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FINAL REPORT

Aqueous Photodegradation of
¹⁴C-Rotenone

Prepared for:

U.S. Department of the Interior
Fish and Wildlife Service
National Fishery Research Laboratory
Prime Contract #14-16-0009-81-042

Prepared by:

Biospherics Incorporated
4928 Wyaconda Road
Rockville, Maryland 20852

October 1982

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10/26/82

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10/26/82

Project No.: 82-E-076-P
Protocol No.: EC-87
EPA Contract No.: 14-16-0009-81-042

Amended to define complete degradation of Rotenone. W.C. Spare 1/4/83
W.C. Spare

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ABSTRACT

Rotenone was studied to determine its photolytic rate constants and half-life as well as its photodegradation products in aqueous solution exposed to artificial sunlight. Rotenone was found to photolyze very rapidly in aqueous solution with a half-life much shorter than 30 days. Half-life calculations yielded a 1.4 hour half-life and rate constant of 0.489 when exposed to light at an intensity of 13,500 $\mu\text{W}/\text{cm}^2$ at 22°C. The expected complete degradation of Rotenone would be 10 half-lives or approximately 14 hours in this reactor. Degradation resulted in at least 2 photolysis products one of which chromatographed at an R_f of 0.595 as opposed to Rotenone R_f of 0.749 by single dimensional TLC in benzene:methanol (9:1) solvent system. The second photolysis product was unextractable from the aqueous buffer and did not chromatograph by TLC.

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1.0 PURPOSE

The purpose of this study was to determine the photolysis rate constants, half-life calculations and photolysis products of ^{14}C -Rotenone^a in aqueous solution.

2.0 SUMMARY

^{14}C -Rotenone was added to an aqueous buffer solution at a concentration of approximately 1.0 ppm with final pH of 7.16. Approximately one-half of the test solution was placed in the photolysis reactor which was equipped with a 450 watt U.V. light with a 280 nm and lower cut-off sleeve in a quartz immersion well surrounded by two water jackets, which kept the system isothermal at 22°C. The other one-half of the dosed test solution was placed in a 1 liter bottle which was covered with aluminum foil and kept at room temperature, to determine if appreciable hydrolysis of the test chemical took place in a dark reactor. Duplicate aliquots were removed according to a predetermined schedule from each reactor and the amount of rotenone was determined by radiochemical and autoradiographic methods.

Three photolysis runs were performed. The first two runs established a half-life for Rotenone of less than 24 hours and enabled perfection of the extraction technique. The third run was the definitive run and is reported.

3.0 METHODS

3.1 Test Chemical

Rotenone and Rotenone-6 α - ^{14}C were received from Litton Bionetics for conduct of the study. Rotenone (25 g) was analytical grade (97% purity) manufactured by Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin

^a ^{14}C -Rotenone = Rotenone-6 α - ^{14}C , specific activity 5.28 mCi/mM.

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with lot number 100287 and catalog number R200-1. Rotenone-6 α -¹⁴C was synthesized by Pathfinder Laboratories Incorporated, St. Louis, Missouri with lot number 820209. Approximately 1.6 mCi crystalline Rotenone-6 α -¹⁴C at a specific activity of 5.28 mCi/mM and 394.16 molecular weight was received. Pathfinder Laboratories confirmed chemical and radiochemical purity by liquid chromatography.

A stock solution was prepared by dissolving ¹⁴C-Rotenone in acetonitrile to achieve a final concentration of 10.0 mg/ml. To each liter of buffer solution, 100 μ l of stock solution was added to yield a final concentration of approximately 1.0 ppm. Buffer solutions were sterilized by autoclaving at 15 lb pressure for 30 minutes at 121°C and cooled to room temperature before rotenone addition. Buffer solutions were additionally filtered through a 0.45 μ m Millipore filter before use.

3.2 Test System

The pH 7.16 buffer was prepared with 33 ml of 0.067M NaH₂PO₄ mixed with 67 ml of 0.067M K₂HPO₄ and diluted 10x. Photolysis solutions were sampled according to the following schedule:

<u>Sample</u>	<u>Photolysis Period</u> <u>Hours</u>
1	0
2	0.25
3	0.50
4	0.83
5	2.0
6	4.0
7	8.0
8	24.0

The loss of the chemical with respect to time was monitored by partitioning the samples with methylene chloride and quantification of the test compound by thin layer chromatography (TLC) and autoradiography. Loss of radioactivity from the photolysis chamber was monitored by means of an ethylene glycol trap which was monitored at the 0, 2, 4 and 24 hour sampling intervals.

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3.3 Sample Extraction and Identification

At each sampling period, duplicate 10 ml aliquots of each test solution, irradiated and control, were collected and the radioactivity partitioned into methylene chloride after the addition of 1.0 ml of saturated sodium chloride solution. Subsamples of methylene chloride extract were deposited on TLC plates (Merck precoated silica gel plates 60F-254) and developed in 9:1 benzene:methanol (TLC #1) or 5:1 ethylacetate (saturated with 10% ammonium hydroxide):methanol (TLC #2). After development, non-radioactive spots were visualized under UV light at 254 nm. Radioactive spots were determined by autoradiography.

Confirmation of parent was performed by comparative R_f to supplied standards. The retention factor and relative retention factor of photodegradation products were determined.

3.4 Artificial Sunlight Source

The light spectrum approximated natural sunlight. Light from the lamp (450 watt UV lamp, Ace Glass Company) was filtered through a pyrex glass sleeve which removed all wavelengths 280 nm and lower. The exposed solution was $\frac{1}{2}$ to 1 inch in depth. The exposure intensity of the lamp in the photolysis reactor was $13,500 \mu\text{W}/\text{cm}^2$ (Blak Ray Longwave Ultraviolet meter - Ultraviolet Products, Incorporated, San Gabriel, California). Natural sunlight on a clear day during the summer months at the Biospherics Laboratories provides an intensity of $4700 - 4900 \mu\text{W}/\text{cm}^2$. Thus our lamp intensity was approximately three times natural sunlight. The exposure solution received light directly from the lamp which was approximately $\frac{1}{2}$ inch from the solution.

3.5 Radioactive Analysis

All radioactive spots were scraped from the plates and quantified. Radioactivity in ^{14}C -volatiles trap was determined.

All measurements of ^{14}C were made with a Beckman LS-230 liquid scintillation counter (LSC). TLC scrapings and samples from all volatiles traps were mixed with 18 ml of Maxifluor (Baker) and counted directly.

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All counting was done for 5 minutes, two 5 minute counting channels per sample. Efficiency was computed for each sample using external standard channel ratio values and curve fitting to previously established quench standard sample data.

4.0 CALCULATIONS

Assuming pseudo first order reactions, the photolytic rate constant can be calculated from the following equation:

$$\ln C = -kt + \ln C_0 \quad (y = mx + b)$$

where k = rate constant

C = chemical concentration

t = time (hours)

C₀ = initial concentration

The half-life of the test chemical is calculated by the following equation:

$$T_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

5.0 RESULTS AND DISCUSSION

The radiocarbon balance from aqueous photodegradation of ¹⁴C-Rotenone for exposed and unexposed solutions at seven time periods is presented in Table 1. Volatile traps (ethylene glycol) yielded no volatile products greater than 1% of the dose up to the 4 hour sampling interval. Half-life calculation of the photodegradation of ¹⁴C-Rotenone is presented in Table 2. Calculations indicated a 1.4 hour photolysis half-life and rate constant of 0.489 from exposure of ¹⁴C-Rotenone to artificial sunlight at an intensity of 13,500 μW/cm² and at a temperature of 22°C. The expected complete degradation of ¹⁴C-Rotenone would be 10 half-lives or approximately 14 hours in this reactor. The plot of the natural log concentration vs time is presented in Figure 1.

Parent molecule and photodegradation were monitored on two TLC systems. However, TLC system #1, benzene:methanol (9:1), was the system used in half-life calculation and all tabular data since TLC system #2

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did not adequately separate ^{14}C -Rotenone from its photodegradation products. The average R_f for Rotenone and its major metabolite were 0.756 and 0.595 respectively (Table 3).

The various TLC components were monitored as a function of time (Table 4). From this data the minor constituents which develop in the remainder portion of the TLC plate accounted for up to 16.4% of the dose. These constituents appear as two or three faint spots on the x-ray films and could not be accurately quantitated. Copies of the x-ray films are presented in Figures 2 and 3.

The aqueous fraction of the partition retained significant radioactivity in the irradiated samples e.g., 37.9% of the dose at 4 hours implying that major photodegradation products were polar and not extractable into the organic layer. To further support this conclusion the concentration of radioactivity in the aqueous layer increased as a function of time as presented in Table 1. In order to attempt identification or characterization of these metabolites aliquots of the 24 hour - exposed partitioned aqueous samples were spotted and developed by TLC. The radioactivity remained at the origin and no other major spots were observed (Figure 4).

6.0 PROTOCOL DEVIATION

Due to rapid photolysis of rotenone in the study the exposure period was shortened from 30 days to 24 hours with the sampling times adjusted accordingly.

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TABLE 1

Radiocarbon Balance from Aqueous Photodegradation
of ¹⁴C-Rotenone

Sample Time (hours)	MeCl ₂	% of Dose Recovered ^a		Total % Dose Recovered
		Aqueous	Ethylene Glycol Trap	
0 Dark	98.1	1.9	< 1.0	100
0.25	94.9	8.8		103.7
0.50	91.4	14.3		105.7
0.83	83.4	17.8		101.2
2	68.3	32.8	< 1.0	101.1
4	57.5	37.9	< 1.0	95.4
8	b	b		
24	29.8	60.9	2.6	93.3
24 Dark	100.8	5.0		105.8

Average recovery for light samples = 100.1, standard deviation ± 4.8.

Average recovery for dark samples = 102.9, standard deviation ± 4.1.

^aDose calculated based on 0 time total DPM = 438435 DPM/10 ml.

^bLab accident, sample lost.

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TABLE 2

Half-life Calculation of Aqueous
Photodegradation of ^{14}C -Rotenone

% of Initial Radioactivity as Rotenone

<u>Sample Time (X) (hours)</u>	<u>% Sample Extractable in MeCl_2</u>	<u>% Parent Recovered by TLC</u>	<u>Corrected for % Extractable</u>	<u>ln Corrected % (y)</u>
0	101.2	88.2	89.3	4.49
0	95.1	89.8	82.4	4.41
.25	98.5	68.1	67.1	4.21
.25	91.2	66.6	60.7	4.11
.50	91.6	62.3	57.1	4.04
.50	91.1	61.6	56.1	4.03
.83	84.0	55.2	46.4	3.84
.83	82.7	56.1	46.4	3.84
2.0	68.3	35.8	24.5	3.20
2.0	68.2	36.1	24.6	3.20
4.0	57.5	19.4	11.2	2.42
4.0	57.6	19.8	11.4	2.43

Best line obtained from least squares calculation.

Correlation = -0.9907

Slope = $-k = -0.4891$

Intercept = 4.303

Rate Constant = $k = 4.89 \times 10^{-1}$

Half-life = $t_{1/2} = 1.4$ h

Reactor Temperature = 22°C

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TABLE 3

TLC Retention Factors and Relative Retention Factors
for Rotenone and its Photodegradation Product in
Benzene:Methanol (9:1 v:v) Solvent System

<u>Sample Number</u>	<u>Time (hours)</u>	<u>Rotenone (R)</u>	<u>Degradation Product (DP)</u>	$\frac{R_f \text{ DP}}{R_f \text{ R.}} (rR_f)$
1	0	0.782		
2	0	0.774		
3	0.25	0.758		
4	0.25	0.762		
5	0.50	0.764	0.626	0.819
6	0.50	0.768	0.634	0.826
7	0.83	0.777	0.657	0.846
8	0.83	0.784	0.670	0.855
9	2.0	0.738	0.579	0.785
10	2.0	0.732	0.562	0.768
11	4.0	0.719	0.544	0.757
12	4.0	0.719	0.545	0.758
17	24.0		0.560	
18	24.0		0.570	
19	24.0 Dark	0.749		
20	24.0 Dark	0.762		
Mean		0.756	0.595	0.802
% Std. Dev. \pm		2.9	8.0	4.9
Var.		4.4×10^{-4}	2.0×10^{-3}	1.4×10^{-3}

TABLE 4

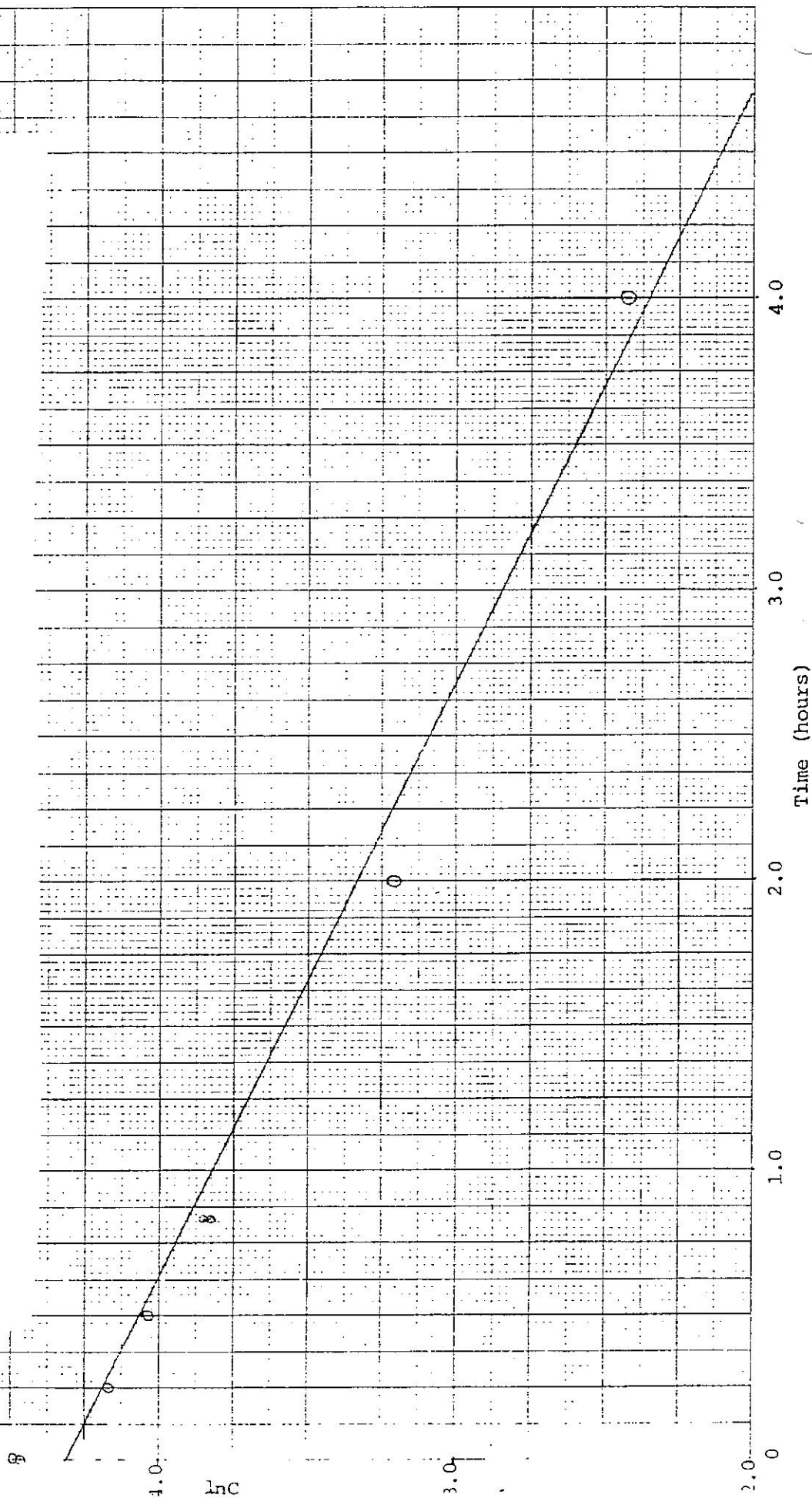
% TLC Component vs Time (hr)
 (Corrected for % Extractable)
 Solvent System: Benzene:Methanol (9:1 v:v)

TLC Component	Time in Hours													
	0		0.25		0.50		0.83		2.0		4.0		24.0	
Origin	<3	<3	3.4	<3	3.4	3.3	3.2	3.0	6.0	5.2	4.7	4.9	2.0	2.0
Remainder	5.6	6.0	15.2	15.4	16.3	16.4	15.6	14.9	16.2	16.0	15.4	15.2	7.9	7.6
Major Degradation Product	4.9	3.1	11.6	14.5	14.8	15.4	18.6	18.6	21.7	22.5	26.8	26.1	17.1	16.6
Rotenone	87.8	89.0	64.6	65.0	56.9	56.3	46.0	46.8	24.5	24.7	11.2	11.4	<1.5	<1.5

Figure 1 - Plot of lnC vs Time (hours)

C = Concentration corrected to % of
initial radioactivity as ¹⁴C-Rotenone.

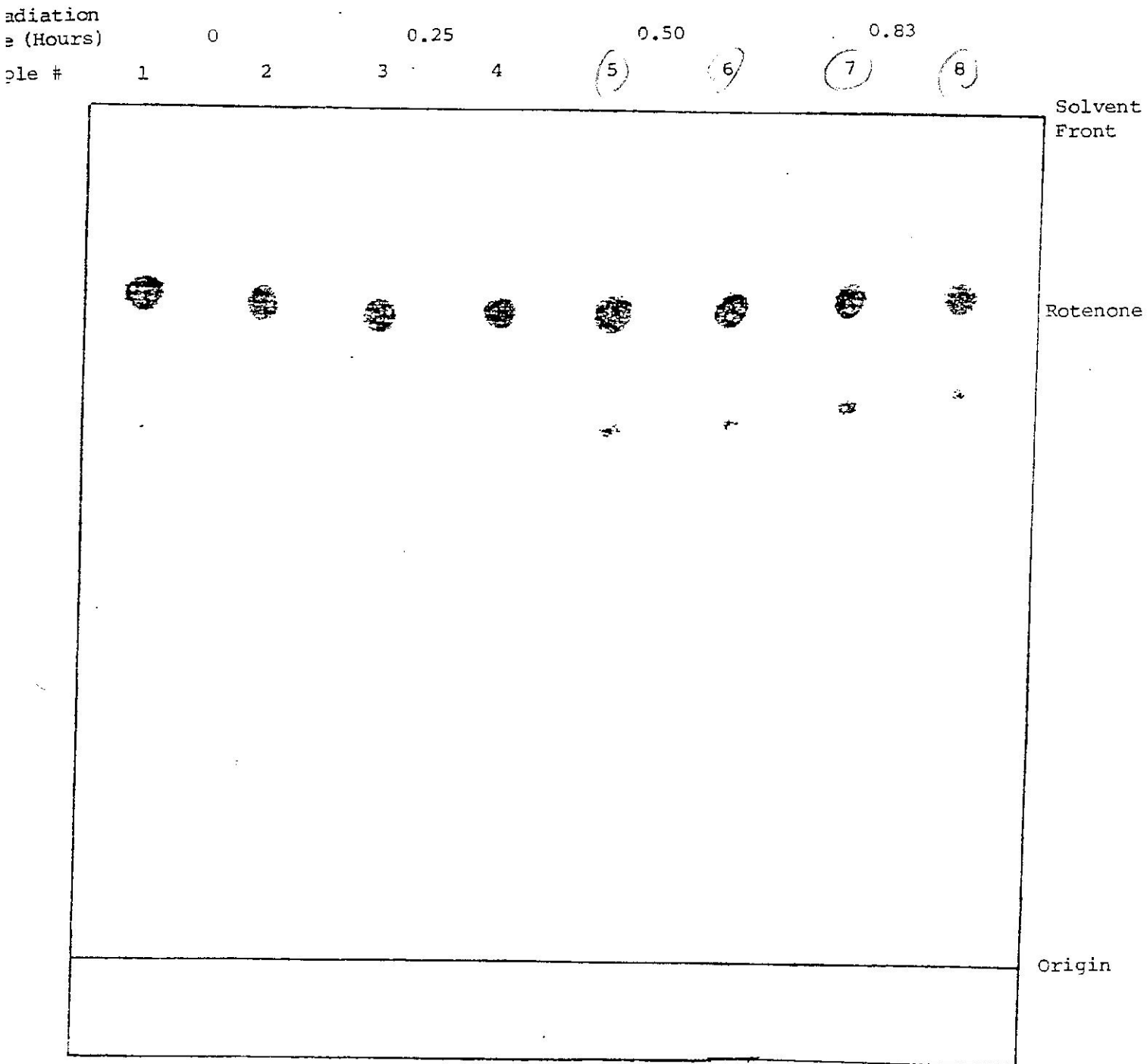
Correlation = -0.9907
Slope = -0.4891
Intercept = 4.303



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FIGURE 2

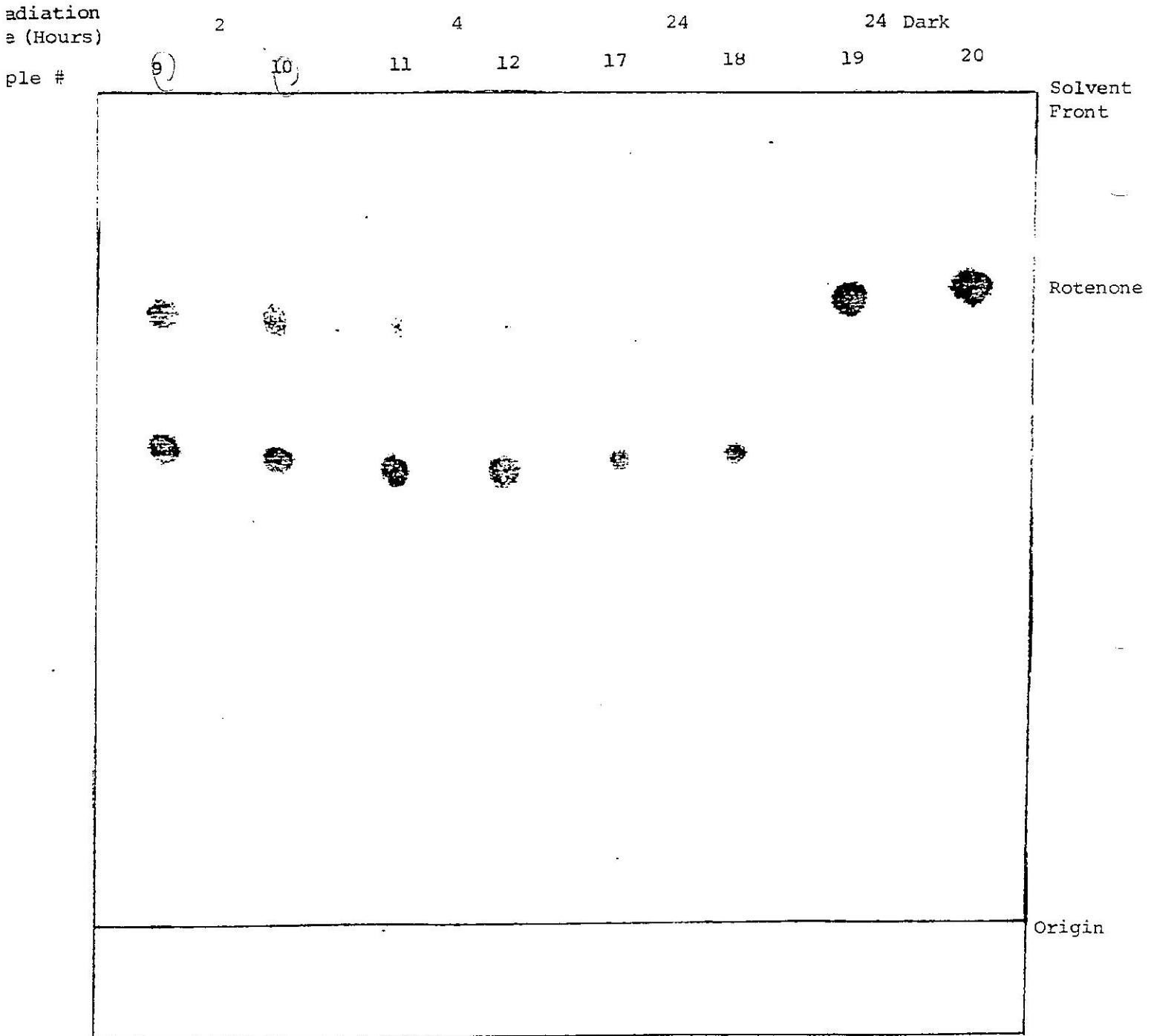
TLC Autoradiogram of Methylene Chloride (MeCl₂) Partition of Photolysis Samples Exposed from 0 to 0.83 Hours, Developed by Single Dimension TLC in Benzene:Methanol (9:1 v:v) Solvent System



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FIGURE 3

TLC Autoradiogram of Methylene Chloride (MeCl₂) Partition of Photolysis Sample Exposed from 2 to 24 Hours, Developed by Single Dimension TLC in Benzene:Methanol (9:1 v:v) Solvent System



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FIGURE 4

TLC Autoradiogram of Aqueous Remainder Rotenone 24 Hour Photolysis
Sample Extracted with Methylene Chloride, by Single Dimension TLC -
Solvent System Benzene:Methanol (9:1 v:v)

Irradiation
Time (Hours)

24

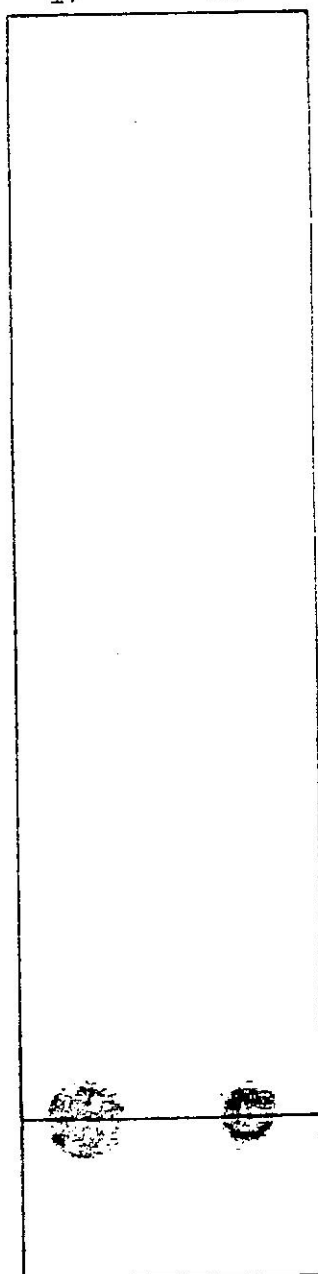
Sample #

17

18

Solvent
Front

Rotenone



Origin

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Certification of QA Inspection

Project Number: 82-E-076-P
Date of Inspection: May 10, 1982
Date Findings Reported to
Management and Study Director: May 10, 1982

Based on the observations made during the study inspection and the QA audit of the raw data and this report, the reported results accurately describe the methods, standard operating procedures and raw data of this study.

Fran Dillon
QA Officer
Biospherics Incorporated

10/26/82
Date

ADDENDUM TO
FINAL REPORT

Aqueous Photodegradation of
¹⁴C-Rotenone

Prepared for:

U.S. Department of the Interior
Fish and Wildlife Service
National Fishery Research Laboratory
Prime Contract #14-16-0009-81-042

Prepared by:

Litton Bionetics, Inc.
5516 Nicholson Lane
Kensington, Maryland 20895

November 1982



BIONETICS

Litton

SPONSOR: Fish and Wildlife Service, USDI

MATERIAL: Rotenone

SUBJECT: FINAL REPORT

Analysis of Rotenone Photodegradation
Products by Mass Spectrometry

1. OBJECTIVE

The purpose of this study was to add structural information to a study of the photodegradation of rotenone.

2. EXPERIMENTAL DESIGN

A. Samples - the following samples were received from Biospherics, Inc. for analysis:

1. Rotenone Standard
2. 4 TLC plate scrapings from the 4 hour reaction mixture (sample #'s 1, 2, 3 and 4)
3. 4 TLC plate scrapings from the 23 hour reaction mixture (sample #'s 5, 6, 7 and 8)
4. 1 TLC plate scraping of the parent compound (sample #9)
5. 1 TLC plate scraping for background determination (sample #10)
6. 2 CH₂Cl₂ extracts - 1 each from the 4 hour and 23 hour reaction mixtures
7. 2 extracted aqueous samples - 1 each of the 4 hour and 23 hour reaction mixtures (sample #'s 11 and 12)

B. Procedures

1. Extraction

TLC plate scrapings (dry) were poured into stoppered 15 mL centrifuge tubes to which 2 mL absolute ethanol was added for extraction. Samples were vortexed then centrifuged at 1200 rpm for 5 minutes. The ethanol layer was removed with a pasteur pipette and evaporated to dryness with a stream of nitrogen. The residue was taken up in 20 μ l absolute ethanol. Two 5 μ l aliquots of each sample were transferred to capillaries and evaporated to dryness.

2. GC/MS Analysis

A minute amount of the rotenone standard was placed in a capillary and analysed using solid-probe mass spectrometry. TLC scraping extracts were then analysed in the following order: blank (#10), rotenone (#9), reaction mixture/rotenone (#7), ¹⁴C-degradation product (#6), non ¹⁴C-degradation product #1 (#5), and non ¹⁴C-degradation product #2 (#8).

Mass Spectrometer parameters were as follows:

ionization mode: electron impact
scan range: 35-600 AMU -1 , $+1$
seconds/scan: 3.5
electron energy: 70 eV
electron multiplier: -1600
emission current: .5 ma
source temperature: 250°C
probe heating: ballistic
sensitivity: -7

The second capillary of each sample was then analysed using a sensitivity setting of -8.

The extracted aqueous sample of the 23 hour reaction mixture (#12) was concentrated on a rotary evaporator to approximately 100 μ l. Two 5 μ l aliquots of this sample were also placed in capillaries and evaporated to dryness with a stream of nitrogen. Mass spectrometer parameters were the same.

3. RESULTS AND DISCUSSION

For the purposes of this portion of the study, the photodegradation, extraction and TLC steps were performed as before with the following exceptions:

- A. Only non-radioactive rotenone was used. This would not be expected to change the photodegradation reactions.
- B. Ten times the volume of reaction mixture was used for extraction, affording a 10-fold concentration of components.
- C. The spots on the TLC plate were visualized by UV light rather than radioautography. As a result of these changes, four spots were seen on the TLC plate rather than the two reported by Biospherics, Inc. previously. Composition of the two new spots is described below (samples #5 and #8).

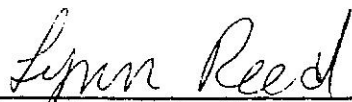
The mass spectrum of the rotenone (see Figure 1 for structure) standard exhibited major peaks at M/z 394 (the molecular ion M⁺), 379, 203, 192 (base peak), 177 and 161. Smaller peaks were present at M/z 351, 219, 147, 121, 93, 91, 77, 69 and 65. The major fragmentation pathway (production of M/z 192) seems to consist of two broken bonds in the central of the 5 rings of the molecule (to the carbonyl and ether of that ring, see Figure 2), with the charge retained on the non-carbonyl portion of the molecule. There was very little interference from the TLC background for ions above M/z 100.

Since the thin-layer chromatograms from the 4 hour and 23 hour reaction mixtures appeared to contain the same components in different concentrations, only the TLC scrapings from the 23 hour reaction mixture were analysed. The sensitivity was very good. The molecular ion and base peak of the parent compound were easily discerned in sample #7; concentration calculated from the amount of radioactivity present as .05 μg (5 μl of 20 μl used; 0.2 μg total, assuming 100% recovery). All three of the other TLC scrapings (samples #5, #6 and #8) gave mass spectra indicative of at least portions of the rotenone molecule. The individual samples could conceivably have contained more than one compound each. Some fractionation of the sample does occur during heating of the probe, and slightly different spectra occurred over the course of analysis of any one sample. Samples #5 and #8 (non radioactive) almost certainly are fragments, since the carbonyl carbon (C_6 - labeled ^{14}C) is missing. These fragments would correspond to the half of the molecule shown in Figure 3. Sample #6 (containing ^{14}C) might include dehydrorotenone (Figure 4) or rotenolone (Figure 5), as evidenced by higher molecular weight peaks (i.e., M/z 392), and a smaller fragment whose spectrum is consistent with the presence of tubaic acid (Figure 6).

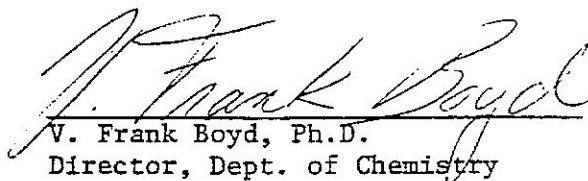
The aqueous sample (#12) contained at least two compounds. The spectrum of the parent compound was clearly present as well as that of a less volatile component. It is more likely that the latter compound is an acid, since it is not extractable into methylene chloride. The amount of parent present is not large enough to account for the amount of radioactivity remaining in solution after extraction. Therefore, the acid component is probably related to tubaic acid, although its mass spectrum indicates a smaller molecule.

Prepared by:

Reviewed by:



Lynn S. Reed, Ph.D.
Head, Mass Spectrometry Section



V. Frank Boyd, Ph.D.
Director, Dept. of Chemistry

Date:

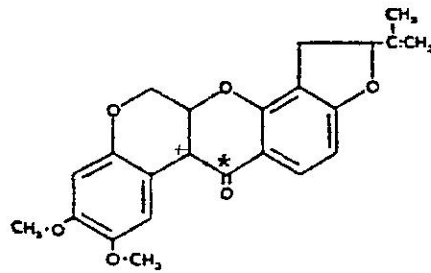
11/11/82

Date:

11/11/82

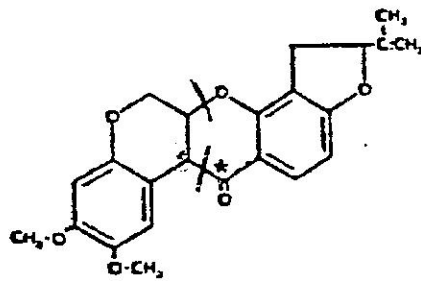
cc: R. Weir
H. Giss
S. Mazursky
W. Spare, Biospherics, Inc.
M. Beckman

FIGURE 1



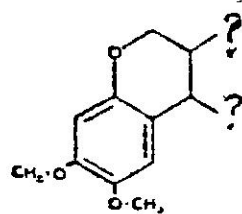
Rotenone

FIGURE 2



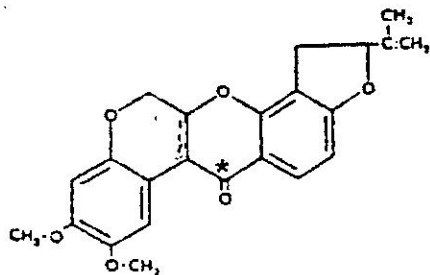
Fragmentation of Rotenone

FIGURE 3



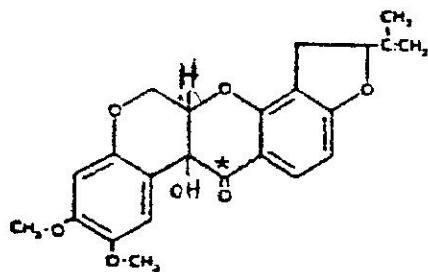
Core Structure of
Non-radioactive Fragments

FIGURE 4



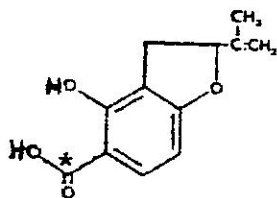
dehydrorotenone

FIGURE 5



Rotenolone

FIGURE 6



Tubaic Acid

Q. A. INSPECTION STATEMENT
(Reference 21 CFR 58.35(b)(7))

PROJECT 22147-01

TYPE OF STUDY Analysis of Rotenone Photodegradation Products by Mass Spectrometry


This study was inspected by the LBI Quality Assurance Unit and findings were reported to the study director and to management. Date of inspection and reporting were as follows:

DATE OF INSPECTION

November 18, 1982

DATE OF REPORT TO MANAGEMENT & STUDY DIRECTOR

November 19, 1982



Mitchell L. Ehrlich
Sr. Q. A. Auditor

MLE:af