264		0.1283		39.8		169.88		19.08
leaf-stem		114.5	1.330		0.0116		21.35		28.40		3.19	
flower		3.3	0.274		0.0831		12.70		3.48		0.39	
seed		79.0	2.660		0.0336		52.00		138.00		15.50	
CI-623	13			6.678		0.0999		30.0		200.50		22.49
leaf-stem		178.0	1.665		0.0093		12.70		21.18		2.38	
flower		6.7	0.313		0.0468		7.41		2.32		0.26	
seed		107.4	4.700		0.0438		37.60		177.00		19.85	

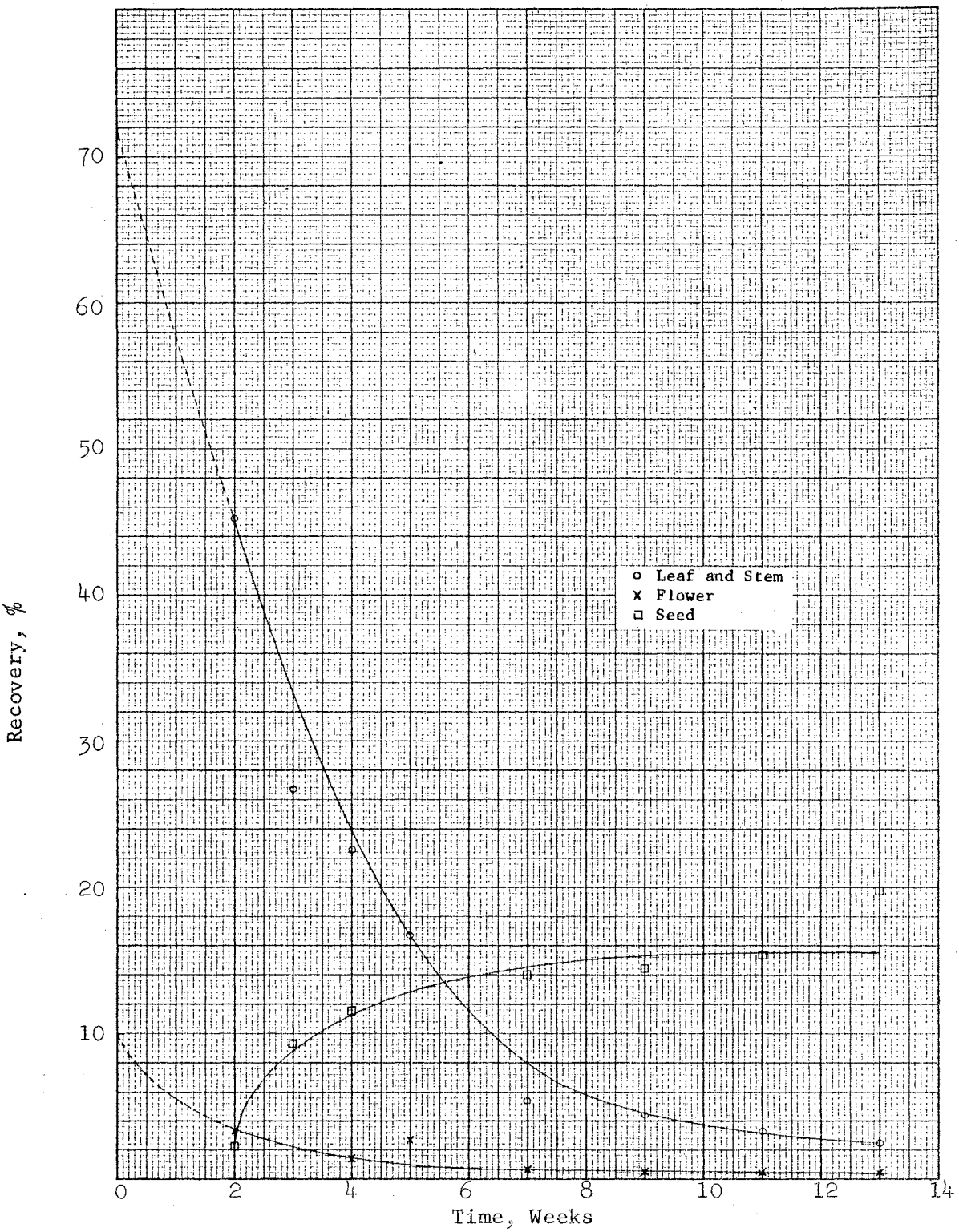

Figure 1. Comparison of the Rate of Disappearance of Ricinine in Different Parts of the Castor Plant.

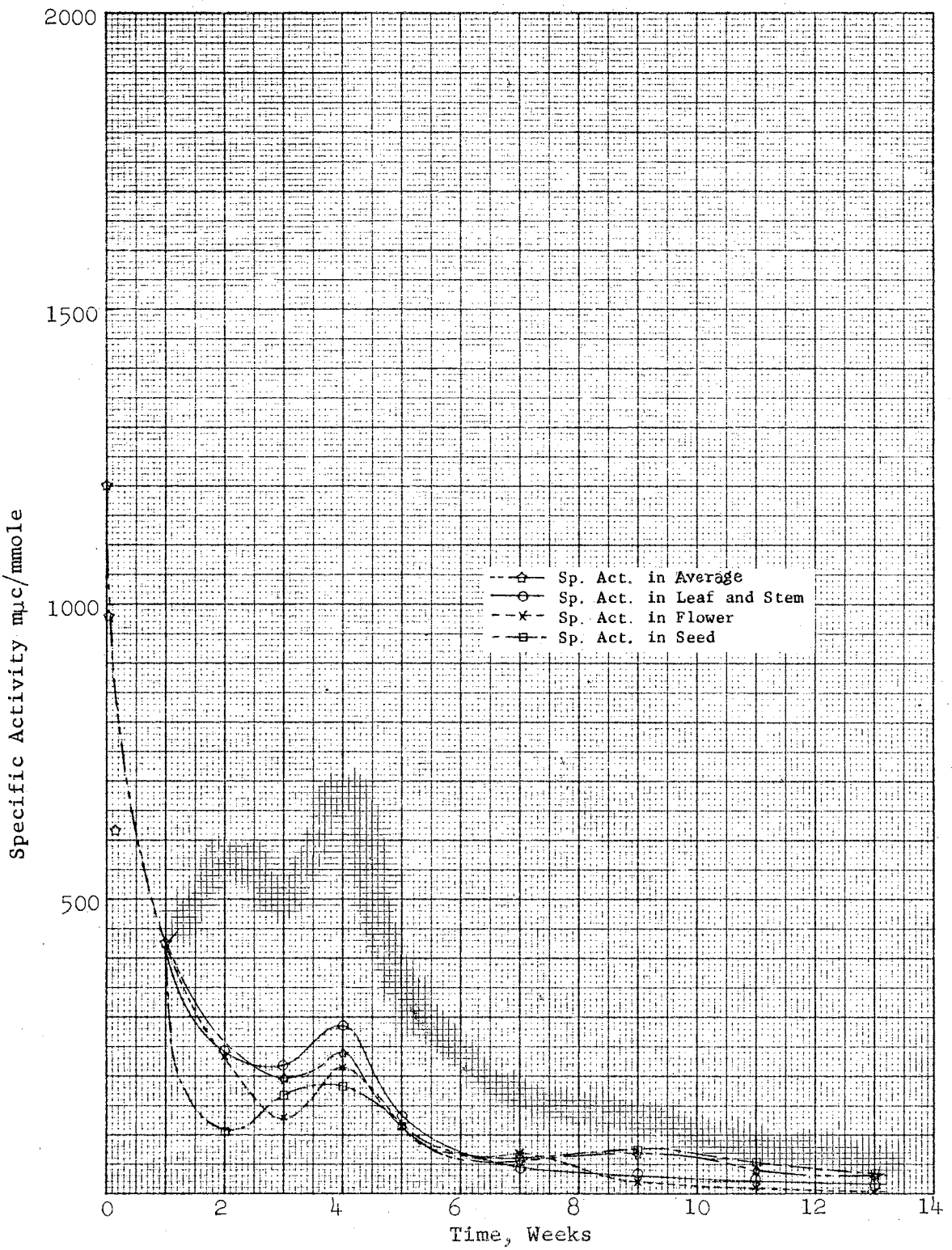

Figure 2. The Specific Activity of Ricinine in Different Parts of the Castor Plant During Development.

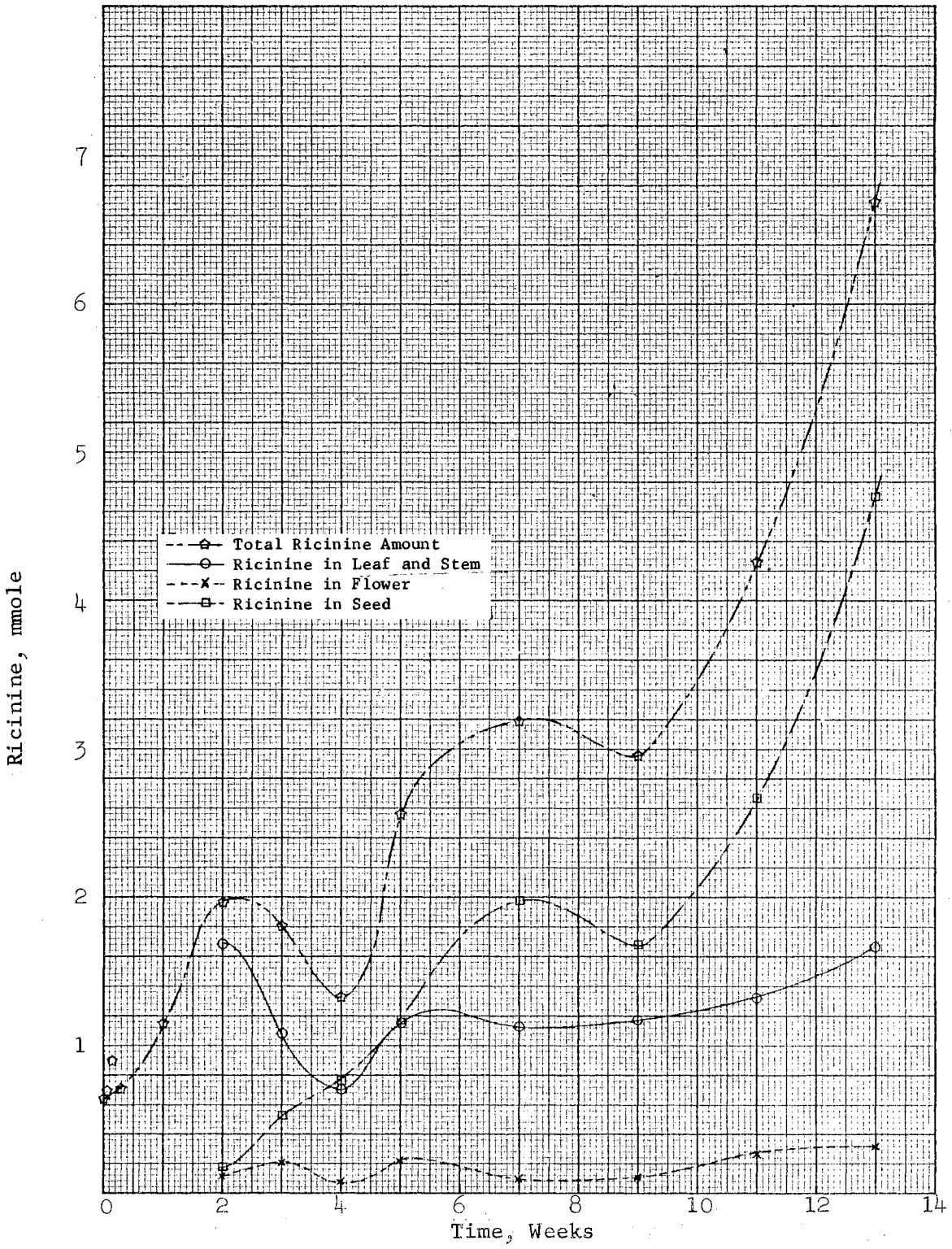

Figure 3. The Rate of Ricinine Formation in Different Parts of the Castor Plant

period, and slowed down later, especially after seven weeks (Figure 4). This can also be seen in Figure 3 which shows that the total amount of ricinine in seeds increased while the ricinine content of leaves and stems increased only up to two weeks and then remained constant. The ricinine content of the flowers remained constant through the whole experimental period. After five weeks, the content of ricinine in the seed was greater than that in the other tissues. However, when compared on a percentage basis, the flower contained about 0.85 per cent ricinine, which was the highest, on a moisture free basis. As can be seen in Table III, the rate of ricinine production in the flower was less constant than in the seed. The ricinine content of leaves and stems showed a decrease during the period of fruiting, i.e. from the second week (Figure 5). This finding also supports the idea that the alkaloid was formed in the younger parts of the plant.

There is a question concerning the source of ricinine in the seed. From the data discussed above, it is clear that translocation of ricinine from other parts of the plant occurred continually throughout the course of this experiment as shown by the increased total activity in the seed. Nevertheless, since the quantity of ricinine contained in the seed was large and increased significantly and its specific activity was greater than in other parts and the total activity of ricinine in the flowers, leaves and stems decreased very slowly after seven weeks, it seems unlikely that the increase in alkaloid in the seed was due solely to translocation from other tissues. In other words, ricinine was also synthesized by the developing seed.

In Figure 1 the rate of ricinine decrease in flowers, and leaves and stems was extrapolated to zero time in order to estimate the half

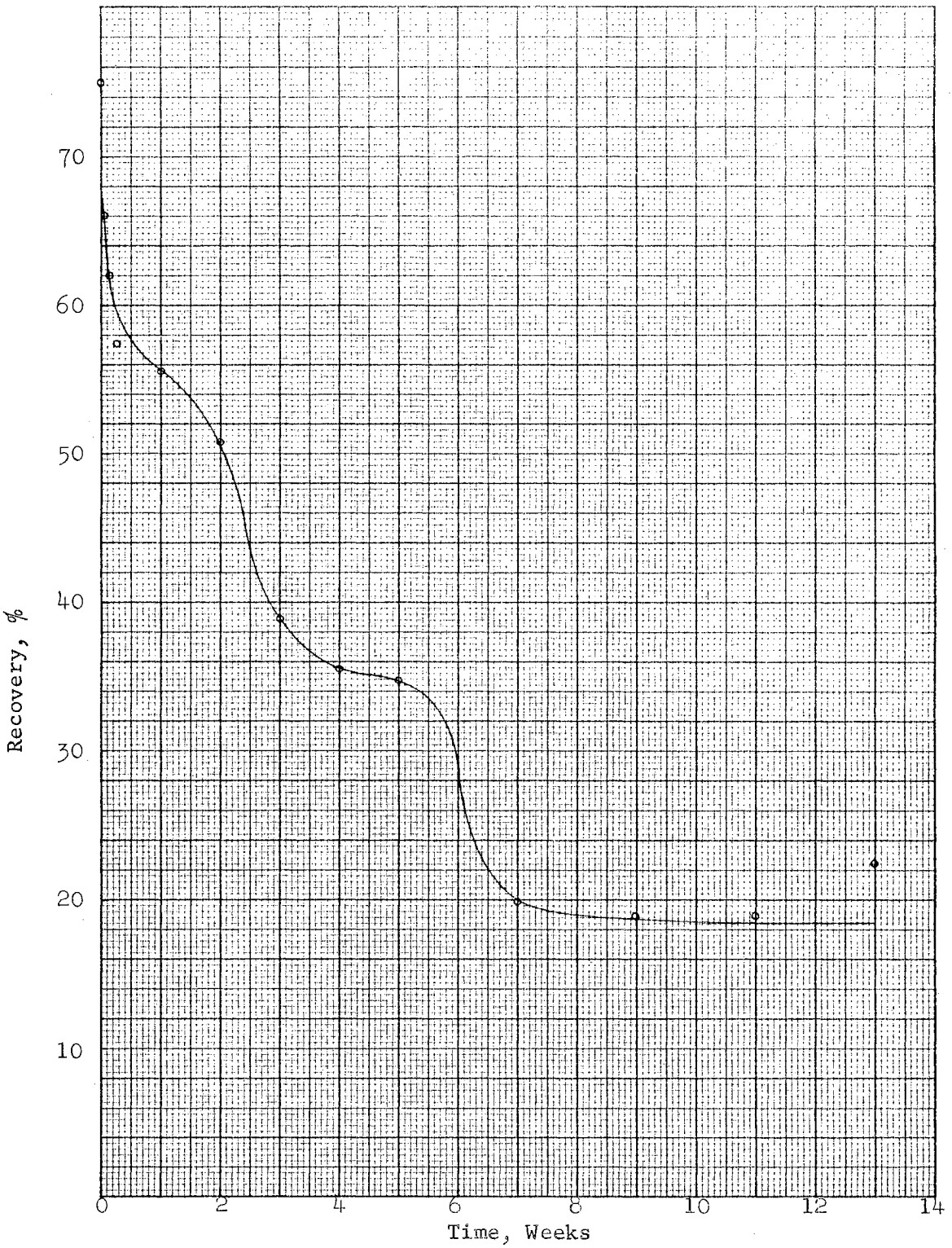

Figure 4. Rate of Disappearance of Ricinine-^{3}H From the Ricinine Pool of the Castor Plant.

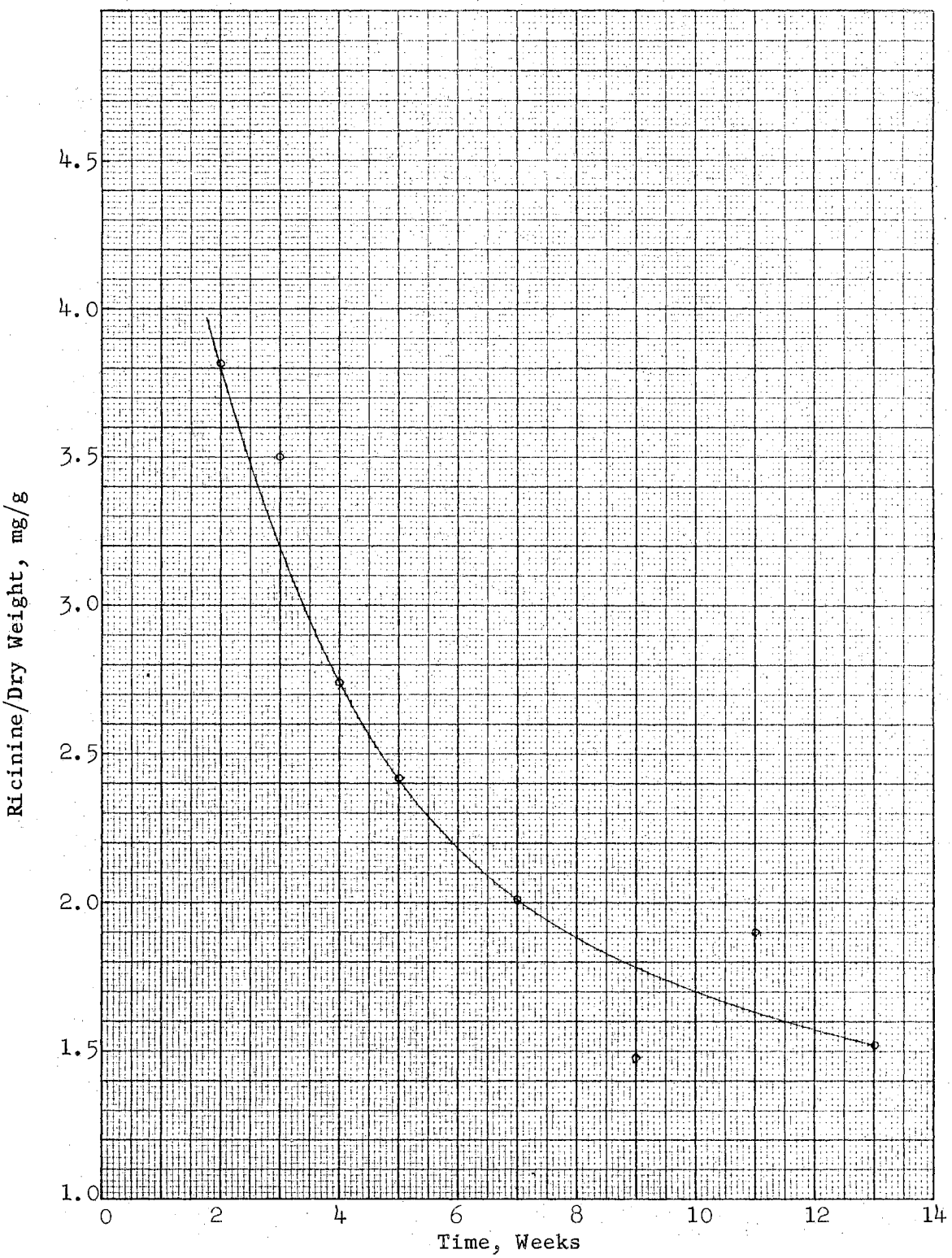

Figure 5. The Decrease in Ricinine Content of Leaves and Stems After Seed Formation.

life of ricinine in these tissues. This time was about twenty days for the leaf and stem, and 7.0 days for the flower, which indicates that the rate of ricinine metabolism in the flower is faster than in the leaf and stem.

B. The Relationship Between Ricinine and Nicotinic Acid in Whole Castor Plants

1. Ricinine and free nicotinic acid

Plants from Experiment 2 (Table I) were used in this study. After injecting ricinine-^{3}H, it was found that the amount of free nicotinic acid increased and reached a maximum at 48 hours, after which time it decreased. Forty-eight hours after injection of the ricinine, the nicotinic acid content was 68.5 per cent higher than it was 15 minutes after injection (Table IV).

The free nicotinic acid was assayed for tritium but no radioactivity could be detected in any sample.

2. Ricinine and total nicotinic acid

Plants from Experiment 3 (Table I) were used in this study. Figure 6 shows that the influence of ricinine on total nicotinic acid was almost identical with its effect on free nicotinic acid. Total nicotinic acid content increased up to 96 hours, and then declined at 96 hours. It was 18.9 per cent higher than at 15 minutes (Table V).

When the free nicotinic acid value is substrated from the total nicotinic acid value, a difference curve results (Figure 6) which shows that the main increase in nicotinic acid in the castor plant was due to free nicotinic acid and not to nicotinic acid in a bound form, such as DPN or TPN.

In order to compare total nicotinic acid with that in the uninjected

TABLE IV

THE EFFECT OF ADDED RICININE ON THE CONTENT OF FREE NICOTINIC ACID IN WHOLE CASTOR PLANTS

Plant No.	Physiological Stage (nodal)	Experiment Duration (hours)	Dry Weight (gram)	Free Nicotinic Acid (μgm)	Free Nicotinic Acid/ Dry Weight (μgm/gm)
1	7	1/4	4.9	189	38.6
2	8	1/2	6.5	268	40.8
3	6	1	3.5	185	52.1
4	7	2	5.4	265	49.0
5	7	12	5.4	296	54.6
6	7	24	4.4	271	61.5
7	6	48	3.8	247	65.0
8	8	96	6.3	332	52.6
9	9	144	6.3	252	40.0
10	10 (flowering)	168	8.9	368	41.3
11	12 (flowering)	336	19.0	666	35.0

TABLE V

THE EFFECT OF ADDED RICININE ON THE CONTENT OF TOTAL NICOTINIC ACID IN WHOLE CASTOR PLANTS

Plant No.	Physiological Stage (nodal)	Experiment Duration (hours)	Dry Weight (gram)	Total Nicotinic Acid (μgm)	Total Nicotinic Acid/ Dry Weight (μgm/gm)
1	8	1/4	8.61	539	62.5
2	8	1/2	7.00	438	62.6
3	8	1	6.50	416	64.0
4	9	2	10.30	707	68.6
5	9	8	13.90	956	68.8
6	7	12	5.80	415	71.6
7	9	24	9.30	642	69.1
8	8	48	6.10	439	72.0
9	7	96	5.80	431	74.3
10	10	144	12.30	830	67.5
11	8	168	6.20	341	55.0
12	11 (flowering)	336	14.60	678	46.4

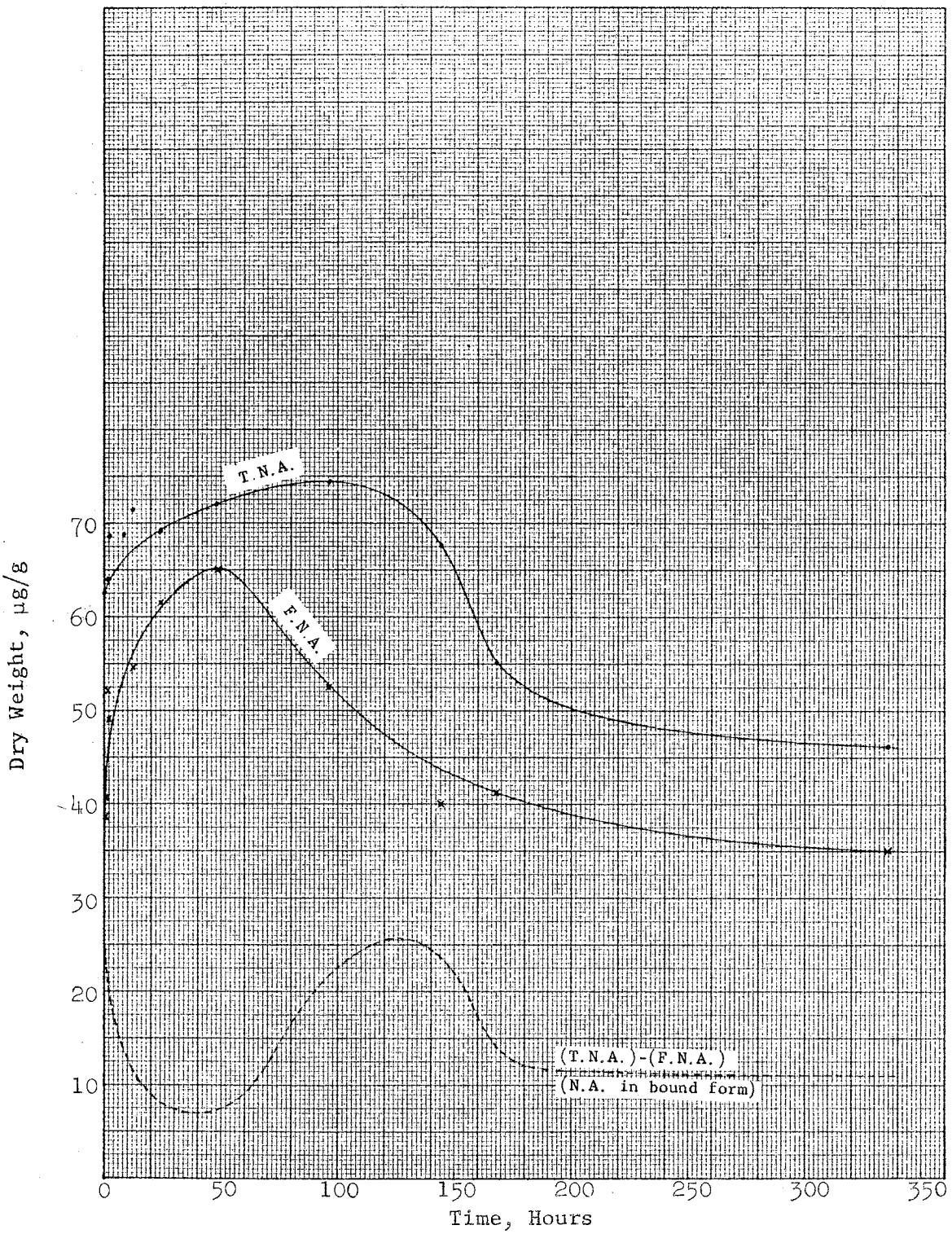

Figure 6. The Influence of Ricinine on the Content of Free and Total Nicotinic Acid in the Whole Castor Plants.

plants, the findings of Waller et al. (40) are shown in Table VI. It appears that total nicotinic acid increased with age but that the relative content decreased after the six nodal stage. However, the relative content of total nicotinic acid in castor plants after administration of ricinine increased up to 96 hours, then decreased (Table V).

Aliquots of the total nicotinic acid solution and of the 95 per cent alcohol extract were subjected to paper chromatography. The only radioactive spot found corresponded to ricinine.

3. Isolation of nicotinic acid and trigonelline

Plants grown in the laboratory (Experiment 4, Table I) were used for this experiment. No carbon-14 radioactivity could be detected in the isolated nicotinic acid and trigonelline.

It can be concluded that ricinine may have a stimulating effect on nicotinic acid production, although neither nicotinic acid nor trigonelline could be detected as metabolites of ricinine.

C. In Vivo Conversion of Ricinine-8-^{14}C to $^{14}CO_2$ by the Castor Plant

The loss of total activity of ricinine-^{3}H would indicate that this alkaloid can be metabolized by the castor plant. However, the loss of activity might be due to an exchange of tritium from the ricinine-^{3}H with hydrogen in the plant cells. Ricinine might also be excreted into the soil; however, several attempts were made to obtain ricinine from the soil without success.

If ricinine is not an end product of metabolism then the possibility exists that it might be degraded to CO_2. Consequently an experiment was performed to test this hypothesis. The results showed that approximately 2.5 per cent of the radioactivity from ricinine-8-^{14}C was lost as

TABLE VI

TOTAL NICOTINIC ACID CONTENT OF CASTOR PLANTS

Sample No.	Harvest Date	Physiological Stage (nodal)	Total Nicotinic Acid	
			Amount/Plant (mg)	µg/gm Dry Weight
CB-60	--	Younger than 6 nodal stage	0.100	140
CB-61	--	Younger than 6 nodal stage	0.255	120
CB-62A	6/26/61	6	0.438	595
CB-62	6/27/61	7	0.235	462
CB-63	6/28/61	7	0.583	503
CB-64	6/29/61	8	0.607	318
CB-65	6/30/61	8	0.730	388
CB-66	7/3/61	9	1.160	---
CB-67	7/10/61	11 (flowering)	2.520	---

respiratory $^{14}CO_2$ after 28 hours (Table VII). This clearly indicates that ricinine can be metabolized by the castor plant, but that it is a slow process. The route of formation of $^{14}CO_2$ is not known. It probably was not formed by a direct cleavage of the nitrile group from ricinine since in vitro experiments (41) failed to yield any N-methyl-4-methoxy-2-pyridone. It is possible that ricinine may be broken down into small molecules by a pathway excluding nicotinic acid and trigonelline.

TABLE VII

$^{14}CO_2$ RELEASED BY THE CASTOR PLANT AFTER ADMINISTRATION OF RICININE-8-^{14}C

Experiment No.	Volume Injected (μl)	Quantity Injected (mμc)	CO_2 Activity (mμc)	Per Cent of ^{14}C Converted to $^{14}CO_2$	Duration (hours)
A	50	5.0	0.14	2.80	28
B	75	7.5	0.19	2.54	28

CHAPTER V

SUMMARY

Tritium and carbon-14 were used as tracers to study the metabolism of ricinine in castor plants.

When tritium-labeled ricinine was injected into young castor plants, the total radioactivity recovered in the ricinine fraction was found to decrease with increasing experiment duration. Alkaloid synthesis was continually occurring and the site of synthesis was in the young parts of the plant as suggested by the observation that rate of ricinine metabolism in flowers was faster than in leaves and stems.

It was also observed that ricinine can be transported from the leaves, stems and flowers to the seeds. However, the major portion of the alkaloid of the seed was synthesized in the seed. Ricinine in the seed was shown to be very inactive and almost unexchangeable.

Injection of ricinine appeared to stimulate an increase in both free and total nicotinic acid. However, the main increase in nicotinic acid in the castor plant was due to free nicotinic acid and not to nicotinic acid in a bound form such as DPN or TPN.

The only radioactive spot detected on paper chromatography of both the "free nicotinic acid" and "total nicotinic acid" extracts corresponded to ricinine.

Following injection of ricinine-8-^{14}C into the castor plant no radioactivity could be detected in the isolated nicotinic acid and

trigonelline. However, respiratory $^{14}CO_2$ was released by the castor plant after administration of ricinine-8-^{14}C.

From these data, it is concluded that ricinine can be metabolized to CO_2 by the castor plant through an unknown pathway which may not include nicotinic acid and trigonelline.

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