The isolation and characterization of a toxic principle of Tetradymia glabrata
by Jerome Cornelius Hurley

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Chemistry
Montana State University

Abstract:
Tetradymadiol 6-isobutyrate, 4(R)[3,4a(S), 5(S)-trimethyl-4(R), 8a(S)-dihydroxy-4,4a,5,6,7,8a,9-octahydronaphtho-2,3-b furanylj (2'-methylpropanoate (I), has been isolated from Tetradymia glabrata, a desert plant of the great Salt Lake Basin, which is the cause of extensive range losses of sheep. This compound was shown to be a liver toxic material by sheep and mouse feeding experiments.

The structure of the compound was firmly established by a combination of solvent induced nmr shifts, nuclear Overhauser effects, and lanthanide shift reagents on the isolated toxin (I) and derivaties.

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ABSTRACT

Tetradymadiol 6-isobutyrate, 4(R)[3,4a(S),5(S)-trimethyl-4(R),8a(S)-dihydroxy-4,4a,5,6,7,8a,9-octahydronaphtho-2,3-b furanyl] (2'-methylpropanoate (I), has been isolated from Tetradymia glabrata, a desert plant of the great Salt Lake Basin, which is the cause of extensive range losses of sheep. This compound was shown to be a liver toxic material by sheep and mouse feeding experiments.

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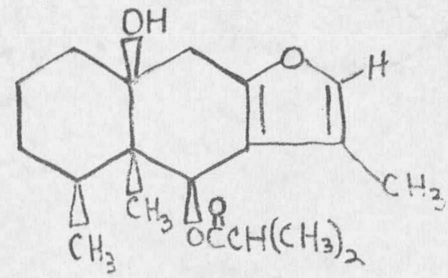

(I)

INTRODUCTION

"Since plants are entirely unable to flee from their predators, it is probable that they have been under continuous evolutionary pressure to solve their survival problems by chemical means."[1] Plants have accumulated many secondary substances--chemicals that have unknown roles in the basic metabolism of the plant. Among these secondary substances are many that serve to repel or discourage the use of the plant by insects, microorganisms, nematodes (roundworms), grazing animals and man. Many of this set of chemicals are toxic or poisonous. A rough outline of the toxic secondary substances in plants according to their types of physiological action leads to five general categories:

1. Enzyme inhibitors;
2. Physiological irritants;
3. Allergens;
4. Hormonal activity alterators;
5. Vitamin and amino acid antagonists;
6. Miscellaneous toxins.

The first and most common of these, the enzyme inhibitors, include protease inhibitors as found in many legumes, cereals, and throughout the plant kingdom.[2] The favism substances in legumes,[3] cholinesterase inhibitors in the tomato family,[4] cyanogens (found in plants as cyanogenetic glucosides)[5] and a variety of toxic alkaloids are examples of these enzyme inhibitors widespread in the plant kingdom.[6]

The second most common group is the physiological irritants. This group includes the saponins, which form foams in the gut and occur in approximately eighty families of plants,[7] the hemaglutinins, which disrupt the function of red blood cells and occur commonly in legumes (grasses) and euphorbes (trees and shrubs);[8] the lathyrogens, which disrupt collagen structure, are found in peas;[9] the irritant oils, including many cyanogenic compounds that occur most commonly in the cabbage family,[10] but also in many other food plants including manioc;[11] and raphides, crystals that may serve as irritants in the mouth or gut, such as the oxalate raphides in beet and rhubarb.[12]

The third group, the allergens, are not of general toxicity, but rather toxic only to the individuals with allergic reactivity. The sources include cereal grains, vegetables, fruits, and the most notorious pollens.[13]

The fourth group includes compounds that alter hormone systems. Substances exhibiting estrogenic activity (stimulation of growth of the vagina, uterus, and mammary gland) are very widespread and are found in such plants as carrots, soybeans, wheat, rice, cottonseed oil and coconut oil. The compounds isolated have in general been isoflavones.[14]

The fifth group, the vitamin and amino acid antagonists, are of lesser toxicity and include citral, a competitor of vitamin A, and solanine, an inhibitor of cholinesterase.[15]

A sixth group, every other toxin known, is little other than a holding pen for the toxins that are not understood well enough to actually classify them.

The system of interest in our research is a presumed hepatotoxin. Hepatotoxins are included in the sixth group and these toxins comprise a list of compounds that number in the thousands with a great variety of structural types ranging all the way from carbon tetrachloride to steroids and alkaloids. Some of these compounds are listed in Appendix A.

As we will be dealing with hepatotoxicity, a general review of liver structure, liver function and hepatotoxicity in general seems desirable.

The liver is in one sense several organs in one. The liver consists of a complex circulatory system, biliary passages, a collection of reticuloendothelial cells of various types and the liver cells, or polygonal parenchymal cells, themselves.[16] A brief description of their individual, but interrelated, anatomic structures and physiologic activities will aid in the later understanding and correlation of the diagnostic tests that are available for the evaluation of overall liver function or dysfunction.

I. Circulatory System (Figure 1). The liver is unique in that it has a dual blood supply consisting of the hepatic artery and the portal vein, both of which participate in the transport of oxygen and foodstuffs to this organ for assimilation. Sinusoids surrounding the liver cells merge to form central veins, which empty into sublobular veins, large collecting veins, then into the hepatic veins, and finally into the vena cava. The blood vessels are accompanied by lymphatics and nerve fibers. Lymph channels carry liver lymph with its high protein content from the liver into the general circulation and aid in the transport of material from the splanchnic bed to the systemic circulation. Nerve fibers transmit sensory impulses and help to regulate intrasinusoidal vascular pressure and blood flow.[17]

II. Biliary Passages (Figures 1 and 2). The biliary passages consist of a series of thin-walled tubes into which conjugated bilirubin, cholestral, certain drugs, and other substances are secreted by the liver cells. Within the liver cell, the system originates with the Golgi apparatus which consists of lamellae and vesicles adjacent to microvilli of the bile canaliculi lying between the liver cells. Bile canaliculi represent the biliary cell wall or plasma membrane of the liver cells. They empty into bile ductules which in turn, lead to interlobular bile ducts (small), septal bile ducts (medium), large intrahepatic bile ducts, and finally, main branches of the common bile duct.[18]

III. Reticuloendothelial Cells (Figure 3). The reticuloendothelial system is widely distributed; however, sixty percent of its elements are found in the liver, five percent consists of reticular cells in the spleen, and the remaining thirty-five percent are present in the lymph nodes and other tissues. Reticuloendothelial, or sinusoidal cells in the liver are of three varieties: twenty-five percent protrude as stellate phagocytic (Kupffer) cells; fifty percent are flat phagocytic cells; and twenty-