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GOSSYPOL AS A BIARYL POLYPHENOLIC SESQUITERPENOID ATROPISOMER OBTAINED FROM COTTONSEED OIL USED IN INFERTILITY

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ABSTRACT
Gossypol is a polyphenolic compound isolated from cottonseed. There are two optical enantiomers of gossypol, (−)-gossypol and (+)-gossypol. Gossypol exists as three different tautomers, aldehyde, ketone and lactol. Gossypol is toxic and provides a protective mechanism for cotton plants against pests. Gossypol was used as a male contraceptive in China in the 1970s. It was eventually abandoned due to noticeable side effects, disruption of potassium uptake and incomplete reversibility. Gossypol has gained considerable research interest due to its attractive biological activities, especially antitumor and antivirus. Gossypol derivatives are prepared by a structural modification to reduce toxicity and improve their therapeutic effect. This review depicts the bioactivity and regulation mechanisms of gossypol and its derivatives as drug lead compounds, with emphasis on its antitumor mechanism. The design and synthesis of pharmacologically active derivatives based on the structure of gossypol, such as gossypol Schiff bases, apogossypol, gossypolone, are thoroughly discussed. This review aims to serve as a reference for gossypol-based drug discovery and drug design. Polyphenols are naturally occurring compounds found in fruits, vegetables, cereals, and beverages. Polyphenols occupy a unique place in biological science for their pharmacological properties. Gossypol is a polyphenolic compound that has attracted attention because of its biological effects. Gossypol is reported to exhibit antifertility, antioxidant, anticancer, antivirus, antiparasitic, and antimicrobial properties and lower plasma cholesterol. These are summarized with attention to the mechanisms of activity. The results of these studies provide a comprehensive understanding of the biological action of gossypol and its potential for the prevention of and therapy for resistant tumors and chronic human diseases such as HIV, malaria, and psoriasis.

KEYWORDS: polyphenol, sesquiterpene, biaryl, chiral, infertility.

INTRODUCTION

Roger Adams (born Jan. 2, 1889, Boston—died July 6, 1971) was a chemist and teacher known for determining the chemical constitution of such natural substances as chaulmoogra oil (used in treating leprosy), the toxic cottonseed pigment gossypol, marijuana, and many alkaloids.

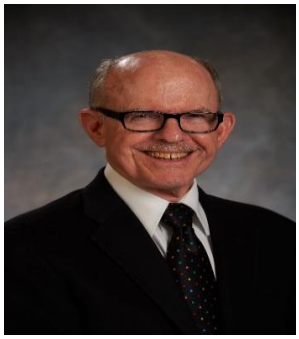

Figure-1: Inventor [Gossypol].

Gossypol [CAS: 303-45-7] is a yellow polyphenolic compound naturally occurring in different parts of cotton plant with the highest concentration in the seeds. It acts as a natural defensive agent against predators, and provoks infertility in insects. The molecular formula of gossypol is $C_{30}H_{30}O_8$. The chemical structural formula of gossypol is 2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene).[1-3] Gossypol is a sesquiterpenoid produced naturally by cotton *Gossypium hirsutum*, which has antifungal properties that demonstrated strong growth inhibition activity against many economically important fungi, such as *R. solani* and *F. oxysporum*. Gossypol is a natural phenol derived from the cotton plant (genus Gossypium). Gossypol is a phenolic aldehyde that permeates cells and acts as an inhibitor for several dehydrogenase enzymes. It is a yellow pigment. The structure exhibits atropisomerism [Atropisomerism is stereochemistry arising from restricted bond rotation that creates a chiral axis. Atropisomers are subject to time-dependent inversion of chirality via bond rotation, a property which in drug molecules introduces complexity and challenges for drug discovery and development processes], with the two enantiomers having different biochemical properties. An atropisomer is a type of molecule that exhibits chirality due to hindered rotation around a sigma bond, commonly found in axially chiral biaryls and natural products. Among other applications, it has been tested as a male oral contraceptive in China. In addition to its putative contraceptive properties, gossypol has also long been known to possess antimalarial properties.

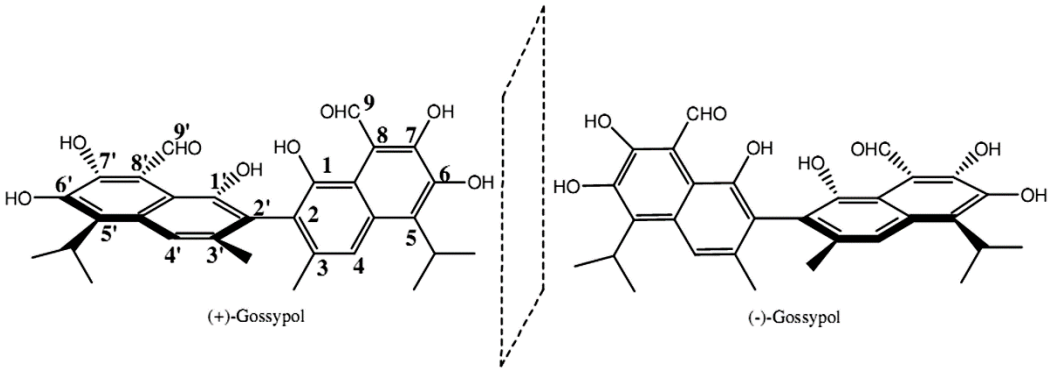

Figure-2: Gossypol chiral centre with mirror image.

Chemistry: Molar mass: 518.562 g•mol−1, Water Solubility: 0.00385 mg/mL, logP: 4.49, logP: 8.02, logS: -5.1, pKa (Strongest Acidic): 7.8, pKa (Strongest Basic): -6.1, Physiological Charge: -1, Hydrogen Acceptor Count: 8, Hydrogen Donor Count: 6, Polar Surface Area: 155.52 Å2, Rotatable Bond Count: 4, Refractivity: 147.61 m3•mol-1, Polarizability: 55.98 Å3, Number of Rings: 4, Bioavailability: 0, Rule of Five: No.

Gossypol is one of the several polyphenolic compounds found in the pigment glands of whole cottonseed. Some gossypol is bound to protein in the stomach contents of ruminants. Cottonseed cake remains after oil is extracted from the seeds. In the cotton plant, it acts as a natural defensive chemical that can lead to infertility in insects. People most commonly use gossypol for birth control. It is also used for cancer, HIV/AIDS, and many other conditions, but there is no good scientific evidence to support these other uses. Gossypol is one of the several polyphenolic compounds found in the pigment glands of whole cottonseed. Some gossypol is bound to protein in the stomach contents of ruminants. Cottonseed cake remains after oil is extracted from the seeds. Gossypol (GOS) is a polyphenolic compound derived mainly from cottonseed oil, which has been found to have anti-fertility effects in males. It has been reported to induce disturbances of the hypothalamicpituitary axis, disruption of spermatogenesis in the testes, and inhibition of postejaculatory spermatozoa motility.[4-7] Gossypol is a toxic compound found in the cotton plant. It is concentrated in the cottonseed but can also be found in other parts of the cotton plant such as hulls, leaves, and stems. Gossypol exists in two forms: free and bound.

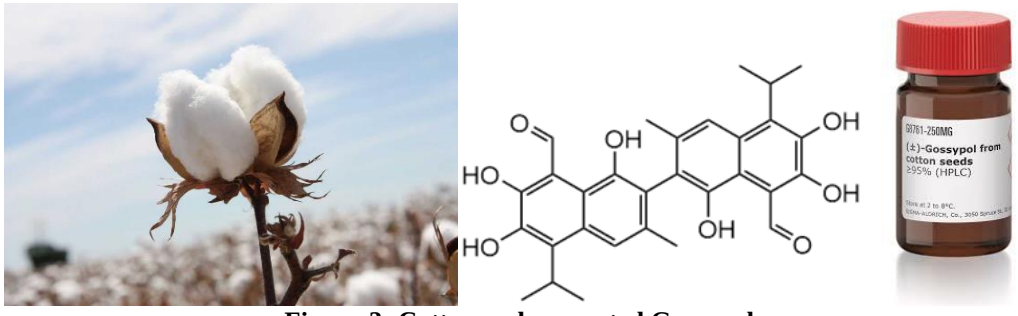

Figure-3: Cottonseed generated Gossypol.

Unrefined cottonseed oil contains a toxin called gossypol. Gossypol has been found to have several negative side effects, including: infertility and reduced sperm counts and motility, pregnancy problems, including early embryo development. Gossypol has been shown to induce also cell apoptosis through oxidative stress. Gossypol treatment has been demonstrated to induce the production of reactive oxygen species (ROS) in tumour cells. Elevated levels of ROS can trigger oxidative stress, DNA damage, and the activation of apoptotic pathways. The most widely used technique to separate oil and gossypol from cottonseed is solvent extraction although mechanical fractionation, liquid cyclone process, adsorption, membrane separation and super critical CO2 extraction have also been applied to recover gossypol. A proper combination of acetone and water enhances the solubility of gossypol, and in our study, the combination of 90:10 of acetone and water mixture showed higher removal of gossypol. By increasing the volume of acetone in the mixture, more gossypol can dissolve in the acetone phase. Humans can't eat typical cotton seeds because they contain a toxin called gossypol. Gossypol is toxic to red blood cells. Consuming gossypol can cause anemia and even death. High concentrations of free gossypol may be responsible for acute clinical signs of gossypol poisoning which include respiratory distress, impaired body weight gain, anorexia, weakness, apathy, and death after several days. However, the most common toxic effects is the impairment of male and female reproduction. Gossypol was soluble in ether and acetone, but only sparingly soluble in other common organic solvents. It was insoluble in water, but dissolved readily in dilute ammonia and sodium carbonate.[7,8] High concentrations of free gossypol may be responsible for acute clinical signs of gossypol poisoning which include respiratory distress, impaired body weight gain, anorexia, weakness, apathy, and death after several days. However, the most common toxic effects is the impairment of male and female reproduction. Gossypol is a natural phenol derived from the cotton plant (genus Gossypium). Gossypol is found in cottonseed as both protein-bound and free forms; only the free form is toxic. Gossypol content of cottonseeds varies from a trace to >6% and is affected by plant species and variety and by environmental factors such as climate, soil type, and fertilization. Gossypol is known to cause problems to the reproductive system in mammals by affecting the reproductive tissues directly or pituitary and gonadal hormone secretion. Gossypol was first identified by Longmore in 1886, purified by Marchlewski in 1899, and named gossypol due to its origin from Gossypium and phenolic compounds (ol). The gossypol is extracted in the presence of 3-aminopropan-l-ol either by a mixture of propan-2-ol and hexane for the determination of free gossypol, or by dimethylformamide for the determination of total gossypol. Gossypol (GOS) is a polyphenolic compound derived mainly from cottonseed oil, which has been found to have anti-fertility effects in males. It has been reported to induce disturbances of the hypothalamicpituitary axis, disruption of spermatogenesis in the testes, and inhibition of postejaculatory spermatozoa motility. Gossypol is non-steroidal and does not affect hormone levels, but does inhibit sperm production and motility in male animals and humans. It acts as a contraceptive by inhibiting enzyme systems that effect energy metabolism in sperm and spermatogenic cells. It is a yellow-colored phenolic aldehyde derived from seeds, roots and stem of the cotton plant. It inhibits the antiapoptotic proteins Bcl-2 and MCL-1 by working as a BH3 mimetic and interferes with the heterodimerization of Bcl-2 proapoptotic interaction. A type of cell death in which a series of molecular steps in a cell lead to its death. This is one method the body uses to get rid of unneeded or abnormal cells. The process of apoptosis may be blocked in cancer cells. Also called programmed cell death.[9-11]

Biological properties

Antifertility/contraceptive: Gossypol is non-steroidal and does not affect hormone levels, but does inhibit sperm production and motility in male animals and humans. It acts as a contraceptive by inhibiting enzyme systems that effect energy metabolism in sperm and spermatogenic cells. Human lactate dehydrogenase (LDH) has five isoenzymes. Under anaerobic glucose conditions, pyruvate is reduced to lactate by LDH in the presence of NADH. Numerous reports suggest that the antifertility properties of gossypol are associated specifically with the (−)-isomer. (−)-Gossypol is a non-selective competitive inhibitor of NADH binding with LDH. Yu et al. (Citation2001) attribute its antifertility action to inhibition of mitochondrial LDH-C4 (LDH-X), which is present only in the testes and sperm and is essential for energy production. However, the mode of action is complex and involves the inhibition of a number of essential enzyme systems, including ribonucleotide reductase, malate dehydrogenase (MDH), glyceraldehyde-3-phosphate dehydrogenase (GA3PDH), and cytoplasmic phospholipase A2 (cPLA2). The latter enzyme plays an important role in the acrosomal reaction during sperm maturation.

Antioxidant properties: Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens. Like many other aromatic phenolic chemicals, gossypol is an effective and potent natural antioxidant. For example, gossypol was found to protect carotene against preformed fat peroxides in vitro and also act as a carotene protecting antioxidant in vivo. Gossypol was reported to inhibit rat liver microsomal peroxidation caused by incubation with ferric/ascorbate ($IC50 < 0.1\ \mu M$). In some cases, the modification of phenolic hydroxyl groups on gossypol significantly decreases the chemical antioxidative abilities of free radical scavenging activity, reducing power assay, DNA damage prevention, and demonstrating that the hydroxyl groups are critical to antioxidation.

Antitumor properties: Researchers have assessed the anticancer properties of gossypol against many types of cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) or flow cytometry cell-viability assays. The effect on cells of (−)-gossypol at a lower concentrations was more potent in comparison with (+)-gossypol or racemic gossypol. The effects included inhibition of cytoplasmic and mitochondrial enzymes involved in energy production, uncoupling oxidative phosphorylation and depletion of cellular adenosine triphosphate.

Gossypol was also shown to inhibit key nuclear enzymes responsible for DNA replication and repair, including DNA polymerase α and topoisomerase II, and to block DNA synthesis in HeLa cells Rao reported that inhibition of DNA synthesis was achieved with 10 μM gossypol by blocking the G1/S checkpoint in MCF-7 cells after 24 h of incubation.

Gossypol has shown in vitro to inhibit cell cycling by modulating regulatory proteins Rb and cyclin D1, elevating TGF-β1 gene expression and inhibiting protein kinase C activity. Telomerase is a reverse transcriptase which helps to stabilize the length of telomeres. The absence of telomerase activity causes replication senescence and cell death. Gossypol has been shown to induce apoptosis and repress telomerase activity via transcriptional downregulation and posttranslational modification of hTERT in human leukemia cells. Transcriptional downregulation involves the inactivation of c-Myc and posttranslational modification that of Akt. Gossypol also downregulates the expression of NF-kappaB-regulated gene products, including inhibitors of apoptosis such as the proteins IAP-1, IAP-2, and X-linked IAP.

(−)-Gossypol induces complete cytochrome c release from mitochondria, increases caspase-3 and caspase-9 activity, and causes apoptotic death. It is found that (−)-gossypol acts as a BH3 mimetic and binds to the BH3-binding domain in pro-apoptotic proteins of the Bcl2 family, displacing pro-death partners to induce apoptosis. It enhances the antitumor activity of X-ray irradiation and chemotherapeutic agents such as docetaxel that exert antitumor activity via inhibition of the antiapoptotic protein Bcl-xL and increasing proapoptotic Noxa and Puma.

Antiviral properties: Gossypol has been reported to possess antiviral properties against enveloped viruses, including HIV-1, HSV-2, influenza, and parainfluenza. Although the compound is significantly less potent than AZT, it lacks the serious side effect on bone marrow toxicity that is associated with AZT treatment. It is not clear whether the inhibition of HIV-1 reverse transcriptase by gossypol is the primary mechanism of action.

Antiparasitic properties: Malaria is a mosquito-borne infectious disease caused by protozoan parasites of the genus Plasmodium. Four species (*Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax*) can infect and be transmitted by humans. Gossypol derivatives with ethyl, propyl, or isopropyl side chains and gossylic nitrile 1,1-divalerate have shown stronger inhibition than other gossypol derivatives against the growth of *P. falciparum*.

Gossypol also exhibits activity against *Entameba histolytica and Trypanosoma cruzi*. The molecular mechanism behind gossypol antiparasitic activity could be the selective inhibition of vital and essential enzymes in the anaerobic life cycle of parasites.

Antimicrobial properties: Demonstrated the antibiotic properties of gossypol against sporeformers and lactobacilli by testing its inhibitory effect on microorganisms in cottonseed meal-fed animals. The result showed that gossypol caused a fundamental change in the equilibrium of the microflora of the gastrointestinal tract. It is reported that gossypol is a more potent antibacterial agent against Gram positive organisms (*Streptococcus aureus,* Bacillus spp., *and Staphylococcus aureus*) as opposed to Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae, Shigella spp., Proteus spp*., and *Escherichia coli*. This may originate from structural differences in the cell wall and cell membrane of Gram-positive and Gram-negative groups. For example, Gram positive bacteria have more peptidoglycan in their cell walls and lack the outer membrane found in Gram-negative organisms. This possibly influences the transport of gossypol to its target site. Plasma cholesterol reduction properties.

Lipid lowering property: Cholesterol is a lipid produced by the liver and is vital for normal body function. Elevated levels of low density lipoprotein (LDL) have been linked to an increased risk of heart disease. Shandilya et al. found that gossypol administered orally at 10 mg/kg/day for 6 months to adult male Cynomolgus monkeys caused a significant decrease in total plasma cholesterol and LDL without a significant decrease in high density lipoprotein (HDL) levels. The possible mechanisms of this action can be attributed to a reduction in intestinal absorption of dietary cholesterol and a decrease in hepatic synthesis of LDL.

CONCLUSION

The numerous studies outlined above show that gossypol has the potential for prevention and therapy of various cancers and chronic human diseases. Gossypol is a versatile molecule with an abundance of biological properties. It has the potential for use in the development of drugs for disorders as varied as resistant tumors, HIV, malaria, and psoriasis. Further investigation on the mechanisms, the nature of the active compounds and

appropriate dose levels are needed for therapeutic exploitation of gossypol.

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