Naturally Occurring Fish Poisons from Plants

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It has been our experience that students have a real interest in compounds derived from natural sources that have biological activity. Unique examples pique their curiosity and provide a connection between "classroom chemistry" and the real world. One such class of plant-based natural products that has received very little attention in this regard is fish poisons or piscicidal compounds used by native people for fishing. A survey of the literature reveals that piscicidal plants are distributed throughout the world; and some are still used in the more remote areas of the world. Burkill *(1)* and Altschul *(2)* each identified more than a hundred different piscicidal plants. In their 1981 book entitled *Secondary Plant Metabolism* Vickery and Vickery *(3)* claimed that over 150 species of plants had been identified as showing piscicidal activity, an estimate that is now believed to be very low. In 1994, Neuwinger catalogued 284 fish poisoning plants from 171 genera and 62 families found in tropical areas of Africa alone *(4, 5)*.

There probably is no simple answer to the question of why plants produce fish poisons; many of these piscicidal compounds possess other biochemical activities beyond their ability to kill fish. For example, the same plants that are poisonous to fish are often used in traditional medicines as well as to prepare poison arrows in parts of Africa *(4)*. Some plants are used as insecticides and aphrodisiacs *(6, 7)*.

Active compounds isolated from these plants have potential use for management of unwanted species in both waterways, fish culture operations, and sport fishing. Many of these plants that are used as traditional medicines hold promise of containing useful medicinal compounds. Some of these piscicidal compounds have insecticidal activity and may be potential sources of new insecticides. Despite the cultural interest and the potential of commercial possibilities, chemical knowledge of fish poisons from plants is still at an early stage.

Plant Sources of Fish Poisons

Most fish poisons have been identified from plants in use by native fishermen. For example, the Chiriguano Indians of South America use the bark of *Myrsine pellucida (7),* a tree that grows wild in Bolivia and other Andean countries, and the Tukanoan and Kubeo Indians of the northwestern Amazon area use *Caryocar microcarpum* as fish poisons *(8)*. Fishermen in parts of the Philippines use *Heritiera littoralis (9),* and Samoans and other Pacific Islanders use the seed of *Barringtonia asiatica (10)*. All of the species reported by Neuwinger *(4)* were identified through field studies and records of plants used as fish poisons by Africans. Some of the more common piscicidal plant species reported in the literature include the following:

Schima mertensiana (11)
Iris germanica (6)
Pellia endiviifolia (12)
Dryopteris fragrans (13)
Camellia sinensis (14)
Madhuca butyracea (15)
Edgeworthis chrysantha (16)
Maesa ramentacea (17)
Pimelea spp. (18)
Aegiceras cerniculatum (19)
Iris japonica (20)
Cylicodiscus gabunensis (21)
Mundulea sericea (22)
Myrsine pellucida (7)
Caryocar microcarpum (8)
Heritiera littoralis (9)

The active components may be found in the root, bark, stem, or leaves of the identified plant. In addition, other plants with fish poisoning properties have been discovered in plants closely related to known piscicidal species.

How Fish Poisons Are Used for Fishing

Extensive descriptions of how piscicidal plants have been traditionally used are found in Neuwinger's articles *(4, 5)*. Typically the plant is thoroughly pounded using a rock and the macerated material is thrown into a shallow pool of slow moving or stagnant water. Sometimes plant sap is collected and dropped into small pools, or branches are scratched open and weighted down with stones to keep them in the water.

The seeds of *Barringtonia asiatica* are used in the Pacific Islands by scraping the seeds on lava rocks by shallow tidal pools, and washing the scrapings into the water *(10)*. This method and the methods described above result in dead or stunned fish rising to the surface in a short time where they are collected by hand, then cooked and eaten with no apparent harm to the consumer.

Cissus quadrangularis is used in Nigeria both as a fish poison and as arrow poison to kill small birds *(4)*. This poison is often used in conjunction with other local plant poisons (e.g., *Balanites aegyptiaca* and *Tephrosia vogelii*) and the mixture is more toxic than any one of the poisons used alone. The fruit of the plant *Balanites aegyptiaca,* which is an effective fish poison, is eaten, however, by goats, camels, and other animals without apparent adverse effects *(4)*.

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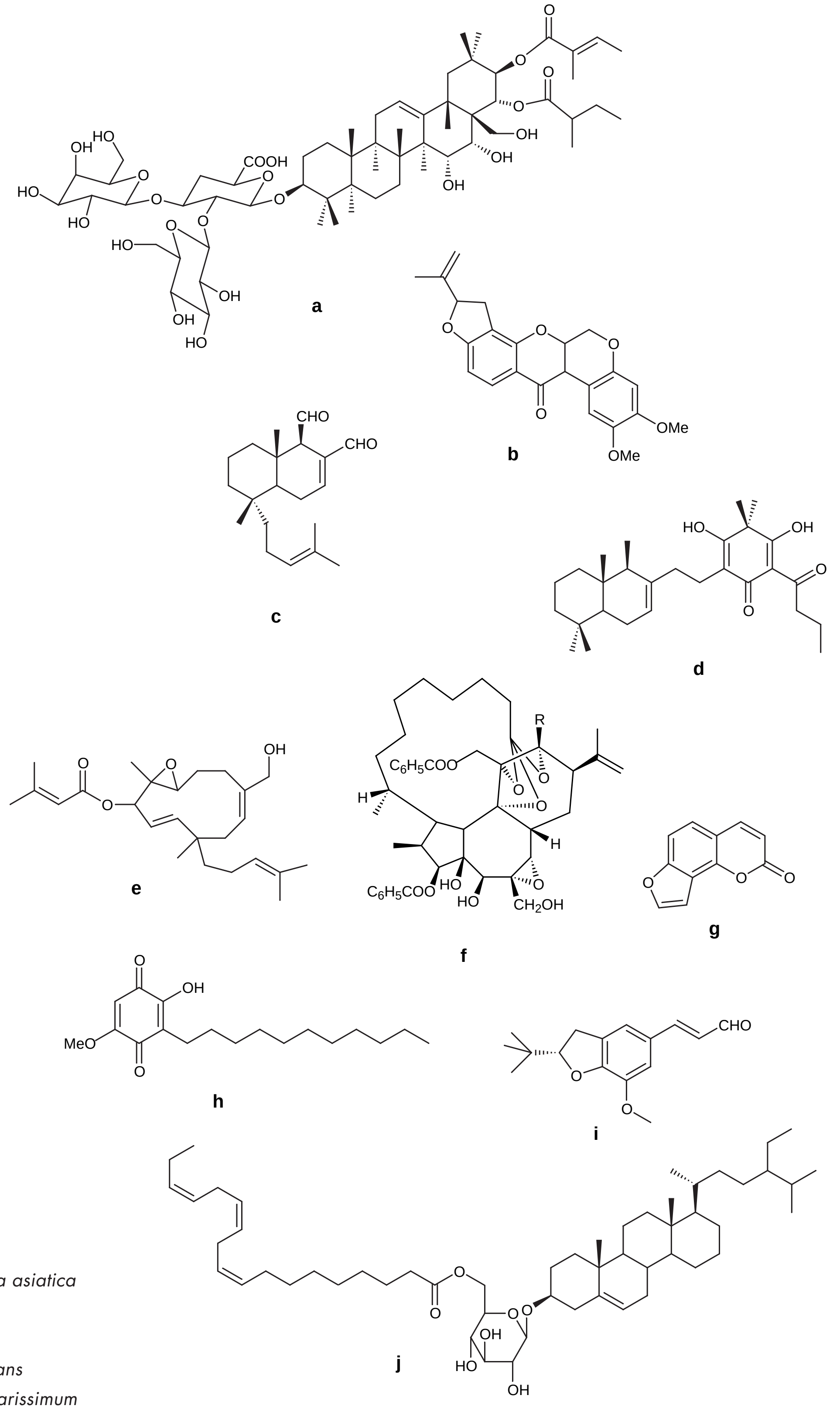

Figure 1. Representative structures of plant-based fish poisons of various chemical classes:

(a) Ranuncoside VIII from *Baringtonia asiatica*

(b) Rotenone from *Derris eliptica*

(c) Sacculatal from *Pellia endiviifolia*

(d) Dryofragin from *Dryopteris fragrans*

(e) Vibsanine A from *Viburnum odorarissimum*

(f) 1-Alkyldaphnmane orthoester from *Pimelea spp.*

(g) Furanocoumarin

(h) 2-Hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone from *Aegiceras corniculatum*

(i) Wutaiensal from *Xanthoxylum wutaiense*

(j) Sterol acylglucoside from *Edgeworthia crysantha*

Compounds Found in Piscicidal Plants

The most commonly found classes of fish poisons are triterpenoid saponins (Figure 1a) *(7, 11, 15, 17, 21)* and rotenoids *(4, 22)* with rotenone (Figure 1b) *(1)* being the most widely used commercially. Some other poisons include sesquiterpenes (Figure 1c) *(9, 13, 23)*, diterpenoids (Figure 1d and e) *(4, 12),* including diterpenoid orthoesters (Figure 1f) *(18, 24, 25),* triterpenes *(6),* furanocoumarins (Figure 1g) *(1),* 2-hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone (Figure 1h) *(19),* prenyl phenyl propanoids (Figure 1i) *(26),* and sterol acylglucosides (Figure 1j) *(16).* Some of the natural products that have been extracted, and whose structures have been elucidated, are listed in Neuwinger's book *(5).* The great variety of chemical structures presents many interesting questions regarding the relative toxicity, biochemical mechanisms, and environmental effects of fish poisons.

Relative Toxicity of Piscicides

Several different methods of reporting the toxicity of piscicides are found in the literature. The median tolerance limit ($\mathrm{TL_m}$) is defined as the average concentration required to kill a number of fish in a given period of time. A more common designation (e.g., $\mathrm{LD_{50}}$) indicates the dosage required to kill a fixed percentage (in this case 50%) of the fish in 48 or 96 hours. Other research workers simply report a concentration that killed all the fish in a few minutes or hours, or alternately report a comparison of the toxicity of a piscicide with that of a well-known fish poison such as rotenone. The reported toxicities of several poisons are summarized in Table 1. A very important parameter of toxicity studies is the residual or long term effect the substance has on a pond or lake. This is crucial if a fish poison is to be used in waterways management. In an extensive study of "tea seed-cake", which contains a high concentration of saponins, De et al. found that the residual toxicity of the saponins in their pools was less than 7 days *(14).*

Table 1. Reported Toxicities of Some Common Piscicides

Chemical Class	Name and Source	Reported Toxicity
Sesquiterpenoids	Dryofagin from *Dryopteris fragrans (13)*	$\mathrm{TL_m}$ (24 h) 1.2–1.5 ppm
	from *Buddleja davidii (23)*	Specific toxicity not reported
	Heritonin from *Heritiera littoralis*	10% mortality in 12 hours at 100 ppm
Sterol Acylglucosides	from *Edgeworthia crysantha (16)*	Complete mortality at 0.1 ppm in 3 hours
Furanocoumarins	Heritol from *Heritiera littoralis (33)*	Complete mortality at 20 ppm in 90 min
Diterpenoids	1-alkyldaphnane orthoesters from *Pimelea spp (18)*	Complete mortality within 24 h at 30–60 μg/L while rotenone was lethal at 60 μg/L in the same test
	Vibsanine A from *Viburnum odoratissimum (34)*	$\mathrm{TL_m}$ (24 h) 0.18 μg/mL
	1 β-hydroxy sacculatal and sacculatal from *Pellia endiviifolia (12)*	Complete mortality at 1 ppm in 20 min
	Huratoxin from *Hura crepitans (24, 35)*	$\mathrm{TL_m}$ (24 h) 0.0014 ppm, (48 h) 0.011 ppm. Rotenone had $\mathrm{TL_m}$ of 0.013 and 0.012, respectively
Rotenoids	Rotenone from *Derris eliptica (3)* and members of the family *Leguminosae (36)*	Apart from the toxicities listed above, rotenone has an $\mathrm{LD_{50}}$ of 132 mg/kg when given orally to rats; it is more toxic when inhaled *(31)*
Quinones	2-hydroxy-5-methoxy-3-undecyl-1, 4-benzoquinone from *Aegiceras corniculatum (19)*	Toxic at 1 ppm within 75 min
Prenyl Phenyl Propanoids	from *Xanthoxylum wutaiense (26)*	Weakly piscicidal
Saponins	Boninsaponin from *Schima mertensiana (11)*	Toxic at 1.5 ppm
	from *Madhuca butyracea (15)*	$\mathrm{LD_{50}}$ 11 ppm, $\mathrm{LD_{90}}$ 14 ppm
	Saponin A from *Maesa ramentacea (17)*	Crude saponin fraction had $\mathrm{LD_{100}}$ 1 mg/L
	from *Camellia sinensis (14)*	100 ppm of crude saponin extract was lethal in 5–6 h; the residual toxicity lasted less than 7 days
Triterpenes	Irisgermenical A	$\mathrm{TL_m}$ (24 h) 0.1 μg/mL
	Irisgermanical B and C from *Iris germanica (5)*	$\mathrm{TL_m}$ (24 h) 0.5–0.9 μg/mL

Mechanism of Action

Relatively little is known about how most fish poisons work on a molecular level, with the possible exception of rotenone. Rotenone is known to inhibit NADH-Q reductase in the mitochondrial electron transport chain *(27),* and this prevents the mitochondria from using NADH as a substrate. Electron transfer is virtually halted, and the organism cannot produce enough ATP, which leads to asphyxia and paralysis, followed by death *(4).* It would appear likely that other rotenoids act by the same mechanism.

There is more uncertainty about the way saponins act as fish poisons. A study of the hemolytic and moluscicidal activities of saponins showed no simple correlation between molecular structure and bio-activity, although the hemolytic and moluscicidal activities showed some correlation with each other *(28).* Neuwinger postulated that saponins permeabilize the gill epithelial cells of fish allowing essential electrolytes to escape *(4).* The fact that not all saponins have piscicidal activity does not preclude this hypothesis. Rao and Sung found that a difference as small as one sugar unit can greatly affect the activity of a saponin *(29).* Although not true of

Figure 2. The seeds of *Barringtonia asaiatica:* the top image shows a whole seed (5–6 cm long); the bottom image shows split seeds.[1]

all, many saponins only stun fish *(3).* Vickery and Vickery claim that saponins are generally only toxic to cold-blooded animals *(3),* and if this is true, saponins may offer an attractive alternative for rotenone in fish management projects since the latter is somewhat toxic to small mammals. The authors also quote a study of saponins produced by sea cucumbers in which it was found that the saponins had strong membraneotropic activity against any cellular or model membrane containing Δ^5-sterols. This may in part explain the wide spectrum of activities possessed by saponins. Considerably less work has been done on the terpenoid-based fish poisons.

Sakata and colleagues have studied some of the factors that affect the toxicity of huratoxin, a diterpene orthoester with a long aliphatic chain (see Figure 1f for a similar structure) *(24).* Upon acetylation of the hydroxyl groups, toxicity vanished, and removal of the aliphatic side chain made huratoxin 10,000 times less toxic. From these observations, Sakata et al. suggested that the hydroxyl groups are involved in the active sites of toxicity, and that the aliphatic chain may be involved in penetration of the cell membrane and in facilitating the introduction of the poison to the enzymes *(24).*

Potential Uses of Piscicides

Fish poisons are widely used for cultural, commercial, and environmental reasons (4, 7, 8, 21, 22, 30). Although most countries no longer allow the use of piscicides in large scale killing of fish, they are used to catch fish for food in certain areas of Africa *(4, 5),* South America, the Philippines *(9),* and the South Pacific. They are also used throughout the world in waterways management for the control of nongame fish species *(30).* Rotenone is the most commonly used piscicide in these applications, and although rotenone is known to be toxic to nearly all animals, it decays in sunlight and in air *(31),* thus enabling it to degrade without causing much harm to mammals and other non-target species. When rotenone is used to treat large reservoirs, the treatment is carried out at times of low water, and outflow from the reservoir is cut off so that the rivers downstream are not poisoned.

Piscicidal Plants as Sources of Medicinal Compounds

Discovery of many other biological applications of fish poisons has broadened the interest in the study of piscicides. For example, piscicidally active compounds extracted from *Iris germanica* and *Iris japonica* show antiulcer properties *(20),* and rotenoids discovered in the bark of *Mundulea sericea* were found to inhibit ornithine decarboxylase activity in mouse epidermal cells, induced by phorbol esters *(22). Dryopteris fragrans* is used in northeastern China to treat skin diseases *(13),* and inhabitants of several West African countries and the Polynesian Islands use liquid from the crushed bark of *Barringtonia asiatica* to treat chest pains and heart troubles. The same plant is used in Papua New Guinea to treat stomachaches; the top leaves from this tree are squeezed into water and the liquid taken orally *(32).* Daphnane orthoesters extracted from *Pimelea sp.* exhibit antineoplastic activity against *in vivo* murine P-388 lymphocytic leukemia *(18).* Some of the other interesting biological properties claimed for these compounds include characterizations as insecticidal *(3, 6, 20, 22),* aphrodisiac *(6, 20, 22),* tumor promoting *(6),* antitumor promoting *(6, 13, 20),* and cytotoxic *(13).*

An Example: *Barringtonia asiatica*

One of us, R. A. B., developed a keen interest in ethnobotany and natural products chemistry as an undergraduate student. When an opportunity arose to spend part of a summer in Western Samoa, he searched the literature for an interesting project that would further these interests. A reference was found to the use of seeds of the futu (or vutu) plants as fish poisons by indigenous Polynesian peoples. While on the island of Upolu, Western Samoa, he collected seeds of this tree, *Barringtonia asiatica* (Figure 2), and tested them in the field for piscicidal activity using small fish from a river in Western Samoa. Some futu seed samples were placed in 70% ethanol and shipped back to Brigham Young University for subsequent testing, including the isolation and the bio-assay testing of the active ingredient, as well as the structure determination. Following grinding in a water/ethanol solvent mixture, removal of the pulp, and acidification, the liquid extract was exhaustively extracted with *n*-butanol. The solvent was removed and the material was separated into its components using preparative reversed phase HPLC. Using brine shrimp from the Great Salt Lake (Utah), the most retentive fraction was found to be most toxic. A literature search revealed that there had been very little work reported on compounds extracted from *Barringtonia asiatica.*

Following careful purification of the active material, the structure of the most active piscicidal compound from the futu seed extract was determined. The high resolution mass spectrum of the compound provided the molecular weight. Gas chromatographic analysis was used to confirm the presence of one glucose, one galactose, and a glucuronic acid unit. One-dimensional and two-dimensional NMR spectral experiments were utilized to complete the full structure. The most active component of the futu poison was determined to be a new compound: 3-*O*-{[β-D-galactopyranosyl-(1→3)-2-β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyloxy}-21-*O*-{[(2*E*)-2-methyl-1-oxo-2butenyl]oxy}-22-*O*-(2-methyl-1-oxobutoxy)-15,16,28-trihydroxy-(3β,15α,16α,22α)-olean-12-ene (Ranuncoside VIII). Figure 1a shows the structure. Recently, two other new saponins were isolated from the seeds of the futu plant, but neither was credited with piscicidal activity *(37).* Thus, as a result of his interest in the use of fish poisons in Polynesia, an undergraduate was able to gain valuable experience in many different areas of chemistry.

Summary

A brief review of fish poisons derived from plants used throughout the world, not only as piscicides, but for a range of other uses, including insecticidal and in folk medicines has been presented in the hope of providing a useful background for students interested in natural products. As more research is carried out on the bio-active compounds produced by these plants the potential for discovering new medical drugs increases. Much work is still to be done on the chemistry of these interesting plants.

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Note

1. The nut is grated on a piece of coral or rough lava rock. The gratings are then wrapped inside the leaves of a wild hibiscus tree and holes are poked through the leaves. The packet is then placed in the water to stupefy the fish.

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