



Metabolic fate and toxic effects of one of the components of *Tetradymia glabrata*
by Sandra Keller Holian

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Chemistry

Montana State University

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Abstract:

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Acute poisoning studies in mice have shown tetradymol caused dose dependent, centralobular necrosis. The death time in control mice was 7.5 hours. The death time and the hepatic necrosis could be altered after pretreatments with various compounds that altered the action of either the mixed function oxidase enzymes or the conjugating enzymes.

Spectral binding studies have shown tetradymol to be a Type I binder to cytochrome P-450. This, along with pretreatment studies indicated that it is metabolized via the mixed function oxidase system. Pretreatment studies have shown the metabolite formed is more toxic than tetradymol.

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Date

May 16, 1975

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by
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A thesis submitted in partial fulfillment
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TABLE OF CONTENTS

	page
LIST OF FIGURES	v
LIST OF TABLES	vii
ABSTRACT	viii
INTRODUCTION	1
RESULTS AND DISCUSSION	33
Summary	64
EXPERIMENTAL SECTION	68
Reagents	68
Instruments	69
Mice	70
Pretreatment of mice	70
Histology slides	72
Isolation of microsomes	73
Experiments	74
Quantitation of tetradymol	74
Stability of tetradymol	76
Distribution study	76
Elimination	78
Histology studies	78
Death time study	81
Spectral studies	83
APPENDIX	
A. Structures of pretreatment compounds	85
B. Table of histology slides	87
C. Death times	98
LITERATURE CITED	105

List of Figures

	page
1. Tetradymol mercuric chloride	5
2. Classic hexagonal lobule	7
3. Flow diagram for liver	8
4. Quadrant of a liver lobule	9
5. Structure of Ngaione	16
6. Structure of Acetaminophen	18
7. Mixed function oxidase reactions	21
8. Electron transfer and substrate oxidation	24
9. Conjugation scheme for glutathione	30
10. Conjugation scheme for UDP-glucuronic acid	31
11. Structure of glutathione	32
12. Reaction of tetradymol with Ehrlicks reagent	33
13. Tetradymol, four hours	41
14. Tetradymol, six hours	41
15. Tetradymol, eight hours	41
16. Tetradymol, ten hours	41
17. Normal mouse liver	42
18. Sublethal tetradymol, eight hours	42
19. Hexobarbital, aniline, tetradymol spectral binding	44
20. Tetradymol spectral binding	45
21. Hydroxylation of salicylamide	49
22. Salicylamide spectral binding	50

	page
23. Phenobarbital, four hours	52
24. Phenobarbital, six hours	52
25. 3-methylcholanthrene, six hours	52
26. SKF-525A, six hours	52
27. Piperonyl butoxide, eight hours	55
28. Salicylamide, six hours	55
29. Cysteine, six hours	55
30. Diethylmaleate, eight hours	55
31. Olive oil, eight hours	57
32. Ethanol, eight hours	57
33. Plot of death time <u>vs</u> SKF-525A dose	59
34. Double reciprocal plot of death time <u>vs</u> tetradymol dose	61
35. Death time <u>vs</u> reciprocal of Cyt. P-450	63
36. Metabolic cage	71

	List of Tables	page
1.	Plant extract sheep feeding	4
2.	Mouse feeding	6
3.	Mechanistic classification of hepatotoxins	12
4.	Direct hepatotoxicity criteria	12
5.	Indirect hepatotoxicity criteria	13
6.	Hypersensitivity criteria	13
7.	Substrates and pathways for cytochrome P-450	22
8.	Compounds causing Type I or II spectral changes	28
9.	Acid lability of tetradymol	35
10.	Tetradymol stability in stomach	36
11.	Tetradymol recovery from organs	37
12.	Elimination of tetradymol	39
13.	Death time	47
14.	Varying SKF-525A dose	58
15.	Varying tetradymol dose	60
16.	Death times and Cyt. P-450 concentration	62
17.	Elution pattern for Alumina Column	74
18.	Tetradymol stability at different pH	77
19.	Tetradymol recovered from organs	79
20.	Elimination of tetradymol	79

Abstract

Tetradymol is an hepatotoxin of moderate toxicity. It can survive in the animal system for at least seven days and was located in all the organs examined.

Acute poisoning studies in mice have shown tetradymol caused dose dependent, centralobular necrosis. The death time in control mice was 7.5 hours. The death time and the hepatic necrosis could be altered after pretreatments with various compounds that altered the action of either the mixed function oxidase enzymes or the conjugating enzymes.

Spectral binding studies have shown tetradymol to be a Type I binder to cytochrome P-450. This, along with pretreatment studies indicated that it is metabolized via the mixed function oxidase system. Pretreatment studies have shown the metabolite formed is more toxic than tetradymol.

INTRODUCTION

Tetradymol is a toxic constituent isolated from Tetradymia glabrata by Dr. Sam Reeder. It is a member of the Compositae family, Senecio tribe, resembling sage brush and found in a broad region covering an area north to Washington, east into Wyoming, west to California and south to the Utah-Arizona border. It was first shown to be responsible for death in sheep on the Nevada ranges by Fleming.¹ Further, it was known at that time to cause a reversible phenomenon called "Big Head", the symptoms of which were facial and ear tissue swelling. For more information on "Big Head" the reader is referred to Brown.²

From 1918 to 1922 Fleming and his colleagues conducted feeding experiments and a brief chemical study in which the more pertinent facts necessary for killing sheep were ascertained: (1) under scarce food conditions sheep would eat the new growth of T. glabrata, normally they would not, and an adult sheep could eat up to 2% of its body weight per day without apparent harm; (2) since the lethal dose could be fed over a relatively long period it was thought the toxic principle was slowly eliminated; (3) death was attributed to hepatodysfunction and cardiac failure; (4) the toxic constituent was contained in petroleum ether and acetone extracts from the green plant.¹

Considering this work and a later investigation by

Clawson and Huffman,^{3,4} the problem of isolating the toxic constituent of T. glabrata was undertaken by Drs. S. K. Reeder⁵ and J. C. Hurley.⁶ Two toxic compounds were isolated, tetradymol and tetradymadiol 6-isobutyrate, the following is a brief summary of Reeder's⁵ work with tetradymol.

From whole plant feeding experiments on sheep it was shown that: (1) feeding 1% of body weight for three days resulted in death; (2) bromsulphalein clearance time was greatly lengthened; (3) blood serum ammonia levels were elevated three to six times in poisoned sheep.⁵ The last two points indicate hepatodysfunction. To test cardiac dysfunction electrocardiograms were monitored on all sheep resulting in no marked changes being observed.

Autopsies were performed on all sheep that were poisoned. The results are summarized below:⁶

1. Liver tissue demonstrated panlobular necrosis localized in the centralobular area.
2. Kidney tissue showed some general congestion and swelling and hyperemia especially in the medulary portion.
3. Varying degrees of congestion were reflected in the lungs with some emphysema and bronchiolar hemorrhage.
4. Cardiac tissue was not greatly different from

normal revealing some congestion and a few subepicardial hemorrhages.

It was concluded that the toxic principle was a hepatotoxin and did not greatly effect the heart.

The results of plant-extract feedings are shown in Table 1.⁷ This Table shows the percentage of plant weight to body weight was similar to whole-plant feedings and for the hexane or acetone extracts, the BSP clearance time and blood serum ammonia level changes were comparable. Results of feeding hexane or acetone extracts were similar in dosage level and hepatic damage incurred indicating the toxic constituent was successfully extracted by these solvents.

Since sheep were a large and expensive laboratory animal, other smaller animals were tested resulting in similar gross changes in the livers. It was decided that mice would be used for further toxicity experiments.

Preliminary separation and feeding experiments of the crude extract with mice indicated two different toxins. One of the toxins, tetradymol, was isolated and its structure was confirmed by X-ray crystallographic determination of the mercuric chloride derivative shown in Figure 1.⁸

Extractions with hexane were made with both ground and unground plant material. Since grinding the material did not result in the isolation of more tetradymol, it was assumed the

TABLE 1⁷

Plant Extract Sheep Feeding Experiments

Sheep number	Wt. lbs.	Age yrs.	Extract fed	Extract from pounds	% plant of body weight	NH ₄ ⁺ level*	BSP clearance ^o	Result
H-636	98	1	Acetone	4.4	4.5	3/12 5/18	3/36 5/79	death
H-665	104	1	Hexane	5.0	4.8	3/14 5/13 7/14	5/44 7/39	v. ill ^{oo} sacrificed
H-634	103	1	Pentane of ethanol	2.5	2.5	3/6		release
H-628	104	1	Ethanol <u>remains</u>	2.5	2.5	3/6		release
			<u>Plant extracted with</u>	<u>pounds fed</u>				
H-641	102	1	Acetone	2.2	2.2	3/5		re-use
H-671	90	1	Hexane	2.5	2.8	3/8		release
H-653	100	1	Ethanol	2.5	2.5	3/6		release

*Recorded as a fraction with the day of the feeding experiment when the NH₄⁺ level test was run in the numerator and the g/ml of NH₄⁺ found in the serum recorded as the denominator

^oRecorded as above with the day in the numerator and the T_{1/2} in minutes recorded as the denominator.

^{oo}V. ill meaning very sick, actually down and on the verge of death.

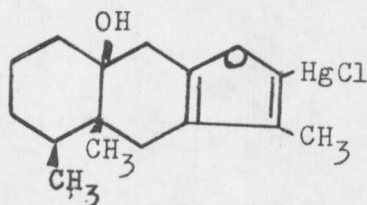


Figure 1. Tetradymol mercuric chloride⁸

toxin was a surface compound and further hexane extractions were made on unground plant material.

An LD₅₀ of 170 to 333mg/kg was determined for the pure tetradymol compound by feeding experiments with mice. The results are shown in Table 2.⁹

Because preliminary feeding experiments indicated tetradymol was a hepatotoxin, the liver would be an important organ in considering what happens to tetradymol and the effects of tetradymol poisoning. Therefore, a brief review of the liver structure and function will be presented.

The liver contains hundreds of lobules which are the basic functional unit. These lobules are basically hexagonal in shape being longer than wide. The liver lobule is constructed around a central vein and is composed principally of many hepatic cellular plates. The plates are usually two cells thick and radiate centrifugally from the central vein. Lying between the hepatic plates are the small bile canaliculi and around the plates are the liver sinusoids. On the periphery of the lobule are portal areas which contain the bile

TABLE 2⁹

Mouse Feeding Experiments

Materials and/or carrier	mg/kg of toxin	ml/kg total volume	Number of animals	% dying in one week
1. Pure (II)* 50% ethanol in n-hexane	460	3.8	9	25
2. " "	360	3.6	8	50
3. " "	280	3.5	8	63
4. " "	190	3.1	8	75
5. " "	160	4.0	8	50
6. 50% ethanol in n-hexane	0.0	3.3	8	50
7. " "	0.0	5.0	9	50
8. Crude extract in propylene glycol	2000-3000	3.0-4.5	5	00
9. Crude extract in n-hexane	2100-3300	4.4-5.8	12	100
10. " "	1100-1200	1.9-2.0	4	25
11. N-hexane	0.0	4.0-4.9	8	00
12. " "	0.0	7.0-8.6	7	00
13. Sublimed (II) in n-hexane	750-580	4.8-6.2	7	100
14. " "	330	3.3	9	100
15. Pure (II) in n-hexane	330	3.3	9	100
16. " "	170	3.3	11	45
17. " "	282	3.9	5	40
18. " "	200	3.9	5	00
19. " "	140	3.9	5	00
20. " "	100	3.9	5	00

*Pure (II) refers to sublimed, base washed tetradymol.

