BORRISTON
LABORATORIES, INC.

FINAL REPORT

THIS PROJECT WAS CONTRACTED FOR BY THE U.S. FISH AND WILDLIFE SERVICE AND PARTIALLY FUNDED USING FEDERAL AID IN SPORT FISH RESTORATION (DINGELL-JOHNSON) ADMINISTRATIVE FUNDS.

HYDROLYSIS OF [6-^{14}C]-ROTENONE

Borriston Project No. 0301A
June 30, 1983

Submitted to:
FISH AND WILDLIFE SERVICE
NATIONAL FISHERY RESEARCH LABORATORY
P.O. Box 818
La Crosse, Wisconsin 54601

Submitted by:
Borriston Laboratories, Inc.
5050 Beech Place
Temple Hills, MD 20748

BORRISTON
LABORATORIES, INC.

Borriston Laboratories, Inc.
5050 Beech Place
Temple Hills, Maryland 20748

Telephone: 301-899-3536
Telex: 248838

SPONSOR: FISH AND WILDLIFE SERVICE

INITIATION DATE: 3-23-82

MATERIAL: [6-^{14}C] -ROTENONE

COMPLETION DATE: 4-22-82

SUBJECT: HYDROLYSIS OF [6-^{14}C]-ROTENONE
Borriston Project No. 0301A
FINAL REPORT

ISSUE DATE: 6-30-83

SUMMARY

The rate of hydrolysis of [6-^{14}C] - rotenone in deionized water and pH 5, 7 and 9 phosphate buffers was determined in the absence of light at 25^{0}C. Rotenone is more rapidly hydrolyzed in neutral and basic solutions than in acidic or unbuffered solutions. The half lives of rotenone in deionized water, pH 5, pH 7 and pH 9 buffers were calculated to be 29.1, 12.6, 3.2 and 2.0 days, respectively. After extraction with ethyl acetate, the major degradation product was found to cochromatograph with 6αβ,12αβ-rotenolone; no other major degradation products were observed.

INTRODUCTION

Rotenone is a natural product which has been widely used as a piscicide and pesticide. The objective of this study was to determine the rate of hydrolysis of rotenone in aqueous solutions (pH 5, 7 and 9) and to determine the resultant degradation products.

EXPERIMENTAL DESIGN AND METHODS

Test Article

[6-^{14}C]-Rotenone, (specific activity 13.1 mC/mmol, lot number 801110) a white crystalline material, was received from the sponsor on 1-18-82. The test article was stored frozen and protected from light. The rotenone solution was found to be chromatographically pure, when checked on Day 1 by autoradiography. Unlabeled rotenone was purchased from Aldrich Chemical Company, St. Louis, Missouri. Rotenolone was obtained from Battelle Memorial Laboratories, Columbus, Ohio. The relative migration of these standards on silica gel thin layer chromatography is shown in Table 1, and chemical structures are presented in Figure 1.

Hydrolysis System and Sampling

Three sterile solutions (autoclaved) of 0.1 M sodium phosphate buffer were prepared and adjusted to pH's 5, 7, and 9 with 0.1N NaOH or H_3PO_4 when necessary. A stock solution of rotenone dissolved in acetone was prepared on Day 0; on the same day, each buffer solution and deionized water were spiked with the ^{14}C-rotenone to a final concentration of 0.1 ppm.

Each test solution was immediately aliquoted (10 ml each x 8 vials) to glass vials that were sealed, wrapped in foil and placed in a 25^{0}C incubator. On days 1, 3, 7 and 30, duplicate vials of each test solution were removed from the incubator for analysis.

Extraction and Radiocarbon Analysis

Each test solution sample (10 ml) was extracted with ethyl acetate (3 x 25 ml) and the combined 75 ml ethyl acetate fractions were dried over

sodium sulfate. The extracted water phase and the ethyl acetate extract were evaporated to dryness using a vacuum rotary evaporator, and residues were dissolved in acetone (1 ml). Aliquots were taken for liquid scintillation counting, and for spotting on thin layer chromatography plates.

Radiocarbon content was determined using a Packard Model 300C Tricarb liquid scintillation spectrometer. External standardization and automatic quench control were used for the calculation of counting efficiencies and conversion of cpm to dpm. Samples were counted in Scint A® liquid scintillation cocktail (Packard Instrument), two times for 10 minutes each.

Thin Layer Chromatography (TLC)

Pre-coated TLC plates, silica gel 60 F-254, with flourescent indicator, were used for chromatographic analysis of test solution extracts. The plates were developed in chloroform:methanol (49:1). Both ultraviolet light and autoradiography were used to locate spots on TLC plates. Following development and air drying, the plates were exposed to Kodak X-Omat AR film for 4-14 days. Areas of the plate corresponding to exposed spots on the film were scraped and analyzed for radioactivity by liquid scintillation counting. The samples were placed into Oxiflour® liquid scintillation cocktail (New England Nuclear, Boston, MA) and the radioactivity was measured in a Packard 300 TriCarb Scintillation Spectrometer equipped with external standardization.

RESULTS AND DISCUSSION

The recovery data for the total radioactivity present in the individual test solutions during the time course of the study are presented in Table 2. Recovery ranged from 73 to 112% and averaged 92% for all extracts. It appears no appreciable radioactivity was lost as $^{14}CO_2$ or other volatile compounds during this study.

The majority of the radioactivity (>73%) was extracted into the ethyl acetate fraction. TLC, followed by autoradiography, was used to separate and locate ^{14}C-labeled degradation products in the ethyl acetate fractions.

The major degradation product cochromatographed with 6αβ,12αβ-rotenolone; other degradation products were not found in sufficient amounts to be identified and no ultraviolet absorbing material was visible on the TLC plates.

The amount of radioactivity remaining in the aqueous phase after extraction increased with time and alkalinity of the test solution, indicating that hydrolysis of rotenone into polar compounds had occurred. Likewise, by Day 30, 12 to 20% of the ethyl acetate extractable radiocarbon applied to the TLC plates was found to be distributed along the sample channel and not associated with the major degradation products. However, there was no increase in the number of exposed spots on the film.

The degradation of rotenone in each test solution followed first order kinetics, and is presented graphically in Figure 2. The half life calculations from this data are shown in Table 4 using the equation $t_{\frac{1}{2}} = \frac{\ln 2}{k}$ where k is the rate contact for loss of rotenone from each test solution (shown in Figure 2). The degradation of rotenone at pH 7 and pH 9 had passed through two half lives by Day 7, and the data from Day 30 were not included in the calculation of the half life at these pH's. Rotenone is more stable at acid pH and in unbuffered water ($t_{\frac{1}{2}}$ = 29.1 and 12.6 days, respectively) than in neutral or alkaline solutions ($t_{\frac{1}{2}}$ = 3.2 and 2.0 days, respectively).

All raw data and the final report are stored in the archives at Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, Maryland 20748. If at some time in the future any of these data are to be discarded, the sponsor will first be notified to obtain permission.

R.D. Thomas, Ph.D.
Study Director

Paul Sundersky 6-30-83
Quality Assurance Officer

TABLE 1

RELATIVE MIGRATION OF ROTENONE AND ROTENOLONE ON SILICA GEL TLC PLATES

COMPOUND	Rf
Rotenone	0.75
6αβ,12αβ-Rotenolone	0.53

Compounds were chromatographed in chloroform:methanol (49:1).

TABLE 2

RECOVERY OF RADIOACTIVITY IN HYDROLYSIS TEST SOLUTIONS

STUDY DAY	% of INITIAL RADIOACTIVITY[a]			
	H_2O[b]	pH5	pH7	pH9
1	89.9	99.2	99.7	95.5
3	92.4	111.8	89.1	86.3
7	86.8	81.2	77.6	73.0
30	101.1	90.7	100.5	99.5

[a]The radioactivity present in the aqueous and ethyl acetate fraction was analyzed by liquid scintillation counting and the sum was expressed as % of initial radioactivity present in that sample.

[b]Deionized water.

TABLE 3

DISTRIBUTION OF RECOVERED RADIOACTIVITY IN ROTENONE AND ITS DEGRADATION PRODUCTS

TEST SOLUTION	COMPOUND	% OF RADIOACTIVITY[a] STUDY DAY 1	3	7	30
H_2O [c]	Rotenone[b]	88.0	85.8	71.3	44.0
	Rotenolone[b]	2.7	5.4	9.3	17.7
	Origin	1.5	1.6	1.3	3.1
	Aqueous	0.2	0.7	0.3	1.1
pH 5	Rotenone	89.4	80.6	65.3	18.3
	Rotenolone	2.6	5.0	8.4	36.6
	Origin	1.4	3.4	3.9	12.8
	Aqueous	0.3	0.3	0.9	3.3
pH 7	Rotenone	76.3	58.8	21.9	2.9
	Rotenolone	11.0	22.6	39.5	39.4
	Origin	1.0	2.6	5.0	2.9
	Aqueous	2.0	2.6	6.6	7.2
pH 9	Rotenone	65.7	39.2	8.4	1.7
	Rotenolone	21.7	38.5	48.7	50.4
	Origin	0.8	3.2	3.7	2.1
	Aqueous	3.2	8.4	20.3	16.4

[a] Samples were extracted with ethyl acetate and the ethyl acetate and aqueous fractions were analyzed for radioactivity. The ethyl acetate fractions were evaporated to dryness and aliquots were spotted on silica gel TLC plates. The data from the TLC plates have been normalized for distribution of radioactivity between the ethyl acetate and aqueous phases, and data is expressed as % of radioactivity recovered (Table 2) for each sample. The remaining channel from each TLC plate probably contained minor components that were not resolved and tailing radioactivity, making these results appear lower than reported in Table 2.

[b] Identity of compounds was determined by cochromatography with known standards; rotenolone is 6αβ,12αβ-rotenolone.

[c] Deionized water.

TABLE 4

DEGRADATION OF ROTENONE

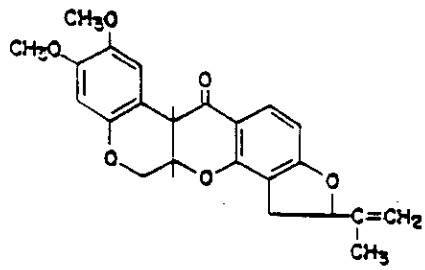
TEST SOLUTION	$t_{1/2}$ (days)[a]	R^2[b]
H_2O	29.1	0.99
pH 5	12.6	1.00
pH 7[c]	3.2	0.99
pH 9[c]	2.0	0.99

[a] $t_{1/2} = \frac{\ln 2}{k}$, where k is the rate constant for degradation determined from the slope of a first order plot of the data presented in Table 3 and Figure 2.

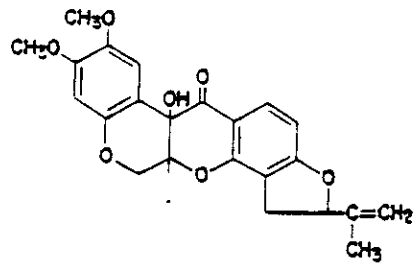
[b] R^2 is the coefficient of determination calculated from the fit of the data to a linear equation, using a linear regression program.

[c] The rate constant was calculated from the data of days 1, 3, 7.

Figure 1. Formulas of rotenone and 6αβ,12αβ-rotenolone.



Rotenone



6αβ,12αβ-Rotenolone

FIGURE 2. DEGRADATION OF ROTENONE IN DEIONIZED H_2O (●); pH 5 BUFFER (x); pH 7 BUFFER (o); pH 9 BUFFER (□).

Areas of silica gel TLC plates which were identified as rotenone by cochromatography with standards were scraped and counted for radioactivity. Data was normalized for distribution of radioactivity as described in the legend of Table 3.