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

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.

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Date May 16, 1975

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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. Reeder[5] and J. C. Hurley.[6] Two toxic compounds were isolated, tetradymol and tetradymadiol 6-isobutyrate, the following is a brief summary of Reeder's[5] 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 actone 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°	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°° sacrificed
H-634	103	1	Pentane of ethanol	2.5	2.5	3/6		release
H-628	104	1	Ethanol remains	2.5	2.5	3/6		release
			Plant extracted with	pounds fed				
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 NH_4^+ level test was run in the numerator and the g/ml of NH_4^+ found in the serum recorded as the denominator

°Recorded as above with the day in the numerator and the $T_{\frac{1}{2}}$ in minutes recorded as the denominator.

°°V. ill meaning very sick, actually down and on the verge of death.

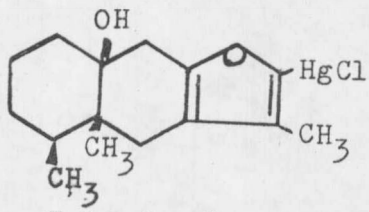

Figure 1. Tetradymol mercuric chloride[8]

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

An LD_{50} 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 centrifugically 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 2[9]

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.