Chemistry of St. John's Wort: Hypericin and Hyperforin

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Many herbal medicines are available in drug stores, in grocery stores, and especially, in "natural foods" stores. This is a growing business in the United States, now accounting for at least $4 billion in commerce per year *(1)*. One herb that has received significant attention recently is St. John's wort. In 2000, Americans spent about $200 million for St. John's wort and 1.5 million people in the United States used it on a regular basis *(2)*. At least five million others have tried it in recent years, making St. John's wort one of the most popular herbal remedies *(2, 3)*. Use of St. John's wort is even more significant in Europe where it is officially accepted and commonly prescribed, especially in Germany *(2b, 3)*.

The major selling point of St. John's wort is its appeal as *natural* antidepressant—it has been referred to as "Prozac from the plant kingdom" *(3)*. It is instructive to examine the science of the constituents of this popular herb. St. John's wort contains two major constituents with significant biological activity, hypericin and hyperforin. Both are complex molecules with unusual features; their chemical and biological properties are summarized below, along with relevant medical applications.

The plant St. John's wort (*Hypericum perforatum*) is a low evergreen shrub with attractive bright yellow flowers. It is grown in many gardens as ground cover and in many regions of the United States it grows wild. St. John's wort is known to be toxic to range animals: ingestion followed by exposure to direct sunlight can cause inflammation of skin and mucous membranes and may lead to death *(4)*. This photosensitivity is called hypericism and the active agent has been identified as hypericin *(4)*. It has been argued that in humans photosensitization is not likely to occur at the recommended dosage *(5)*, but warnings are often issued for fair-skinned individuals.

Medicinal Use

Species of *Hypericum* were known in ancient times—their use as medication was reported by many ancient Greek and Roman authors and has continued ever since *(3, 6)*. In folk medicine, St. John's wort was used internally against depression and "to soothe the digestive system" and was applied externally to wounds, burns, bruises, and sprains *(7)*. It has been used as powder, extract, tincture, infused oil, and cream *(8)*. In modern times, St. John's wort has been collected, dried, ground, and extracted. The powdered extract residue is then filled into capsules or pressed into tablets and sold. St. John's wort is recommended primarily for the treatment of mild depression, but has been used as an anti-inflammatory agent *(5, 8)*. St. John's wort has also been sold as a food additive *(9)* and is available as a "mood enhancer" in soft drinks[1] *(9c, 9d)*. One ingredient of St. John's wort, hypericin, has shown antiviral activity against several types of viruses, including the human immunodeficiency virus (HIV) *(10, 11)* and has been tested as a photosensitizer in the treatment of cancer *(11)*. Much is known about this unusual herb, but the information is buried in a bewildering array of hundreds of scientific papers.

St. John's wort is a complex mixture of chemicals including tannins, flavonoids, xanthones, and phloroglucinol derivatives *(3, 12)*. Since health effects occur at the molecular level, it is important to identify the responsible molecules, the active ingredients. The most commonly cited active ingredients in St. John's wort are hypericin and hyperforin, two chemicals with very different structures. Most commercial samples in the United States contain 300 mg of herbal extract per dose and are advertised as being standardized to contain at least 0.3% hypericin. The chemistry of hypericin is surprisingly complex and quite fascinating: it is a large aromatic molecule in the shape of a propeller; ten tautomeric isomers are in equilibrium; it has a hydrogen deficiency index of 23; it is a potent photosensitizer; and it is very acidic. Recent evidence indicates that another ingredient, hyperforin, might be responsible for the antidepressant activity of St. John's wort. This molecule is totally different: it consists of a bridged eight-carbon ring with four large substituents; it has tautomeric structures; and it is very active in biological systems.

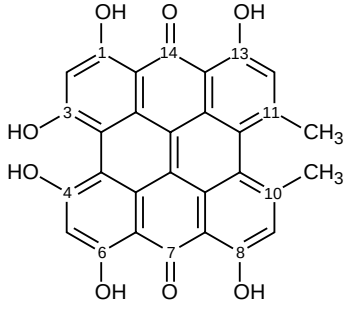

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Figure 1. Structure of hypericin.

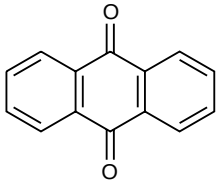

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Figure 2. Structure of anthraquinone.

Hypericin

For many years the main active ingredient in St. John's wort has been identified as the chemical hypericin, **1**, which has the formula $C_{30}H_{16}O_8$ and is shown in Figure 1. The numbering of carbon atoms proceeds counterclockwise around the periphery, counting only carbons that can have substituents.

Pure hypericin was first isolated by Brockmann and coworkers in 1942 *(13),* who proposed an initial structure, which was later modified to that shown in Figure 1 *(14).* This structural assignment was substantiated by the laboratory synthesis of hypericin *(15).* In the plant and in the extract hypericin is normally accompanied by an analog, pseudohypericin, which has a hydroxyl group on the methyl group bonded to C-10 *(16).*

Hypericin is a distant analog of *para*-quinone and its fundamental structure is similar to that of anthraquinone, **2** (Figure 2). In the comparison to anthraquinone, hypericin has a much more extensive aromatic system, where three rings have been replaced by eight rings. The hypericin ring system is fully aromatic and each ring carbon is sp^2 hybridized. Because of this aromaticity, hypericin can be expected to be planar. However, the structure is much more complex, because steric strain, caused by two major repulsive interactions, distorts the molecule, causing a pronounced twist (Figure 3) *(6, 17).* The steric interaction of the two methyl groups on carbons 10 and 11 is the strongest; the angle between the two methyl groups is about 32°. Steric strain between the two hydroxyl groups on carbons 3 and 4 distorts that side of the molecule by about 20°. The deformed ring system can be represented in perspective, **3a**, or in a side-view, **3b**, seen from the bottom of the first structure, looking upwards.

In the conformation shown in Figure 3, carbons 3 and 11 are on opposite sides of the ring plane with C-3 up and C-11 down. Likewise, C-4 is down and C-10 is up. This deformation results in a helical twist of the molecule and is said to be a propeller conformation. The structure has been determined by X-ray crystallography *(17a, 17b)* and is in agreement with molecular modeling calculations *(17c).* This conformation has a mirror image in which the flaps that are up in structure **3a** are down and those that are down in structure **3a** are up. This structure is not superposable on the original and is its mirror image: its enantiomer. The energy barrier to interconversion of the two propeller conformations has been calculated to be about 80 kJ/mol *(18).*

Another level of complexity is added by the fact that the carbonyl groups in hypericin, shown at C-7 and C-14, are tautomeric with the adjacent hydroxyl groups at C-6 and C-8 and C-1 and C-13, respectively. This tautomeric relationship is illustrated for carbons 1 and 14 of structure **1** in Figure 4. In structure **1** the relevant proton is attached to the oxygen on C-1 and in the second structure, **4**, it is bonded to the oxygen on C-14. These two tautomers are identified by the location of the two carbonyl groups in hypericin as 7,14-dioxo, **1**, and 1,7-dioxo, **4**. Similar proton exchanges give rise to a total of four additional tautomers of hypericin: 1,6-dioxo, 1,8-dioxo, 7,13-dioxo, and 8,13-dioxo *(8, 19).* In addition, the hydroxyl groups on C-3 and C-4 can be tautomeric, giving rise to four more tautomers: 1,4-dioxo, 3,4-dioxo, 3,7-dioxo, and 3,8-dioxo. These tautomers are apparently less stable than the first six *(8, 19).*

The 7,14-dioxo tautomer of hypericin **1** has been calculated to be most stable by at least 45 kJ/mol *(17a, 19b).* This tautomer seems to be present in the crystalline state, because X-ray crystallography has shown the carbon–oxygen bonds of C-7 and C-14 to be the shortest *(17a).* In solution with polar solvents such as DMSO hypericin is present as the 7,14-dioxo tautomer *(19c)* and, surprisingly, in nonpolar solvents like THF the 1,6-dioxo tautomer has been reported to predominate *(19c, 19d).* However, the presence of the 1,6-dioxo tautomer has recently been challenged on the basis of detailed NMR analysis *(20).*

Hypericin is phenolic and phenols are weakly acidic (pK_a = ~10), yet hypericin is very acidic with pK_a = 1.7–2.0 *(17b, 21)*; it is almost as strong an acid as the phenol picric acid (pK_a = 0.4). Hypericin has six phenolic protons; however, the proton on the hydroxyl group on C-3 is lost preferentially *(17b).* The resulting anion is stabilized by two major factors: there is strong hydrogen bonding from the adjacent

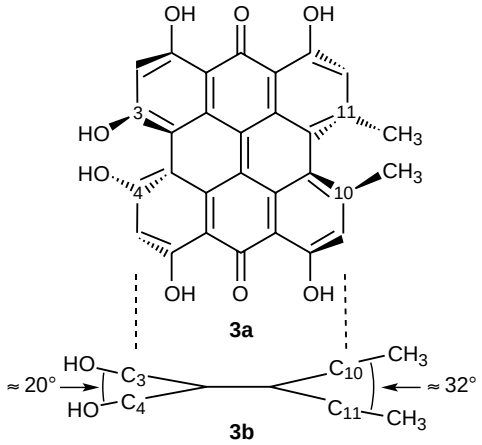


Figure 3. Structure showing the twist in the hypericin molecule because of steric strain between the two methyl groups and the two hydroxyl groups.

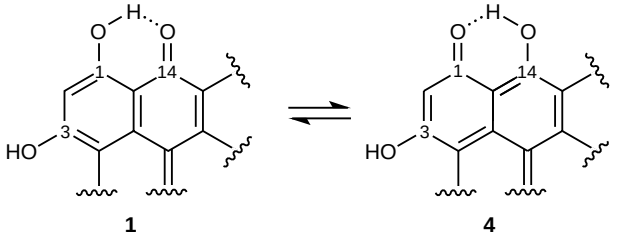
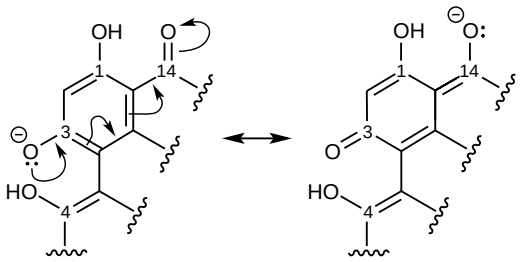


Figure 4. Example of one of the tautomeric relationships observed in hypericin. Only a portion of both structures is shown in this figure.



5a **5b**

Figure 5. Resonance stabilized anion of hypericin. Both **5a** and **5b** are portions of structure **1**.

hydroxyl group on C-4 and the negative charge on the oxygen is delocalized by resonance to the carbonyl group of C-14, which is in the para position of the shared benzene ring *(19b, 21)*. The second effect, the resonance stabilization, is illustrated in Figure 5 for the carbons cited. It must, however, be remembered that, because of the symmetry of hypericin, the same effects result from loss of the proton from the hydroxyl group on C-4 or simply the shift of the hydrogen bonded to the two oxygens on C-3 and C-4. In addition, similar resonance structures of the anion of hypericin can be drawn for the other tautomers. The anion exists primarily as the 7,14-dioxo tautomer, **5a**, and its conformation is almost identical to the propeller conformation of the parent hypericin *(19b)*.

The anion of hypericin is so stable that in nature hypericin occurs primarily as salt, consisting of this anion and a positive potassium ion *(22)*. Hypericin is almost insoluble in most organic solvents, but dissolves readily in pyridine, because it protonates the amine, forming the anion discussed above. The resulting solution is bright red and shows red fluorescence. The salt of hypericin is soluble in polar organic solvents such as DMSO and aqueous ethanol; it shows similar color and fluorescence *(17b)*.

Isolation and Analysis

The isolation of hypericin from plant material is relatively easy, but its purification is more difficult and requires chromatography. Originally, St. John's wort was extracted with methanol and treated with hydrochloric acid. When the resulting precipitate was dissolved in pyridine and acidified, crystals of hypericin formed *(13)*. Recently, optimization of extraction conditions has been studied in detail *(23)* and the best conditions found were found to be extraction with equal quantities of ethanol and acetone for five hours at 55 °C. Many other methods for the extraction have been published, including pressurized liquid extraction *(24a)* and sonication in methanol *(24b)*.

HPLC seems to be the method of choice for the separation of various constituents of the extract of St. John's wort. The most common detection methods seems to be photodiode array *(23, 24b, 25)*, but fluorescence detection has also been used *(26)*. One unusual method for determining the composition of St. John's wort is the direct analysis of the extract, without separation, by two-dimensional proton NMR spectroscopy *(27)*.

The NMR spectrum of hypericin has been studied in detail *(17b, 19c, 19d, 20)*. In deuterated DMSO the following spectrum was observed: four aromatic hydrogens (δ 6.59 and δ 7.46), six benzylic hydrogens (δ 2.75), four phenolic hydrogens (δ 14.74 and δ 14.09), and two phenolic hydrogens (δ 8.1–8.3) *(20a)*. The last signal was assigned to the hydroxyl protons on C-3 and C-4.

Hypericin has an extended aromatic system and absorbs light in the visible region resulting in a bright red color. The strongest absorption occurs at about 590 nm with other bands at lower wavelengths *(11, 19d)*. This absorption is very significant for the role of hypericin as photosensitizer *(11)*.

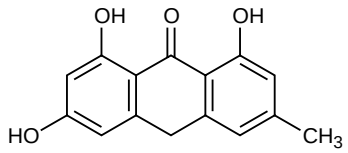


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Figure 6. Structure of emodin anthrone, a key intermediate in the biosynthesis of hypericin.

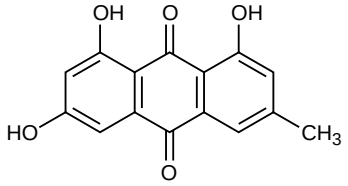

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Figure 7. Structure of emodin, an intermediate in the synthesis of hypericin.

Biosynthesis of Hypericin

The biosynthesis of hypericin from the simple acetyl group seems to be one of those impressive marvels of enzyme productivity and specificity. A series of reactions forms the key intermediate emodin anthrone, **6** (Figure 6) *(28)*, which is exactly half of the structure of hypericin **1**. Dimerization of this compound leads to a direct precursor of hypericin, appropriately called protohypericin (structure not shown). The final step in the biosynthesis, the conversion of protohypericin to hypericin, is a photochemical cyclization that has been observed to occur quantitatively *(28, 29)*. A similar series of reactions has been employed in the synthesis of hypericin.

Synthesis of Hypericin

The symmetry of the hypericin molecule clearly points to the dimerization of a derivative of emodin anthrone, **6**, as an appealing synthetic route and most of the syntheses have been based on this approach. The first synthesis was reported by Brockmann, starting with an emodin derivative *(15)*. In a different approach, emodin, **7** (Figure 7), was dimerized when heated under basic conditions with the reducing agent hydroquinone *(29a, 30a)*. The dimeric product formed hypericin in 25–29% yield, when it was irradiated. Modifications in this synthesis of hypericin have been reported *(30b, 30c)* and many analogs of hypericin have been synthesized in similar reactions *(31)*.

Hyperforin

St. John's wort has been reported to be an effective antidepressant. For many years the active ingredient responsible for the antidepressant activity was assumed to be hypericin, hence all medications are standardized for it, normally to 0.3% hypericin. However, the significance of the role of hypericin had not been established clearly and it was also found that extracts without hypericin retain antidepressant activity *(32a).* In 1998 it was suggested that the antidepressant activity of St. John's wort might be due to another ingredient, the complex molecule hyperforin *(32).* Since then hyperforin has been investigated extensively. The structure of hyperforin bears no relation to that of hypericin: it is bicyclic, oxygenated, and unsaturated, but not aromatic. The formula of hyperforin is $C_{35}H_{52}O_4$ and its structure is shown in Figure 8. It is classified as a derivative of phloroglucinol. In the dried herb hyperforin occurs in a relatively high concentration of 2–4% *(33);* however, it is quite sensitive to air oxidation and content in the herbal drug may vary.

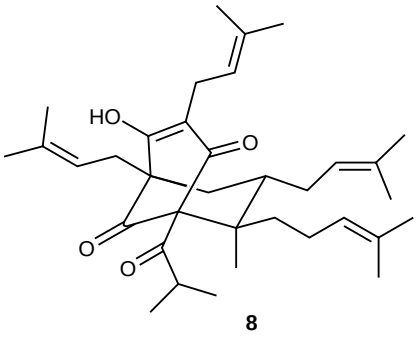

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Figure 8. Structure of hyperforin.

Isolation and Analysis

Hyperforin can be obtained from St. John's wort extract or directly from plant material by extracting with heptane or by using supercritical carbon dioxide *(33).* Purification is achieved through chromatography. The structure of hyperforin was determined by classical methods *(34a)* and the absolute configuration was found by X-ray crystallography *(34b).* All signals in the ^1H and ^{13}C NMR spectra of hyperforin have recently been assigned unequivocally *(35).*

Biosynthesis of Hyperforin

Because of its complex structure, it is intriguing to consider the biosynthesis of hyperforin. This mechanism has been elaborated recently using ^{13}C-labeled glucose, followed by detailed NMR analysis of the resulting ^{13}C-labeled hyperforin *(35).* Apparently, the biosynthesis of hyperforin involves the reaction of acyl phloroglucinol **9** (Figure 9) with five different isoprene units, each formed by the deoxyxylulose pathway. The three ring oxygens of compound **9** are retained and are apparent in the bicyclic system of hyperforin.

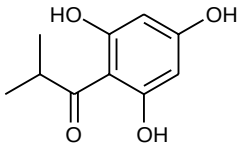

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Figure 9. Structure of acyl phloroglucinol, a reactant in the biosynthesis of hyperforin.

Synthesis of Hyperforin

Surprisingly, the synthesis of hyperforin has not yet been reported. Recently, related bicyclic ring systems have been prepared *(36)* and these efforts may lead to the synthesis of hyperforin in the near future.

Antidepressant Activity

St. John's wort is used primarily to control depression, therefore it is important to understand its neuropharmacology, the biochemical mechanism by which it might exert its purported antidepressant effects. Initially, researchers believed that hypericin in St. John's wort exerted an inhibitory effect on monoamine oxidase (MAO) *(37).* MAO inhibition prevents the breakdown of key neurotransmitters, including norepinepherine and 5-hydroxytryptamine, thus increasing the time in which they remain active in the synapse, able to exert their biological effect. This relates to the amine hypothesis of depression, based on the idea that decreased concentrations of neurotransmitters in the brain lead to depressive disorders. A number of the main synthetic antidepressants act in this manner by effectively increasing the concentrations of these neurotransmitters in the synapses in the brain. However, for St. John's wort this relationship could not be established clearly and MAO inhibition by extract or pure hypericin was found to be too weak to be effective with normal dosage *(38).*

When it was proposed that hyperforin might be the active ingredient in St. John's wort, the molecule was studied in detail. Results indicate that hyperforin's antidepressant activity might also be caused by altering the neurotransmitter balance in the brain. Hyperforin has been shown to be a potent inhibitor for the uptake of several neurotransmitters, including serotonin, dopamine, norepinepherine, γ-amino butyric acid (GABA), and L-glutamate *(32a, 39).* Inhibition of serotonin re-uptake is a mechanism used by a major class of synthetic antidepressants, the serotonin-selective re-uptake inhibitors (SSRI), including such well-known names such as sertraline (Zoloft) and fluoxetine (Prozac) *(40).* However, unlike these substances, hyperforin also inhibits the uptake of the two amino acid neurotransmitters GABA and L-glutamate. This might indicate a different mode of action *(39c)* and several labs are actively pursuing a more definitive mechanism *(41).* Structure–activity studies of hyperforin analogs have shown that the enol system in hyperforin is required for inhibition of serotonin re-uptake *(41c).*

After the many years of using St. John's wort to combat depressive disorders, the question of its clinical efficacy remains open and continues to be discussed at length in the scientific literature. A number of clinical trials have exam-

ined the antidepressant activity of St. John's wort and these studies have been extensively reviewed *(3, 42)*. The results of these investigations seem to indicate that St. John's wort is more effective than placebo for mild to moderate depression, but each review calls for longer-term studies. Another weakness is that the material was not standardized for hyperforin content.

Recently these results have been challenged by two major studies in the United States. The first study was directed by R. C. Shelton of Vanderbilt University and examined 200 patients with major depression for eight weeks in a double-blind study in which patients received either St. John's wort extract or a placebo *(43)*. Scores on the Hamilton depression scale were used for analysis. From the resulting scores it was concluded that the effect of the extract was roughly equal to that of the placebo, rendering it ineffective. In the second study, led by Jonathan Davidson of Duke University, 340 patients with major depression were tested in a similar manner for eight weeks, using extract, placebo, or sertraline (Zoloft) *(44)*. On the basis of scores on the Hamilton depression scale, responses were judged not to be different for the three groups. The results of both tests have been publicized extensively in the press *(2a, 2c, 45)* and discussed in journals *(46)*.

While the results of these two recent studies independently show that St. John's wort extract is not effective for treating *major* depression, it remains to be determined how effective it is in the treatment of *mild* to *moderate* depressive disorders, for which it is recommended. Another confusing issue is that in the Shelton study, St. John's wort was not tested against an established antidepressant and in the Davidson study, the antidepressant sertraline also performed only as well as the placebo. A well-designed, large-scale clinical trial that addresses a population suffering from mild or moderate depressive disorder is an obvious area for further research and some such trials have been reported to be underway *(46a)*.

Antiviral Activity

Hypericin has a wide range of antiviral activity, both in vitro and in vivo. Hypericin has been shown to inactivate a number of viruses and retroviruses, including HIV, influenza A, herpes simplex, and others *(10, 31, 47)*. One common characteristic of all of these viruses is that they are "enveloped" viruses, having a glycoprotein sheath surrounding the protein shell that contains the viral genetic material. In contrast, nonenveloped viruses, such as poliovirus and adenovirus, are not affected by hypericin. The mechanism of the antiviral activity of hypericin has not been established clearly; however the reported antiviral activity is greatly enhanced by light and, therefore, one proposal is that hypericin interferes with viral assembly via light-induced generation of reactive singlet oxygen. This may cause disruption of proteins in the viral envelope *(47, 48)*. Antiviral activity may also be caused by proton transfer from an excited state of hypericin *(49)*.

To fully utilize the antiviral activity of hypericin, light of about 600 nm is needed, but is not available in many parts of the body. In one very imaginative approach, a chemiluminescent light source was attached directly to the molecule: luciferin was bonded to hypericin *(31)*. This light delivery system has been called a "molecular flashlight". However, it has been found to be less effective than a continuous light source *(31)*.

Initially there was much hope that hypericin therapy might be a useful against HIV *(31, 50a)*, however clinical studies have shown no success *(51b)* and it is now believed that hypericin will not be used directly against HIV *(6)*. However, similar therapy might become very significant in the antiviral treatment of human blood for transfusion. When human blood containing hypericin is irradiated, HIV and other enveloped viruses are inactivated *(52)*.

Cancer Treatment Activity

Hypericin is a potent natural photosensitizer and its photochemistry has been studied in great detail; it is considered so significant that recently a whole issue of the journal *Photochemistry and Photobiology* was devoted to the topic *(51)*. Hypericin has been tested extensively as photodynamic agent for the treatment of cancer *(53)*.

As a photosensitizer, hypericin absorbs light and is raised to an excited state. Once excited, hypericin can react with molecular oxygen to form singlet oxygen, which is very reactive and can oxidize different substrates *(53)*. This type of reaction is called a type II photosensitizing mechanism. The highest yield of singlet oxygen is obtained when hypericin is irradiated at about 600 nm. Hypericin in the excited state can also react with molecular oxygen to generate superoxide radicals in a photosensitization reaction called type I, but this seems less significant *(53)*. If hypericin is imbedded in a cell either mechanism can be toxic, because the reactive forms of oxygen can oxidize lipids and damage cell membranes *(53, 54)*. Cell damage may also occur from a significant decrease of pH that occurs after irradiation of cells containing hypericin *(55)*. Other factors may also be involved, for example there could be interference in signaling within the cell *(53, 54)*.

Hypericin molecules can enter cells because they are amphipathic, meaning that they have both hydrophobic portions (the hydrocarbon regions) and hydrophilic portions (the hydroxylated regions). Thus, a hypericin molecule can insert itself into the cell membrane with the hydrophobic end inside the lipid bilayer of the membrane and the hydrophilic end in the aqueous medium of the extracellular environment *(10, 53)*. If hypericin is irradiated when it is in a cell, photosensitization may destroy this cell. The resulting technique is called antitumor photodynamic therapy and has been summarized well in a recent review *(53)*.

In photodynamic therapy in vivo, hypericin is injected into a tumor, which is then irradiated by laser *(11)*. This approach has been used externally *(56)* and was successful against carcinoma in humans *(56c)*. If the tumor is internal, hypericin can be injected into a tumor and irradiated by a laser using microfiber optics *(56d)*. Similar photodynamic therapy is undergoing clinical trials against psoriasis *(53)*. While photodynamic therapy with hypericin is still experimental, it is a unique utilization of natural products chemistry.

Safety of St. John's Wort

The regular use of St. John's wort seems to entail few side effects. One concern is overexposure to sunlight, because hypericin is a strong photosensitizer *(4)*. This effect has been observed in humans when receiving high doses of the herb; with normal use, only a small amount of additional sensitiv-

ity was detected *(57)*. However, warnings against excessive exposure to sunlight while taking St. John's wort are listed on most containers of the extract. As is common, use during pregnancy or while nursing is not recommended.

Interference with Prescription Drugs

One very serious concern is that the use of St. John's wort interferes with the efficacy of conventional medications by increasing their rate of metabolism *(58)*. The U.S. Food and Drug Administration issued a warning against taking St. John's wort with a number of other prescription medications *(59)*, including indinavir for HIV infection, the immunosuppressant cyclosporin for organ transplantation, and oral contraceptives *(60)*.

Hyperforin has been identified as the cause of this interactive effect; it can induce the production of the liver enzymes cytochrome P450, which oxidize many drugs in preparation for excretion *(61)*. Generally speaking, these enzymes function by inserting oxygen into bonds of the chemicals being metabolized, resulting in a variety of breakdown products that can be eliminated more readily *(62)*. Hyperforin activates a particular receptor that induces the production of a specific enzyme that increases the rate of metabolism of medications, thus decreasing their concentration in the human body *(61)*. It has been established that hyperforin has a very high affinity for the receptor mentioned, and the crystal structure of a hyperforin–receptor complex has just been published *(63)*.

Perhaps as many as half of all ingested drugs are broken down by the cytochrome P450 metabolic pathway; all of these medications may be affected by regular use of St. John's wort. Clearly, harmful consequences could result from this effect, especially if the patient were unaware of this interaction. A warning label should alert users to this danger. This finding should also prompt an immediate re-evaluation of the use of St. John's wort as food additive.

Summary

St. John's wort is a common plant that has been used medicinally for over 20 centuries. Only recently has its medicinal activity been investigated scientifically. St. John's wort has been found to contain at least two major constituents with unusual structures, each with pronounced biological and medical properties. At present, the chemistry of St. John's wort remains an exciting and active area for biochemical and medical research. It is most impressive that generations ago people were able to discern this plant's unusual characteristics.

Note

1. An example is the beverage "Wisdom" by SoBe, which is owned by Pepsi Cola. It contains ginko biloba, St. John's wort, and gota kola.

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