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Tomatine and Furocoumarins: Toxins in Commonly Consumed Plants

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1. Introduction

It may seem that all that leafy thing your mom keeps on the windowsill does is pathetically wilt. However, under examination, that poor potted excuse of a pet truly is a fascinating tangle of chemical drama. Plants produce a range of secondary compounds, or secondary metabolites, which are chemicals that are not necessary for the plant's most basic, immediate survival, its primary metabolism.[1] While some of these compounds have currently unidentified functions, we have reason to believe they do serve some real purpose, as the secondary compounds' roles we have recognized are not trivial. Some of these secondary metabolites are used to attract pollinators, while others are defense chemicals. Many plants produce a variety of toxic compounds which aim to harm either herbivores, competing plants, or pests.[2] Some are produced in reaction to some sort of attack or adverse conditions, while others are present continually, latently ready to protect from potential harm. The latter are termed constitutive, as they exist in active forms in healthy plants, although their abundance may increase or decrease depending on conditions. The distribution of these defense chemicals are often tissue specific, targeting places that other organisms may be more likely to invade.[3] In the following chapters, we will explore the constitutive toxic secondary metabolites tomatine and furocoumarins. They both prevalently exist in those parts of plants we as humans consume.

2. A Profile of Tomatine

Tomatine is a glycoalkaloid that is present naturally in all parts of tomato plants. Tomatine, as it was originally isolated and is still sometimes talked about, is actually a mixture of two compounds: α-tomatine and dehydrotomatine. As seen in Figures 1-2, these differ only in one double bond.[4]

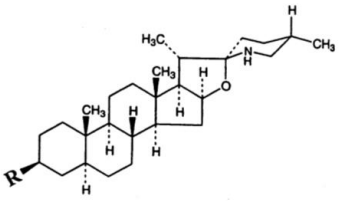

Figure 1. α-tomatine[4]

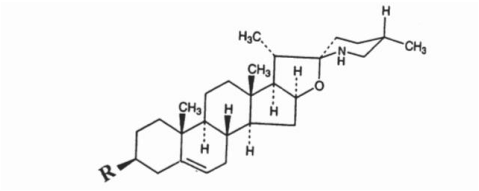

Figure 2. Dehydrotomatine[4]

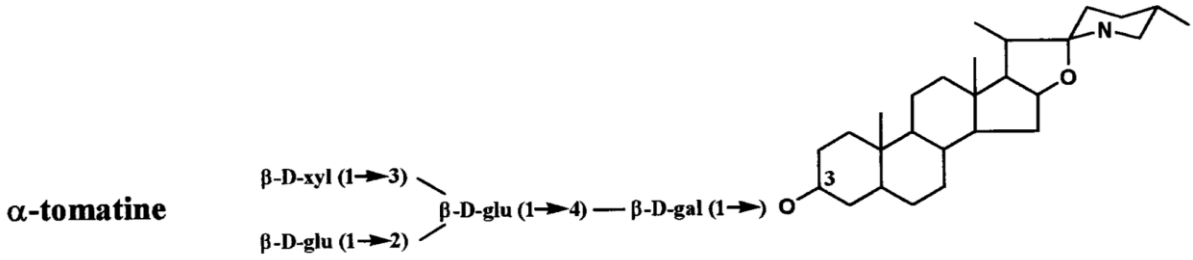

Figure 3. α-tomatine[5]

The R group in both complexes is a solatriose group, $C_{18}H_{32}O_{15,}$ a carbohydrate chain. As can be seen in Figure 3, it is composed of a xylose and glucose attached to another glucose which is bonded to a galactose and finally, by an oxygen, to the alkaloid. However, α-tomatine is the molecule focused on here, as it is better studied and understood. Also, the amount of dehydrotomatine in tomatoes is typically an order of magnitude below that of α-tomatine, further justifying this choice.[4] So, from now on, 'tomatine' will refer specifically to α-tomatine.

Tomatine has interesting chemical properties, having a hydrophilic saccharide side chain, a hydrophobic steroidal moiety, "and a polar–NH group, which can participate in acid–base equilibria."[4] While nitrogen is needed for tomatine's creation, carbon – rather than nitrogen – is the limiting factor in tomatine's synthesis; increasing N concentration in soil decreases tomatine synthesis in tomato plants. A likely explanation for this is that the ring with the substituted O forms first, followed by the glycosylation which adds the N-containing ring to the structure.

Tomatine, as well as other glycoalkaloids, originates as cholesterol, which in plants never accumulates but is immediately converted to other molecules.[4]

Tomatine's toxicity comes from the fact that it can form complexes with membrane-bound 3β-hydroxy sterols, form 1:1 insoluble complexes with these susceptible sterols.[4] When tomatine binds to these sterols, the integrity of the cell membrane is compromised.[5] The extent to which tomatine is able to disrupt membranes has been observed to be correlated with 3β-hydroxy sterol concentration in the membrane, supporting this mechanism of toxicity.

In more detail, as Figure 4 below shows, the glycoalkaloid tomatine (GA) binds to cholesterol in the cell membrane.[4] The alkaloid portion of tomatine interacts with the sterols, while the sugar groups are left outside of the bilipid membrane. Those sugar moieties interact with each other, hydrogen bonding with each other and forming a matrix. Even at this stage, the presence of the glycoalkaloids may be enough to cause a "loss of barrier function" for the cellular membrane. Eventually, when the matrix becomes large enough, a spherical or tubular vesicle may separate, which may immediately cause cell lysing or simply leave part of the membrane in need of repair.[6] Another possibility after tomatine binds with membrane sterols is shown in Figure 5; the tomatine present may cause a pore in the lipid bilayer to form as the sugar moiety matrix splits in two.[7] Increasing porosity of the cell membrane and lack of barrier between the cytosol and extracellular fluid creates a lack of cellular control over the cytosol's chemistry. This can lead to eventual cell death if severe enough or if the cell does not have the resources to repair.

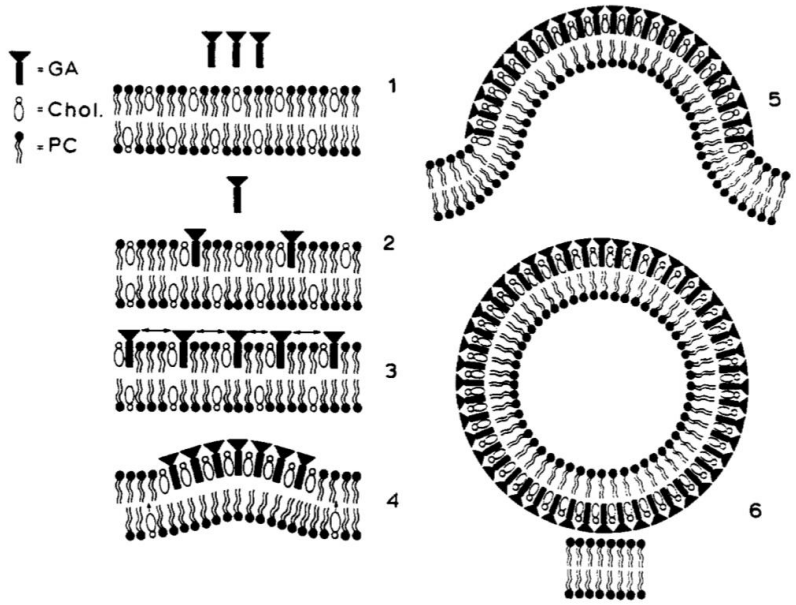

Figure 4. Membrane domain disruption and vesicle formation by tomatine[6]

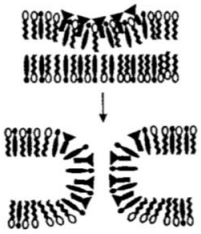

Figure 5. Pore formation by tomatine[7]

Membrane disruption is the most prominent chemical manifestation of tomatine's toxicity; the cell membrane is essentially broken open and extracellular fluid leaks in or cytosol leaks out, damaging or killing the cell.[4]

However, tomatine has been observed as toxic to species that do not contain 3β-hydroxy sterols in there membranes. Therefore, it must be deduced that tomatine is capable of some other method of toxicity.[8] During attempts to find this method, an alternative mechanism of tomatine-induced cell death has been observed, but has not been fully understood or characterized yet. In this additional mode of toxicity, it is theorized that tomatine first induces the production of reactive oxygen species (superoxide radical and hydrogen peroxide) in the target cell – potentially through the mitochondria. These hyper-reactive species do damage to the cell's

proteins. Simultaneously, the tomatine activates tyrosine kinase and G-protein signaling pathways which cause an elevation of calcium ion levels. Osmotic flow of water into the cell increases as the concentration of ions within the cell becomes unusually high. Eventual cell bursting results, releasing the reactive oxygen species to do damage to other cells. Calcium precipitating agents and tyrosine kinase inhibitors blocked this type of programmed cell death from tomatine, supporting this hypothesis.[5] This mechanism of toxicity explains the observation that cells without sterols are affected by tomatine. Both activities of tomatine have been observed to be correlated to rising pH, or indirectly correlated to rising H^+ concentration, indicating that tomatine in its unprotonated form is active, with maximal activity at pH 7.2.[4,9] In an acidic or especially basic solution, tomatine is then rendered benign. Tomatine's chemical toxicity is evident, but only partially understood at this time.

As a plant's secondary metabolite, tomatine's role has been shown to be as a defense chemical. Tomatine is most present in the leaves, flowers, and green fruits of tomato plants.[3] The tomatine in a tomato plant actually degrades at a predictable rate as the plant matures and the fruit ripens.[10] Thinking about the purpose of tomatine, to protect from animals and fungi that could harm the tomato plant, it makes sense that the levels are the most high in these vulnerable areas and before the fruit is mature. Some researchers suppose that tomatine production is under "separate genetic control" in different parts of the plant as an explanation for how varied the levels are between more vulnerable and less vulnerable areas of the plant.[11]

Tomatine defends the plants that produce it by fungal destruction and insect deterrant.[3] Tomatine strongly inhibits development of fungal phytopathogens that grow closely to the tomato plants' own tomatine-containing cells.[11] A couple of years ago, India's Departments of Crop Protection and Agricultural Entomology studied tomatine's insecticidal effects. Their

findings showed that tomatine exhibited antifeedant, repellant effects on all castes of termites when it was added to their food and chamber. This is one specific evidence of how tomatine is effective in keeping insect threats at bay.[12]

Some fungi have developed mechanisms to degrade tomatine with tomatinases – enzymes that break down tomatine – to combat tomatine's toxic effects. One study by the Southern Weed Science Research Unit showed a strong correlation between "tolerance to α-tomatine, the ability to degrade this compound, and pathogenicity on tomato."[8] This correlation points to tomatine as the barrier to fungal attacks on tomato plants; for fungi to successfully subvert tomato plants' defenses they must be immune to and have some way to break down tomatine. In Figure 6, the arrows represent some of the most common tomatinases fungal phytopathogens have developed, and the fungal species next to the arrow is one that contains the enzyme. The arrow points to the bond that the tomatinase cleaves; as can be seen, the mechanisms for action in each of these enzymes are different.[7] This is not a complete list of all fungi or all enzymes that can degrade tomatine, but represents well that they uniformly hydrolyze sugar residues. Some even cleave all four monosaccharides, leaving the aglycone tomatidine.[8] For tomatine, even the cleaving of one of the monosaccharides can eliminate almost all membrane-disruption due to the fact that it inhibits the sugar-sugar interactions which create

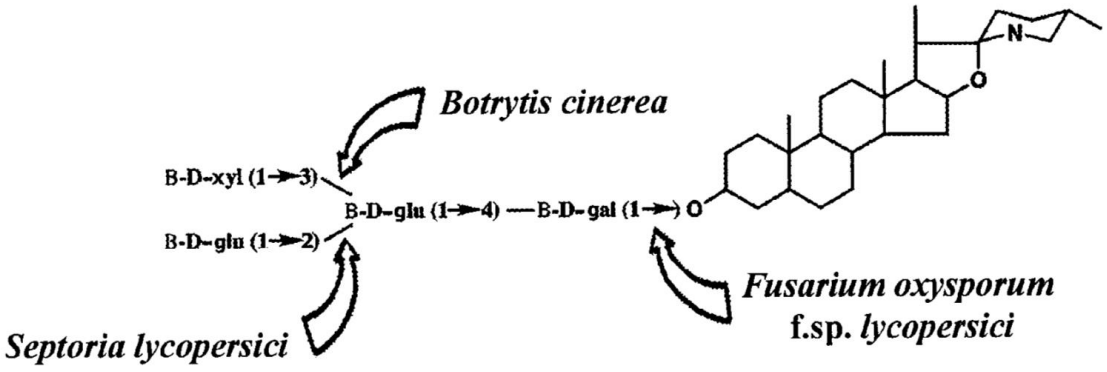

Figure 6. Cleavage sites of some tonatinases in fungal phytopathogens of tomato[7]

the irreversible glycoalkaloid matrix.[6] This is evidence of how integral the hydrogen bonding of the carbohydrate chains is to tomatine's toxicity.

Tomatine's aforementioned reduced presence and therefore toxicity over the course of tomato ripening is not a mistake of nature at all, but rather a brilliant tactic. At the beginning of the fruit's life, it is not advantageous for it to be eaten. It will stop maturing and stop developing the seeds. However, when the fruit is ripe, invasion is actually beneficial for the plant. If insects eat through the tomato's flesh, the mature seeds will probably fall out onto the fertile ground below. Should a larger animal eat the ripe tomato, the seeds will be digested and deposited elsewhere. This provides a mechanism for the tomato species' spread which it absolutely would not be able to supply on its own.[13] Of course, there is the threat of fungal or bacterial infection when the tomato plants lower their defenses. However, the cost-benefit analysis and exact pace of disarming has been fine-tuned by years of natural selection.

Tomatine's toxic effect can indeed take place in human cells if applied, and one should not be injecting this chemical. However, one should not avoid tomatoes at all costs in order to avoid these toxic effects. For one, in ripe, red tomatoes, tomatine levels are at their lowest compared to the unripe fruit.[4] Furthermore, tomatine is not absorbed very well in the human gut, and most of it is hydrolyzed to the harmless aglycone tomatidine.[14] There are not currently FDA guidelines about the maximum daily consumption of tomatine simply because the maximum plausible amount a person could eat does not come close to the toxic threshold for a human.[15] To illustrate that point, *in vitro* studies with mammalian tissue have shown that about 20 µg/mL is the concentration of tomatine needed to do significant damage, with 40 µg/mL necessary to kill the tissue.[16] In an actual tomato, tomatine is about 1,000 times less concentrated than that toxic density.[4] Considering the low concentration in a tomato being lowered even more as it is

spread throughout a human body, it is ludicrous to supposed that from ingesting tomatoes the toxic threshold of tomatine will be reached. With a functioning digestive system, we are not susceptible to tomatine's harmful effects. And of course, tomato consumption leads to many positive health benefits. Tomatoes contribute fiber and antioxidants to human health and also contain lycopene, which is well-established as inversely related to prostate cancer.[4] Interestingly, the counterpart to tomatine's disruption to cell membranes is that it can form its strong, insoluble complex with sterols in blood plasma. In an *in vivo* study on this effect in hamsters the blood plasma LDL cholesterol levels of the hamsters were lowered by tomatine consumption. This at least suggests that the same beneficial effect may take place in humans as well as we consume tomatine.[15] So, there is no need to be scared of bruschetta or reject too many slices of pizza. At a normal rate of consumption, tomato's tomatine is not toxic to humans.[14]

2. A Profile of Furocoumarins

Furocoumarins – also called furanocoumarins – are a class of secondary metabolites and are also defense chemicals. While the term furocoumarin denotes a whole class of compounds with variations, there are hallmarks that all have in common. Furocoumarins contain a furan ring bonded to a coumarin, which contributes the functional group of a ketone as part of its pyrone ring. As the furan may be joined anywhere, there are many isomers of these structures, and many derivatives with different atoms bonded to the core structure, making furocoumarins an overwhelmingly huge and variable class of chemicals. However, there are two isomers that by far dominate the distribution of these variations. Psoralen, shown in Figure 7, and angelicin, shown in Figure 8, are the two most common isomeric forms of the core structure of

furocoumarins. Psoralen and its derivatives are referred to as linear furocoumarins; angelicin and its derivatives are referred to as angular furocoumarins.[17]

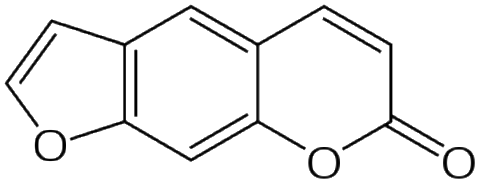

Figure 7. Psoralen[18]

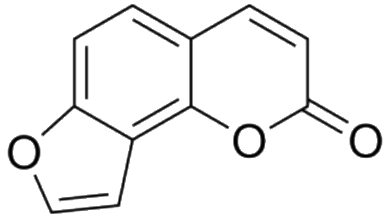

Figure 8. Angelicin[18]

In fact, in nature, furocoumarins are never present as just a single compound, pure and unadulterated. Rather, furocoumarins in plants are a mixture of many isomers and derivatives, some surely dominating, but none alone.[18] Furocoumarins are found in a variety of plants humans consume from the Umbelliferae and Rutaceae families. This includes parsley, carrots, celery, pansnips, and possibly most notably, all citrus contain relatively high concentrations of furocoumarins.[19] Still, each type of citrus contains a different distribution of isomers and derivatives of furocoumarins.[20]

Furocoumarins are phototoxic; UV light, specifically UVA light, initiates mutations in and involving them which lead to increased toxicity.[18] When exposed to UVA light, furocoumarins can be photoionized to radical cations. As is widely known, radicals within living organisms can do considerable damage. However, the most damaging way furocoumarins wreak havoc on cells is their interactions with DNA. Furocoumarins – usually linear – can intercalate in DNA, inhibiting replication or expression. Even without UV radiation, in darkness, furocoumarins form a molecular complex with DNA involving very weak bonds, really just intermolecular forces. Like shown in the Figure 9, the furocoumarin sits in-between the planes formed by the bases.[21] A linear furcoumarin is then in prime position to form bonds with the

DNA strands.[22] Furocoumarins only bind with pyrimidine bases – thymine, cytosine, and uracil, and do not form bonds with purine bases.[23] This preferential bonding may be due to pyrimidine's

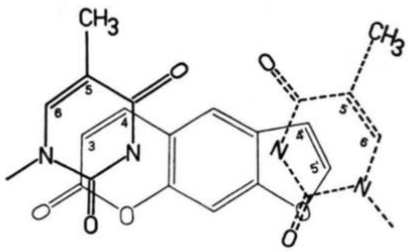

Figure 9. Psoralen intercalating between two thymines[21]

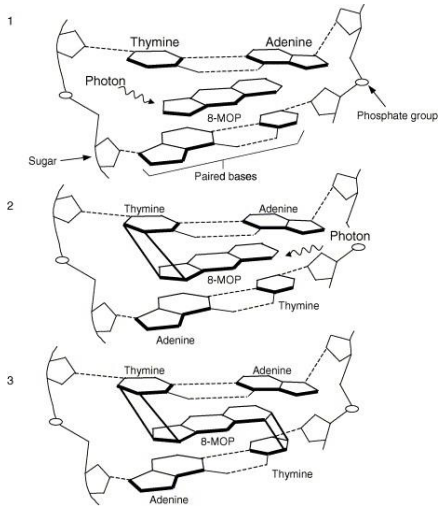

Figure 10. The furocoumarin 8-MOP forming a cross-link between two strands of DNA[26]

electron deficiency paired with the excited, free electron a furocoumarin generates.[24] Monoadducts within DNA form with one pyrimidine base and the furocoumarin; if this is to then form a diadduct there must be another pyrimidine base directly adjacent either above or below.[23] As shown in Figure 10**,** this happens when a photon in the UVA range – 365 nm being most favorable for the reaction – leads to photoinduced electron transfer and binding to the pyrimidine base above.[25] Then, another photon may excite the furocoumarin to bind with the pyrimidine base below, completing a diadduct cross-linkage.[26]

The first linkage between the furocoumarin and the DNA to form is a C_4 photocycloaddition between the double bond at the 3-4 position of the coumarin's pyrone or the 4'-5' position of the furan and the 5-6 position of the pyrimidine base.[23] Whichever double bond in the furocoumarin was not involved in the first linkage may interact with the thymine on the opposite strand to form the diadduct cross-linkage. However, computational studies have determined that the two possible monoadducts differ electronically and that this has implications for if another bond can actually form afterward. If the first bond to a nuclear base involves the pyrone, the bond is formed in the triplet manifold of the system, and the initially irradiated excited singlet state populates the lowest triplet state. This makes the resulting monoadducts relatively inaccessible to excited singlet states and a diadduct is very unlikely to form. However, if the furan side of the furocoumarin is the first to form a bond, it forms in the singlet manifold. After absorbing a photon, the singlet state of the furocoumarin is populated. An intermediate structure which is a conical intersection with the ground state leads to the formation of a ground-state, lowest singlet state monoadduct. From there, absorbing another photon leads to a diadduct. Almost exclusively, furan monoadducts lead to diadducts. However, even if a furocoumarin has formed a furan-side monadduct with DNA, if it has a sterically hindered pyrone, it will not easily form diadducts. The pyrone must also be accessible for cross-linking.[27]

Another aspect of this is that bifunctional furocoumarins are much more likely to cross-link than monofunctional furocoumarins; almost all monofunctional furocoumarins do not form diadducts.[27] Truly monofunctional furocoumarins, or de facto monofunctional furocoumarins due to an inaccessible pyrone, do not form a cross-link and therefore do not block DNA transcription or replication as efficiently as diadducts.[28] Consequently, bifunctional furocoumarins with accessible pyrones are more lethal to cells than monofunctional

furocoumarins. However, at very high concentrations, monofunctional furocoumarins can be just as lethal as bifunctional furocoumarins if they overload the cell's capacity for DNA repair.[29]

Interestingly, both monoadducts and diadducts may split into the original reactants of furocoumarin and nuclear bases if short-wave UV light reaches them. However, this does not guarantee DNA repair.[27] Another unique characteristic of these complexes is that unlike many phototoxic chemicals, furocoumarins are not photooxidative; diatomic oxygen is not necessary for their interactions with DNA.[30] Through their interactions with DNA, furocoumarins far increase the toxicity UV light usually has on organismal tissue.[18]

As defense chemicals, furocoumarins target and repel fungus and "various types of predators ranging from insects to mammals."[17] They even may serve as protection against bacterial threats. A study showed that furocoumarins were able to inhibit bacterial auto-inducers and communications between gram-positive and gram-negative bacteria. The furocoumarins found in grapefruit juice were also able to suppress biofilm formation of *E. coli, Salmonella typhimurium* and *Pseudomonas aeruginosa*.[31] Clearly, furocoumarins are a versatile defense system. Interestingly, like tomatine, furocoumarins do decrease in concentration in fruits as they ripen.[32] For example, it has been repeatedly observed that early-season grapefruit contain more furocoumarins than late-season grapefruit.[33] Again, like tomatine, this is generally attributed to ecological, evolutionary pressures.[34] At the beginning of the fruit's lifetime, while the seeds are still maturing, it is inopportune for their development to be halted. However, after ripeness, the advantage of having the seeds exposed, scattered, and planted outweighs the risk of the seeds being decimated by an herbivore.

Furocoumarins can also have detrimental effects on the human body. They can cause photosensitization toward UV light when administered to skin, which can cause pigmentation,

sunburn, blistering, and increased risk of skin cancer.[35] Ironically, furocoumarins used to be applied to skin therapeutically and added to sunscreens. The theory was that they could increase melanin production to protect against UV radiation. While this sometimes helped in the short-term, it did increase cancer in the long-term, even after furocoumarin contact ceased.[36] Besides sunscreen, furocoumarins used to be rife in cosmetics, where citrus oils are often a key ingredient. Again, applying a phototoxin directly to skin proved dangerous.[20] Resultingly, regulations on the furocoumarin concentration allowed in commercial cosmetics have been passed and enforced.[20] Many governmental regulatory agencies, including the European Commision, have enforced a limit of 1 ppm for furocoumarins in cosmetic products.[37] With this level present, use of cosmetics from legal manufacturers should not contribute to unhealth in the majority of the population.

When foods containing furocoumarins are ingested orally, the bioavailability of the furocoumarins is quite variable, dependent on things like dissolution from and stability in the matrix of the food. Whatever furocoumarins are available are quickly absorbed by the gastrointestinal tract and circulate in the blood. They are detectable in the blood plasma for an average of four hours after ingestion before being almost totally absorbed elsewhere or excreted.[38] The furocoumarins are taken up by the liver, brain, adipose tissue, kidneys, and, most notably, the skin.[36] Clearly, even if not smeared directly onto the skin by contact with a fruit or dermal product, furocoumarins may still travel close to the surface of the skin. There, within reach of UV light, they again become hazardous. To that point, a study last year found an association, albeit a tentative one in a study with a small sample size, between consumption of furocoumarin-containing citrus fruits and incidence of skin cancer.[19] However, independent of UV light, furocoumarins have the potential for harm as well. Furocoumarins interfere with the

uptake and metabolism of certain prescription drugs.[17] Depending on the mechanism by which the drug is delivered to the target cells, the furocoumarins may either cause an overdose or a reduced dose. This is so widely noticed that it has been termed the "grapefruit juice effect" as grapefruit is one of the most common fruits containing the furocoumarins that induce this phenomenon. In-vitro studies have confirmed that this is due to the furocoumarins inhibiting the intestinal cytochrome P450 3A4, which is integral to the metabolism of those drugs that are affected.[39] As far as the metabolism of the furocoumarins themselves, that subject is still cloudy for the scientific community. However, we do know that the human body does metabolize furocoumarins to a certain extent. The furocoumarin derivatives and their ratios found in human urine are different than those found in the food source or in the blood plasma, indicating metabolism in the body.[38]

So, how much is the average person exposed to furocoumarins? Data is not available for all of the world, but is for some developed nations. "Estimates for the average daily intake of furocoumarins via food in adults were published being in the range of 1.3 mg for the United States, 1.2 mg for the United Kingdom, and 1.45 mg for Germany."[18] The phototoxic threshold for furocoumarins is 20-30 ng of furocoumarin per milliliter of blood, or 20 mg of furocoumarin spread throughout the entire body. In a study with humans as subjects, this phototoxic limit "was not reached by the consumption of celery roots and other conventional vegetables under normal dietary habits…. However, the safety factor between the possible actual intake of furocoumarins and the phototoxic threshold dose is about 2–10, which is relatively small."[40] The safety factor in this context is the ratio between the phototoxic threshold and the maximum possible intake of furocoumarins a person could consume in food before encountering other complications, not to speak of personal discomfort. For orally ingested furocoumarins, in one day a person would have

to eat about ten kilograms of grapefruit or celery and even more of other plants to reach the threshold.[15] While this may seem like ridiculously much, and is, it is actually not as far out of reality as for other toxins. For most toxins in foods, like tomatine, the safety factor is typically between 100 and 1,000.[41] The maximum amount of furocoumarins one could ingest from food in a day is only two to ten times the phototoxic limit. If one's diet is even marginally varied, the amount of furocoumarins ingested will not be above the recommended amount.

Still, should one worry about the amount ingested? It's unclear. Our understanding of the metabolism of furocoumarins is currently underdeveloped.[38] Also, the exact mixtures of furocoumarins in plants are various. One study from University of Kaiserslautern in Germany concluded that a "practical threshold" of furocoumarin intake is difficult to decree.[18] My take is that the limit is probably different for every person and probably unknowable. We all have disparate genetic predispositions to skin cancer, unequal exposures to UV light, different levels of protective melanin in our skin. The "last straw", so to speak, to one person getting cancer and another not is often unknowable. For some, furocoumarin intake may be marginally important to a later health development, to others not. To prescribe a rigorous diet aimed at eliminating furocoumarins does not seem helpful. Actually, furocoumarins do have positive impacts on the human body. They are being investigated for potential free-radical scavenging activity. In moderate levels they can promote healthy bone growth and strengthening by activating osteoblast cells and inhibiting osteoclast reabsorption.[42] Additionally, the plants – celery, carrots, parsley, citrus – that contain furocoumarins have myriad health benefits which might outweigh the dubious advantage of cutting such foods out of one's diet.

In reflection, one of the aspects of furocoumarins that I think is most interesting is their varied nature in plants, that there is never just one compound in its pure form present or

produced in a plant.[18] Keeping in mind the furocoumarins' role as defense chemicals, I surmise that this belies the mixture's cure-all nature. The plants have a variety of weaponry that they can use indiscriminately, just as one concoction, and hopefully at least one of the specific compounds included work against the predator or fungi invading the plant. Of course, the trade-off here is that the one specific component that works best is in a lower concentration in the mixture than if it were secreted in pure form. However, it seems that natural selection has decided that the reward of being able to target more than one type of animal is far better than what potency is lost. Another benefit I see to a plant making several derivatives of furocoumarins rather than just one distinct compound is that no matter what elements or small compounds the plant happens to have excesses of at the time, they can be useful in making the defense compounds. If environmental factors mean that the plant cannot make one specific furocoumarin, be it lack of reactants or catalyst, it likely has the resources for the pathway to make at least one of the other derivatives at that time. Essentially, this means that there are rarely stalls in furocoumarin production. As long as psoralen or angelicin can be made, convenient derivatives can be as well.

4. Anticarcinogenic Applications

Ironically, after discussing the toxicity and damage these toxins have the potential to do to living tissue, it is salient to consider the applications tomatine and furocoumarins both have as anticarcinogens. When we consider the reality of cancer, the uncontrolled division of abnormal cells, this makes sense. Treating cancer necessitates killing a part of an organism. As that is what natural toxins already do, they are a promising avenue for scientists pursuing novel and alternative treatments for cancer.

4a. Tomatine

Tomatine is being investigated in various avenues as an anti-cancer drug, showing great potential.[14,43] Tomatine was shown in an *in vitro* study from a Malyasian group to be effective against prostate cancer by increasing cell membrane porosity and eventually inducing apoptosis. Tomatine also inhibited Nuclear Factor kappa-B activation, which contributes to the vitality and reproduction of cancerous cells through a variety of signaling pathways. The tomatine also showed a cytotoxic specificity and was not as toxic to normal human liver and prostate cells as it was to the prostate cancer strains.[44] While the group performing this experiment did not provide a rationale for this, I believe it may have been due to the fact that prostate cancer cells have a higher cholesterol content in their membranes.[45] The increased cholesterol in the membrane would make the prostate cancer cells more susceptible than the normal cells to tomatine's membrane disruption mechanism, which necessitates a binding to a 3β-hydroxy sterol. In fact, across the literature it is evidenced that almost all forms of human cancer involve some type of lipid metabolic reprogramming in the cancerous cells. Often, a symptom of this is increased cholesterol in the membrane. Another of the important manifestations of this metabolic dysfunction is the proliferation of lipid rafts. Sphingolipid and cholesterol tightly pack together and form a microdomain in the cell membrane. The compacted lipids are represented by the green lines and orange hexagons as sphingolipids and cholesterol, respectively, in Figure 11. In a normal human cell, these lipid rafts serve the purpose of compartmentalizing certain membrane functions.

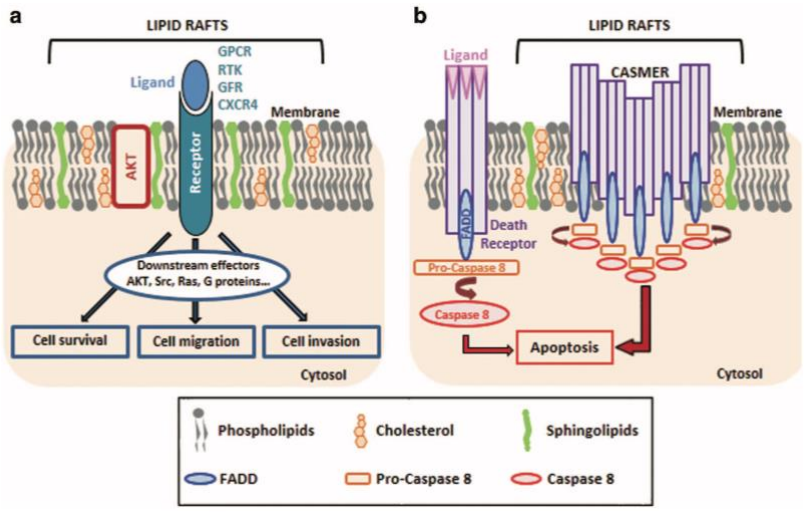

Figure 11. Lipid rafts compartmentalizing a protein receptor and a CASMER[45]

In a cancerous cell, these lipid rafts greatly increase in the membrane and encapsulate crucial receptors that regulate pro-oncogenic (left of Figure 11) pathways. Disrupting the abundant lipid rafts in cancerous tissue can destabilize the receptors that promote survival, migration, and invasion, limiting metastasis and cancerous spread.[46] Tomatine could be the drug that capitalizes on this fact of cancerous tissue. By binding to the cholesterol in the lipid rafts, tomatine would disrupt the structure. When lipid rafts containing these favorable receptors in cancerous cells are disrupted, cancer development and migration are reduced.[46] However, lipid rafts also stabilize CASMERs, 'cluster of apoptotic signaling molecule-enriched rafts' (right of Figure 11), which can lead quickly to apoptosis. While these form to some extent in normal cells, developing drugs to promote CASMER formation in cell membranes is another promising cancer treatment being pursued at this time. Of course, as this CASMER treatment is being applied, cholesterol to form the lipid raft is necessary, and cholesterol-depleting agents, including tomatine, are counterproductive.[47]

Alone, there is more evidence tomatine has been found to be an effective anticarcinogen even *in vivo*. A study by Friedman at the USDA was conducted with rainbow trout in which

tomatine clearly reduced tumor multiplicity. The trout were fed dibenzopyrene (DBP), a cancerous agent, and all developed tumors in the liver and stomach. While the same dosage of DBP was administered, tomatine was added to their daily regimen. After this addition to their diet, the tumor multiplicity was clearly reduced, as shown in Figure 12.

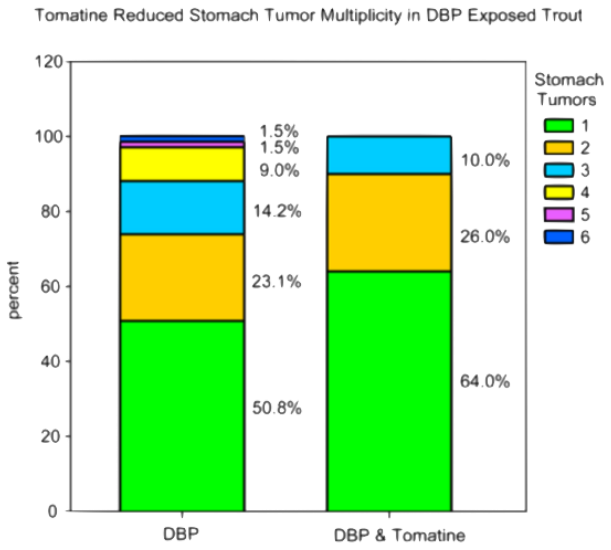

Figure 12. Tumor multiplicity with and without tomatine treatment[42]

In an *in vitro* study, also conducted by Friedman, with human cancer cells, not only pure tomatine, but simply green tomato extract dramatically inhibited cell growth.[43] In a similar study, Sucha *et al.* also found that tomatine had an antiproliferative effect on cancer cells. However, in their study, after a couple of days, the human cancer cells recovered from the initial bout tomatine served them and the levels of tomatine in the solution decreased. As the cells and the culture did not contain chemicals that could transform the tomatine, this disappearance was probably due to the tomatine molecules binding with cholesterol in the media the group used.[48] Tomatine is an exciting possibility on the front in the fight against cancer with built-in targeting for cholesterol-rich cancerous cells.

4b. Furocoumarins

Unlike tomatine, furocoumarins can attack cancerous cells at their source – dysfunctional, mutated DNA. Thanks to their inhibition of DNA replication, furocoumarins are a promising route for anti-cancer therapy. *In vitro* studies have shown that furocoumarins are able to inhibit the growth of breast and lung cancer.[36] Furocoumarins, through forming mono-adducts or cross-links in DNA, slow transcription and can induce p53, tumor suppressor gene, causing apoptosis. Another *in vitro* study with human cells at the University of Michigan found a strong dose-response relationship between the application of furocoumarins and the expression of p53 and resulting apoptosis. Diadducts rather than monoadducts were found to be more effective in inducing apoptosis as they slowed down transcription longer and allowed more time for p53 to be expressed. This sounds promising, but one must consider that p53 inactivation is a hallmark of most cancers.[28] This study was done using healthy cells, and in already cancerous cells the p53 gene may not be so easily activated. Outside of the question of p53, however, furocoumarins have continued to show promise in inhibiting cancerous cell growth. A treatment that has become relatively common is extracorporeal photophoresis. In this process, cutaneous T cell lymphoma has been treated by taking cancerous cells out of the body, adding furocoumarins, irradiating with UVA light, and injecting them back into the body. In long-term studies with human patients, this has improved overall survival and was met with relatively few side effects.[36,49] The exact mechanism of how and why this treatment works is still unclear.[50] What is known is that when these apoptotic cells are injected back into the patient, they are taken up by phagocytes and induce several changes in the phagocyte that may explain the effectiveness of extracorporeal photophoresis. While which change may be responsible is unknown, the changes are: decrease of proinflammatory cytokines; increase of anti-inflammatory cytokines; lowered

effectiveness at inducing T-cell responses, killing T effector cells, and inducing regulatory T cells.[51] This intervention in lymphoma is therefore termed immunomodulator treatment.[52] Using furocoumarins more extensively as anti-cancer agents depends on the development of some safe and specific form of administering them, some way of targeting just cancerous cells. This is especially important as furocoumarins can themselves induce cancer in healthy cells.

5. Consequent Philosophical Considerations

For secondary metabolites, the levels of the compound in the plant are impacted by the environment in which the plant is grown. As a secondary metabolite, variation in tomatine levels in plants can be observed to correlate with external factors. One especially interesting finding is that in one study, organic-grown tomatoes had double the mean levels of tomatine than conventionally-grown tomatoes. This comparison has a p-value of less than 0.001, which is extremely statistically significant.[14]

This result has many implications. For one, it supports the observation that carbon, not nitrogen, is the limiting reactant in tomatine synthesis. Since nitrogen is not available to plants due to photosynthesis, one may assume that it would be the limiting reactant in tomatine synthesis and surely adding more nitrogen would increase tomatine production. In that reality, the human-applied fertilizer would help the tomato plant's resistance to fungal phytopathogens and insect pests. On the contrary, conventional farming techniques supply excess inorganic nitrogen through fertilizer, and still the tomatine levels in such fertilized fields were not higher. However, in organic-style farming, soil microbes release nitrogen gradually, which provides the tomato plants with a more stabilized C/N ratio in the soil and "can result in a more balanced production of primary and secondary plant metabolites."[14] The organic farming – of course

necessitating rich soil with healthy microbes – actually promoted the tomato plants to produce tomatine and protect themselves. This brought the question to my mind: *are we just not allowing plants to create their own armor? Are we usurping nature's regulations?*

I think about pesticides – our attempt to garden better than nature – and how quickly, after a spraying, the herbivore population can bloom without a predator population to control it. Suddenly more spray is "needed," as all we see is the army of animals eating our crops and not the absence of those we have killed that could have restored balance. I consider the sheer amount of deer I have seen in my front yard on a city street. I am not advocating for us to quit killing mosquitoes or let wolves loose in playgrounds, I do not know the first thing about growing a successful crop and I definitely think we should do what it takes to feed as many humans as we can. But I do think that we should at least consider how our best intentions might be inadvertently contrarian to nature's controls.

An extension of that thought is that maybe tomatoes would be alright if we didn't intercede with pesticides. Tomato plants do produce more tomatine in adverse conditions.[14] Maybe the organically-grown tomatoes produced more tomatine not only due to the nitrogen ratio in the soil, but because they were being nipped every once in a while. Maybe the conventionally-grown tomatoes (ironic when you think of Mother Nature being the original farmer) were not producing so much tomatine simply because their leaves were already too chemicalized for any bug to want to nibble. When we do not let the tomato plants stretch their defensive muscles, the crops will need our replacements. I genuinely wonder what would happen if we tried to let tomatine take care of defense.

As Christians, something else worth pondering – although we will not know before death – is whether or not these toxins existed before the fall of man. Tomatine and furocoumarins are engineering with the specific purpose of harming another organism. How could these effects exist before the fall? Did the very chemical makeup of creation shift after sin?

One explanation could be theistic evolution. In this case, death – at least death in non-human organisms – was shaping life for the better. God's hand and will would have allowed fungi to be a little too exposed and animals to consume a little too much of the toxins in order to teach and shape creation into what He desired.

Another explanation could stem from the fact that these toxins decrease when the fruit ripen or when the growing conditions become more advantageous. It could be that in paradise, perfect growing conditions led to a very, very low toxin concentration. It also could be that the pre-fall animals and fungi only feasted on perfectly ripe fruits, having some sense of the right time for anything. In this way, even though the defense chemicals still existed, they would be latent. There would be no need for their use.

Another consideration brought to my attention is that even in a very literal interpretation of Genesis, death may have existed before the Fall. Of course, even herbivores had to eat something before sin, not to mention carnivores. Maybe the punishment of physical death was only new to humans. Possibly physical death for human beings was already part of the plan and the death of punishment was a spiritual death. A very good point by the *BioLogos* group from Grand Rapids is that death is, at least on the Earth as we know it, necessary for a healthy ecosystem.[53] So, maybe even before human sin tainted the world, tomatine and furocoumarins were actively protecting plants.

6. Conclusion

Whether we know when these chemicals first were synthesized or not, it is clear from the study of tomatine and furocoumarins that God has made a brilliantly complex and intelligent creation. Strategically synthesized and distributed, these secondary metabolites portray very disparate mechanisms of protection and toxicity. While tomatine primarily targets cells' outermost membranes, furocoumarins depend on reaching the heart of the cell and disrupting the genetic code itself.[6,21] These compounds are not just frivolously synthesized by the plants that produce them, but serve an important role as defense chemicals. As humans, we are large enough and have a varied enough diet that these toxins are not a concern for us in daily ingestion. But for smaller organisms this is not the case; these toxins contribute to curbing the population of fungal phytopathogens and they regulate predators to consuming and dispersing plants' seeds at maturity.[11,13] Their beautiful and targeted toxicity mean that tomatine and furocoumarins may someday play a role in defeating cancer. [36,43]

These components of the plants around us – not even necessary for plants' most basic needs yet still intricately formed – are evidence of the life, and life abundantly that Jesus sought to bring us. So, let's celebrate the defense chemicals, let's revel in the toxins.

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