5-20-2011

6,6'-Dimethoxygossypol: Molecular Structure, Crystal Polymorphism, and Solvate Formation.

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6,6’-Dimethoxygossypol: Molecular Structure, Crystal Polymorphism, and Solvate Formation.

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Chemistry
Physical

by

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B.S. University of New Orleans, 2005
B.A. University of New Orleans, 2005

May, 2011

Dedication

The following is dedicated to my parents Carlos Alfonso Zelaya Sr. and Dalia Zelaya for their unselfish dedication to their son in all aspects of life. Both of my parents came to the United States, as immigrants, not only to find a better life for themselves but to give their children a greater opportunity for an enriching life. Through their hard work and love, my parents have fulfilled the promise of giving their children a more meaningful life. Without them I would have never had the opportunities offered to me. Without them I would not be. I would also like to dedicate this thesis to Evelyn, Claudia, and Eric Zelaya. Not only have I been blessed with superb parents but with loving siblings. My love for my daughter, Mia Elisia Elfer has given me the desire to want to become a more conscious and humble man. No greater gift can be bestowed upon a man than that of a beautiful child. Mia you have been my greatest joy, and the reason I wish to live my life in full. Most importantly, I dedicate all my work to GOD.

Acknowledgements

I would first like to take the opportunity to thank Dr. Michael Dowd and Professor Edwin Stevens. Dr. Dowd has exhibited a tremendous amount of patience and understanding throughout the years that have made him an excellent mentor. Dr. Dowd's thorough and complete understanding of chemical engineering, crystallography, chemistry, and computational analysis have been impressive. He's ability to examine and solve scientific problems via analysis have had a profound impact on my understanding on what being a scientist entails. Professor Stevens has been a tremendous teacher, mentor, and scientist. His ability to teach mathematics, physics, and its application to physical chemistry, theoretical chemistry, and chemical physics has been exemplary. Professor Stevens' passion for learning is not only integral to his well being but thankfully contagious. I would also like to thank Professor Steve Rick, who has been my principle instructor in the majority of my course work. Dr. Rick always had an open door policy and has taken considerable time, not only to teach but has made sure that I, the student, understood the material thoroughly. Scott Pelitire, from Dr. Dowd's research group, has also taken great care in teaching a multitude of instrumentation, problem solving skills, laboratory techniques, and scientific application. Mr. Pelitire has not only been an outstanding scientist but a genuine friend.

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Abstract

6,6'-Dimethoxygossypol (DMG) is a natural product of the cotton variety *Gossypium barbadense* and a derivative of gossypol. Gossypol has been shown to form an abundant number of clathrates with a large variety of compounds. One of the primary reasons why gossypol can form clathrates has been because of its ability to from extensive hydrogen bonding networks due to its hydroxyl and aldehyde functional groups. Prior to this work, the only known solvate that DMG formed was with acetic acid. DMG has methoxy groups substituted at two hydroxyl positions, and consequently there is a decrease in its ability to form hydrogen bonds. Crystallization experiments were set up to see whether, like gossypol, DMG could form clathrates. The following results presented prove that DMG is capable of forming clathrates (**S1** and **S2**) and two new polymorphs (**P1** and **P2**) of DMG have been reported.

Keywords: Gossypol, x-ray crystallography, clathrates, solvates, hydrogen bonding, functional groups, polymorphs, and space groups

Introduction

Gossypol [1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3-3'-dimethyl-(2,2'-binaphthalene)-8,8'-dicarboxaldehyde] is a natural occurring compound found in the cotton plant *Malvaceae* g*ossypium* that has a multitude of interesting biological, chemical, and crystallographic properties [1,2]. Gossypol has been tested as an oral contraceptive [3], and a number of its derivatives are currently being tested as potential pharmaceutical drugs in human trials [4, 5]. Gossypol acts as a natural insecticide and contains both anti-viral and anti-fungal properties as well [6]. Gossypol contains a number of functional groups that includes six alcohol groups and two aldehyde groups, centered on two naphthalene rings. In addition, gossypol also contains aliphatic side chains consisting of two isopropyl and two methyl groups. The presence of all the functional groups is a primary reason why gossypol exhibits a plethora of biological and chemical activity, and a primary reason why gossypol is currently being researched as a potential medicinal agent in a numbers of areas. The structure of gossypol and how it behaves chemically is vital in understanding the pharmacological behavior of this polyphenolic terpene. Crystallographic data provides a very detailed description of the molecular structure of gossypol [7, 8] and further aids in understanding the intermolecular and intramolecular forces present within the molecule. The following thesis provides information on how one of gossypol's naturally occurring derivatives, 6,6'-dimethyl gossypol (DMG), forms polymorphs and solvates. Like gossypol, 6,6'-dimethyl gossypol has an array of interesting biological and chemical properties that further elucidate the complex interactions between gossypol and its derivatives and the chemical and biological environment.

Gossypol was first discovered and isolated in the late ninetieth century by Marchlewski [9] and Longmore [10] as a natural occurring yellow pigment present in the intercellular pigment glands of leaves, stems, roots, and seeds of the cotton plant. The initial research conducted by Adams and coworkers [11-25] led to the structural arrangement of gossypol through series of diverse chemical reactions that included degradation, esterfication, etherfication, oxidation, and substitution reactions. In the pioneering work of Edwards and coworkers [26-29], a total synthesis of gossypol was realized.

One of the first applications of gossypol was its use as a dye, but it proved unsuccessful because of gossypol's instability in the presence of light. Until the early 20th century, cottonseed meal was used as a feed in livestock because of its high protein content. However, in high dosages, cottonseed meal proved to be toxic and by 1915 it was suggested that gossypol was the main contributor to the toxicity of the cottonseed meal [30-32]. In the following years, further evidence accumulated that the yellow pigment was toxic to monogastric animals which included rodents, poultry, and swine. It was later discovered that cottonseed meal containing gossypol required cooking to promote unbound gossypol to the bound gossypol state which is evidently less toxic. Agriculture research, at this point, focused on methods of decreasing gossypol content in cotton varieties, and methods of removing gossypol from cotton derived products such as meals and naturally occurring cotton seed oil. The overall consensus was that gossypol was a toxic by product of the cotton plant that had little to no use in agriculture and farming. Then, during the 1960's research was began on combating tumor cells with alkaloids that, fortunately, included gossypol. It was this preliminary cancer research that

helped catalyze a shift in the perception of gossypol from being a detrimental agent to a possible pharmaceutical drug [137].

In the 1970's, large scale testing was conducted with gossypol as a reversible oral contraceptive by Chinese scientists. It proved to be a highly successful contraceptive agent for 99.89% of male users where dosages consisted of 20 mg/daily for the first two months followed by 150-220 mg/month the next four months. While the initial studies provided supporting evidence for gossypol as a potential contraceptive in adult men, there were undesirable side effects that raised numerous concerns. The initial studies reported that 10% of patients acquired low potassium levels (hypokalemia) that were thought due to physiological changes in the sodium/potassium pump. Other symptoms included increased fatigue, decreased libido (6%), epigastric discomfort (2.0%), loss of appetite (2.4%), and nausea (1.0%). However, the most undesired effect was sustained or irreversible azoospermia in men (10%) after the initial study [33]. Azoospermia is a medical condition where there is no measurable amount of sperm in semen and is commonly confused with aspermia which is the absence of semen. A number of other researchers conducted similar experiments throughout the following years with conflicting data (34-37). A symposium was held in 1986 from leading gossypol researchers on the viability of gossypol as a potential contraceptive agent. It was concluded because of the irreversible sterilization and the occurrence of hypokalemia, gossypol was not deemed a plausible antifertility drug. Since then, a number of other researchers have continued to study gossypol as a contraceptive agent and newer studies are beginning to shed light on the outcomes presented in the preliminary studies [38, 39]. A recent 10 year investigation, reported by Coutinhou, documents that blood potassium

levels remained unchanged for the majority of gossypol users. It was suggested that additional regional and economical factors that may have contributed to the ailments seen in the initial trials. The initial reports of hypokalemia may have been exaggerated, and mainly due to restrictions in the Chinese diet, which was already deficient in potassium and not a result of gossypol adversely effecting homeostatic renal physiology [40]. Specifically, samples of the testicular germinal epithelium showed no change after gossypol administration. Hormones, such as testosterone, were found at the same concentrations after the gossypol study and no permanent changes were seen in hormonal levels. Irreversible azoospermia was still present in few subjects; however, recent studies have provided that in many subjects, azoospermia was more likely due to subclinical varicocele. These recent findings have sparked renewed interest into gossypol's contraceptive abilities.

Gossypol also exhibits unique anticancer characteristics, and it is being investigated by a number of clinical researchers in combating a number of cancer types. Basic cell theory states that the homeostatic life cycle of tissue is regulated by the precise balance of both cellular proliferation and the death of cells. Defects that do not promote cell death lead to tumorgenesis if left unchecked, and in many cases chemoresistance. In general, gossypol and its derivatives affect the Bcl-2 (B-cell lymphocyte/leukemia-2) family of proteins that promotes the regulation of cellular apoptosis. It has been demonstrated that enantiomeric (-)-gossypol specifically inhibits Bcl-2, Bcl-xl, and Mcl-1 anti-apoptotic regulator proteins [41]. Bcl-2 inhibition or activation of cancer cells relies on the functionality of the mitochondria inner-membrane permeable transition pores which are responsible for Calcium concentrations, pH, voltage potential in the inner

cristea, and the release of cytochrome c in the cytosol. The Bcl-2 gene has been presently linked to a number of cancers which include melanoma, breast, and prostate cancers, and this specific gene lineage has been associated with a number of autoimmune diseases. Gossypol has been also tested at numerous cancer stages (stage 0 -stage IV) and cancer types. Currently, gossypol and gossypol derivatives are being tested as a potential pharmaceutical drug in the treatment of prostate cancer. Both androgen deprivation and chemotherapy have already proved unsuccessful methods in treating prostate cancer because of chemotherapeutic resistance. It has been known that prostate cancer is primarily due to over expression of antiapoptotic members of the Bcl-2 family of proteins which is believed to be the primary reason enantiomeric (-)-gossypol helps in regression of cancer growth. Specifically, Bcl-xL is over expressed in all refractory prostate cancers and further aids in metastasis, recurrence, and shortened survival. In other advanced human cancers, however, gossypol is unlikely to be clinical useful in the regression of cancer cells.

In other recent investigations, apogossypol, a derivative of gossypol where both aldehyde groups have been removed is being investigated in structural studies on cancer research [42]. Bcl-2 anti-apoptotic proteins, Bcl-2, Mcl-1, Bfl-1, Bcl-W, Bcl-Xl and Bcl-2 pro-apoptotic protein members, Bak, Bax, Bad, Bim, and Bid are able to form dimers that negate each other's functions in cell death or cell proliferation. Anti-apoptotic protein members contain a hydrophobic cavity, the BH3 domain, which binds to the pro-apoptotic proteins. Apogossypol mimics and binds to the BH3 domain of anti-apoptotic proteins which decreases anti-apoptotic behavior and promotes apoptosis. Apogossypolone, a derivative of apogossypol where the naphthalene ring is substituted

for 1, 4-Naphthoquinone, is currently being tested for follicular lymphoma (FL), the fifth leading cancer in the United States and the most common lymphoma worldwide [43]. Like apogossypol, apogossypolone acts as an antagonist against anti-apoptotic Bcl-2 protein members.

The gossypol molecule is composed of two naphthalene rings joined by an internapthyl bond at the 2- and 2'- carbon atoms forming a 2-fold axis, and is composed of a number of functional groups and aliphatic side chains (Fig. 1). Six hydroxyl groups exist within the substituted 2, 2' binaphthalene ring structure at positions 1, 1' and 6, 6' and 7, 7' positions. The hydroxyl groups existing at the 1- and 1'- positions are more reactive than the remaining hydroxyl groups. The aldehyde groups are located at the 8- and 8'- carbon positions, and because of aldehyde group's ability to lend pi electron character to the naphthalene rings give rise to the varied and rich chemistry that gossypol and many of its derivatives posses. Both the aldehyde and hydroxyl groups participate in extensive intramolecular hydrogen bonding networks within each naphthalene ring structure (Fig. 2). There exist a strong hydrogen bond between the aldehyde group at C-8 and the C-7 hydroxyl group that forms a pseudo third ring that is coplanar to each naphthalene structure. This particular hydrogen bond is the strongest of the all the hydrogen bonds, and has been estimated to be approximately 10.7 kcal/mol [44]. The locations of the hydroxyl and aldehyde groups form a lipophobic region within the gossypol molecule. Four alkyl groups exist on the other half of the gossypol structure,

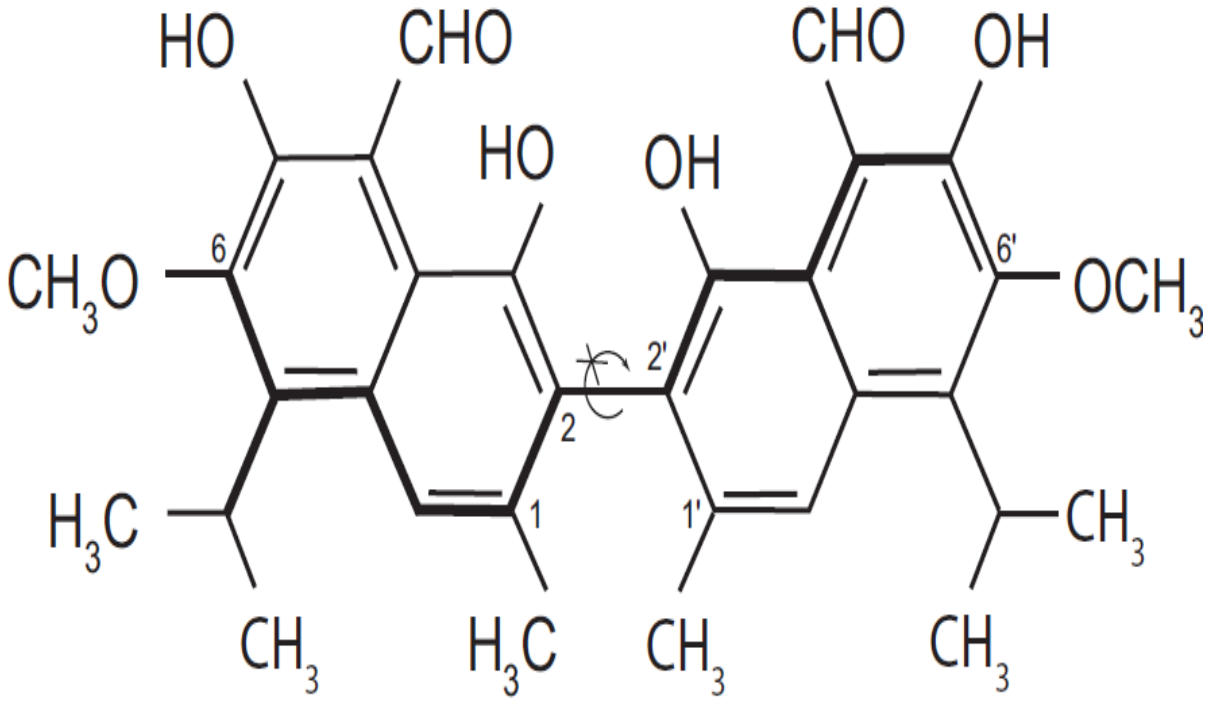

Figure 1. 6,6'-Dimethoxygossypol (DMG) structure. DMG contains a mirror plane between the 2 and 2' carbon atoms forming an internaphthyl bond. DMG consist of two central naphthalene rings and several functional groups: 6 alcohol groups, 2 aldehyde groups, 2 isopropyl groups, and 2 methyl groups. Both the aldehyde and alcohol groups contribute to both inter and intramolecular bonding between adjacent DMG molecules and solvates in crystallographic structures.

two methyl groups at the 3, 3'- positions and two isopropyl groups at 5, 5'- positions. The alkyl groups are usually oriented in the plane of both naphthalene rings, but both the 1, 1' hydroxyl groups and methyl substituents restrict rotation of the two naphthalene planes around the 2, 2'- internaphthyl bond.

The 8, 8'- aldehyde groups contribute to extensive tautomerization present in gossypol and provide for the complex chemistry inherent within the molecule. Gossypol exists in three tautomeric states centralized around its aldehyde groups which consist of an aldehyde tautomer, ketone tautomer, and a lactol or hemiacetal tautomer. The environment and/or solvent that gossypol is immersed in will dictate which tautomeric state is thermodynamically favored. In vitro, gossypol can still exist in two or more states, and depending on which tautomeric state(s) are present, will produce an assortment of undesired reaction products. A simple example can be illustrated with the following example. Suppose a specific chemical reaction, targeted for the aldehyde form reacts to form a single product. If the aldehyde form exists in a 50% equilibrium state with the ketone tautomer, then a number of other reaction products can ensue from the ketone form. Experimentally, this is what is observed with gossypol reactions.

The tautomeric aldehyde (-) - gossypol form has the highest biological activity, and it is believed that reactions with other tautomers, in vitro, produce by products that contribute to the toxicity of gossypol [45]. Gossypol's complex reactionary chemistry is further increased by its ability to form different tautomeric forms on each naphthalene ring simultaneously. As a consequence, it has been suggested that the aldehyde functional groups are the primary cause of toxicity for the gossypol molecule. Recent studies of

apogossypol, a derivative of gossypol, minus the aldehyde group seems to retain a majority of gossypol's therapeutic effects along with decreased toxicity [30].

Gossypol exists as a racemic mixture, naturally, in most cotton species. Both the (+)- and (-)- enantiomeric forms are stable at ambient temperature. The (+)- enantiomer has the S form designation and the (-)- enantiomer is labeled the R form. Crystallographic studies of a pure enantiomeric form have proven difficult because of the difficulty in growing pure R or S enantiomeric crystals suitable for x-ray structure analysis [16]. Certain varieties of cotton plants, however, favor production of either enantiomer, and ratios range from 97:3 to 31:69 for the (+)- and (-)- forms respectively. An abundance of research has suggested that (-)-gossypol is more biological active, but the (+)- gossypol form may serve an advantage in specific cotton varieties. For example, in commercial cotton seed *Gossypium hirsutum*, the ratio between (+)- to (-)- enantiomers is 3:2. In other cotton species such as *Gossypium barbadense*, the (-)- enantiomer predominates. Research conducted on the variety *Thespesia populnea* suggest that it produces enantiomerically pure (+)-gossypol [138]. Interestingly, the (+)- is less toxic to nonruminant animals, and feeds consisting of predominant (+)-gossypol are considered safe in general. More recently, research has focused on whether the (+)-enantiomer is toxic to insects [47] and certain studies suggest that there are benefits of the (+)-enantiomer as an active naturally occurring insecticide. Separation of racemic mixtures of gossypol have proven difficult, but reacting gossypol with amine groups to create Schiff bases and then using reverse phase high performance chromatography has proven a successful technique for separating the S and R forms.

Because gossypol has an extensive number of functional groups, mainly the alcohol and aldehyde groups, the combination of these reactive centers provide for an assortment of rich reaction chemistry. In addition, the conjugated dimeric naphthalene ring system further adds to the complexity of reaction products that gossypol and several of its derivatives exhibit in both chemical and biological environments. As previous stated, the conjugated bonds lend pi character to gossypol's functional groups, primarily the aldehyde groups and their various tautomeric states. In some cases, tautomerization restricts the types of reactions that are feasible with gossypol. This includes alkali-mediated methylation due to gossypol's instability with basic ionic salts, and limitations in esterfication of gossypol's hydroxyl groups because it creates a number of undesired by products. Researchers, however, have made significant progress in developing novel methods of modifying gossypol's central naphthalene framework and functional groups by imposing strict reaction conditions, and by an assortment of regimented processes such as protecting alcohol groups and the implementation of multiple catalysts for desired products.

There exists extensive research published not only on gossypol's rich chemistry but also on its derivatives. In many cases, the chemical processes are well understood but in other cases they are not. Structural studies of derivatives produced by various reactions are vital in aiding the understanding of reaction mechanisms and pathways. Furthermore, chemical reactions and crystallographic analysis of modified forms of gossypol, like dimethyl gossypol, facilitate an understanding of how specific functional groups affect the overall chemistry of gossypol.

Etherfication reactions were vital in deducing the structure of gossypol in the early to mid twentieth century [48-52]. Adams and coworkers lead some of the first pioneering work dealing with modifying and understanding basic principles of gossypol chemistry. Their initial starting point consisted of the synthesis of hexamethyl gossypol ether that was subjected to reduction, oxidation, alkylation, esterfication, hydrolysis, and Schiff's base reactions that aided in elucidating gossypol's complex and dynamic structural arrangement. One of first methods used in etherfication was methylation of the aldehyde groups and 7, 7' alcohol groups with dimethyl sulfate and methanol, forming gossypol tetramethyl ether, and with further changes in reaction conditions, methylation of the 6, 6' alcohol groups, forming gossypol hexamethyl ether. These reactions served as the basis for the synthesis of more elaborate ether products. Synthesis of other ether derivatives involved replacement of hydrogen atoms with methyl groups at alcohol group locations that lead to the creation of gossypol dialdehyde hexamethyl ether, creating a new hexamethyl ether form altogether. Seshadri and coworkers [50, 51] focused on selectively methylating particular hydroxyl groups. These modifications lead to the formation of gossypol containing four, six, or eight ether groups that were positioned symmetrically or asymmetrically across the naphthalene rings. Ether synthesis has not been limited to methylation, and has included silylation with various combinations of gossypol's hydroxyl groups. Selective methylation of gossypol's 6, 6' hydroxyl groups with sodium tetraborate has yielded the ability to synthesize 6-methoxy gossypol and 6, 6'-dimethoxy gossypol, which are naturally occurring gossypol products in *Gossypium barbadense.* Biological research has also been conducted with ether based gossypol products. Specifically, gossypol tetramethyl and hexamethyl ethers were tested on

whether they decreased metabolic fructose degradation in human sperm cells [53]. While research shows that gossypol ethers are biological active, they are not as active as gossypol. In addition, various reviews in the literature support the proposal that for gossypol derivatives to be biological active, a number of free hydroxyl groups must exist on the molecule [54].

Reactions promoting ester group synthesis on gossypol has proven difficult because of the electron delocalization present in gossypol's ring system. A number of esters have been synthesized that include the hexaacetate, hexabenzoate, and hexapmitate esters [55-58]. Acetylated gossypol has been also successfully separated via preparative HPLC analysis. However, gossypol acetylate groups have proven to be very unstable and degrade in multiple pathways.

Oxidation of gossypol is a relatively well known reaction because it degrades so easily in nature and at ambient conditions. Since the first large scale processing of cottonseed oil, oxidation has been observed and created undesirable and degraded cottonseed oil components [59]. Gossypol's conjugated alkene rings are very susceptible to absorbing electromagnetic radiation within the visible spectrum. This creates the potential for highly energetic electron states that aid in the production of free radicals. In general, many oxidation reactions require protecting groups, such as acetyl groups and dithiane derivatives, on all six alcohol groups of gossypol [60]. A number of alkaline solutions also elicit favorable oxidation reactions. Reacting gossypol with ferric chloride in an acetic acid/acetone solution with heat oxidizes gossypol to gossypolone [61, 62]. This oxidation converts gossypol's naphthalene rings to 1, 4-napthoquinone ring

structures. Recent studies have also shown that gossypolone is optically active and has biological activity [63].

The chemistry resulting in the synthesis of apogossypol was first discovered by Carruth in the late 1910's while investigating gossypol extractions with fatty acids and reacting gossypol with hot alkali solutions [64, 65]. The apogossypol reaction involves the removal of both aldehyde groups, and as previous stated, eradicates gossypol's tautomeric properties. Apogossypol formation is also feasible with sodium hydroxide, potassium hydroxide, and other strong bases under nitrogen atmospheres for prolonged periods of times at moderately high temperatures [66]. Apogossypolone is produced by reacting apogossypol with aqueous ferric chloride in an acetone/acetic acid mixture with mild heat that removes two hydrogen atoms and replaces them with two ketone groups on both naphthalene rings creating a 1, 4-napthoquinone central backbone. Zhan and Jia [67] were able to convert apogossypol to apogossypolone using protecting groups of pyridine in acetic acid and subjecting the protected apogossypol structure to a Kiliani's solution, creating the quinone. Removal of the protecting groups on apogossypolone was achieved with a 20% sodium carbonate solution, dioxane, and a 4M hydrochloric solution at 80 degrees Celsius. Apogossypolone has significant biological activity, especially in cancer research. However, it's not known whether apogossypolone is more suitable for cancer studies than apogossypol. While apogossypol seems to be more unstable than apogossypolone at ambient temperatures, it's not well known whether this instability also exists in vitro.

Pharmacokinetical and metabolic analogs have been tested on gossypol, apogossypol, and apogossypol hexaacetate to better understand stability in vitro. A

pharmaceutical study conducted by Lee and coworkers [68] supported evidence that gossypol, apogossypol, and apogossypol hexaacetate are stable in vitro and clear from human plasma. Analogs were tested quantitavely and ascertained with liquid chromatography-mass spectrometry (LC/MS/MS). All three gossypol forms didn't exhibit any permanent conjugate binding to blood proteins but apogossypol binding to mono- and di-glucuronide conjugates were observed. Apogossypol was the most stable showing the lowest amount of metabolites but the slowest clearance rate. Interestingly, 20-40% of apogossypol hexaactetate was converted to apogossypol and the hexaacetate derivative formed various penta-acetate forms. Both gossypol and apogossypol had similar intravenous and oral pharmacokinetic rates and profiles. Apogossypol hexaacetate when administered orally converts to apogossypol and lacks any oral bioavailability [68].

Gossypol reactions involving ammonia and primary amines are some of the most researched and published studies [69-71]. Amination of gossypol usually involves a condensation reaction involving gossypol's aldehyde group. In general, the carbonyl bond on the aldehyde is replaced with a carbon-nitrogen double bond, where the nitrogen is bonded to a **R** group containing of a carbon backbone. The **R** groups themselves can consist of other functional groups such as alcohols, benzyl groups with complex aliphatic carbon chains, such as $-(CH_2)_{17}CH_3$ and aliphatic chains with their own functional groups. **R** groups that consist of aromatic groups have also been extensively studied and synthesized, ranging from phenolic to multi substituted alkene ring systems.

Reacting gossypol with amines ($R\text{-}NR_1R_2$ where R_1=H or C, R_2=H or C, and R=C) that react with its aldehyde groups, in general, forms Schiff's bases. Substitution of gossypol's aldehyde groups removes a degree of tautomerization and thus, decreases

gossypol's toxicity while retaining its therapeutic effects. Gossypolone Schiff's bases, however, have been found to be more toxic than their gossypol counterparts [72-74]. Substitutions with primary amines that form Schiff's bases are characterized with tautomerization that exists in the imine and enamine forms, and have been extensively studied by NMR, IR, and semiemperical molecular modeling [75-85]. Certain amine R groups, such as anilinogossypol ($R=C_6H_5$) register signals on NMR analysis indicating that the enamine structure is favored. Schiff's bases consisting of R groups belonging to hydrazines ($R=NHCH_2CH(OCH_2CH_3)_2$ favor a shift to the imine form. Thus, the identity of the R group and the chemical environment determines which tautomeric structure is chemically favored. It has been postulated that the degree of electronegativity that the R group imposes on the primary nitrogen of the Schiff's base determines the nucleophilicity of the nitrogen. The nitrogen group has a decreased electron environment creating an environment less likely to accept a proton required for tautomerization. Schiff's Base tautomeric equilibrium is also influenced by the presence of monovalent and bivalent metals, and involves complex electronic interactions from d-orbitals [86-89].

In many cases, synthesis of gossypol and gossypol Schiff's base derivatives require complete saturation of starting material in alcohols such as ethanol and methanol. An amine group is then added with other reagents to ensure amination, followed by heating. In recent studies, gossypol Schiff's bases have been synthesized with amino acids, specifically L-phenylalanine methyl ester, L-tyrosine methyl ester, and L-histidine methyl ester [90], adding to the family of gossypol amine substitutes . Other methods of synthesizing Schiff's bases include catalyzing agents such as N, N-dimethyformamidine

[91], and the use of solid-state methods that have been successful in derivatizing only one aldehyde group [92].

Gossypol containing azo derivatives have been extensively studied and for good reason. Diaznonium ions or diazonium salts, RN_2X (X=an organic or inorganic anion) were first discovered by reacting sodium nitrite with phenolic compounds, specifically aniline, by Peter Griess in the 1850's as an intermediate in the production of aryl sulfonyl compounds. Gossypol's substituted naphthalene ring structures serves as an ideal candidate for diazoniation and the choice of the R groups is extensive which includes aliphatic and aromatic groups [93-98]. In general, the reaction mechanism of diazonium compounds with gossypol involves both aldehyde groups, where electrophilic aromatic substitution occurs. The aldehyde group is kept intact and substitution occurs at the 6, 6'-, and 7, 7'- hydroxyl groups, and the 4, 4'- hydrogen atoms of the aromatic ring. These reactions are light sensitive due to u-v degradation of the salts themselves. The azo derivatives of gossypol, generally, aren't soluble in aqueous solutions unless $–SO_3$ and carboxylic acids are introduced into the azo groups, but are soluble in organic media. Azo gossypol compounds are well known as dyes in many cotton varieties where they serve as intermediates in aromatic chemistry [138]. Aryl azo derivatives of gossypol also convey tautomerization between quinohydrazo and hydroxyazo forms and depending on the identity of the R group and chemical environment determines which form dominates. Aryl azo derivatives also seem to posses biological behavior by inducing interferon activity [30].

Hexamethyl apogossypol has been the central starting material for halogenation reactions involving gossypol compounds. Bromination of hexamethyl apogossypol has

yielded a number of interesting derivatives, including the formation of brominated 5-membered ether rings in acidic conditions [99]. The reagents and the experimental conditions determine where bromines cleave on the starting material which include brominating the 3-, 3'- methyl groups, and direct cleavage of the phenyl rings at the 4-, 4'-, and 8-,8'- carbon positions. Halogenation has not been exclusive to just bromine and has included a limited number of products containing fluorine residues. Fluorination, in general, begins with the hexamethyl apogossypol already reacted with bromine. Potassium fluoride or silver fluoride is introduced with the appropriate experimental conditions to yield a replacement reaction where bromine is exchanged for fluorine. Attempts to add fluorine directly to hexamethyl apogossypol have proved unsuccessful. So far, all fluorinated gossypol derivatives require use of brominated apogossypol derivatives.

Reacting gossypol with nitrile groups has been actively studied because of the removal of gossypol's aldehyde groups, which reduces tautomerization. Royer and coworkers have extensively researched the chemical processes in the synthesis of various nitrile groups [100]. Nitrile based reactions consist of reacting gossypol dioxime with acyl anhydrides, followed with heat and carboxylic acid sodium salt. Research into these compounds has concluded most biological active nitrile compounds are doubly substituted. Biological active nitrile derivatives consist of aldehyde groups that are substituted for simple nitrile groups but also the adjacent phenolic hydroxyl group, located at the 1-, 1'- carbon position, is substituted by acetyl groups [101]. Specifically, gossylic nitrile 1, 1'- diacetate exhibits antimalarial activity in *Plasmodium falciparum* by competitively inhibiting lactate dehydrogenase. Other nitrile derivatives, gossylic nitrile

1, 1’-dibutyrate substantially decrease the cellular metabolic pathway of malaria by binding to NADH.

Gossypol exhibits a wide range of reactions involving metals, ranging from light metals such as sodium and aluminum, to the lanthanides and even uranium [102-107]. Gossypol’s ability to form an abundant number of metal complexes has presented vested interest in the areas of molecular biology, analytical chemistry, and genetic research [108-110]. For instance, gossypol complexed with copper (+2), mediates DNA cleavage by the reduction of copper to the plus (+1) state, and infers the possibility of using gossypol metal complex as catalytic precursors in future genetic studies [111-112]. Numerous theoretical studies, with density function theory, have also been conducted on gossypol metal complexes, and how the presence of metals affects the tautomeric equilibrium within gossypol. Like much of gossypol’s rich chemistry, the ability for gossypol to form metal complexes is heavily dependent on the reactive hydroxyl groups present at the 1-, 1’- carbon positions and aldehyde groups.

Currently researchers are developing new reaction methods for the synthesis of new gossypol derivatives as future drug candidates for a variety of human ailments. The possibilities in modifying gossypol and its derivative’s functional groups and ring system’s have yet to be exhausted [113-119]. Amination, azo derivatives, and nitrogen based chemistry are the most abundant papers published on gossypol chemistry. Biological research with gossypol and its derivatives, in the last twenty years, has increased dramatically and yielded promising results. Research is also continuing on how to attain better yields of gossypol, gossypol enantiomers, and gossypol derivatives [120-122]. Recently, Dowd and coworkers have developed a method for separating gossypol

and its methylated derivatives using an acetone extraction and separating the compounds in an acetonitrile/potassium phosphate buffer on a reverse phase high performance chromatography. Apogossypol and apogossypolone are currently being used as therapeutic agents and are the most investigated form of gossypol tested for medicinal usage. A great deal of the literature, to date, reports low yields for both apogossypol and apogossypolone. Dowd and coworkers are currently developing methods for higher yields of both apogossypolone and apogossypol. Preliminary studies on increasing yields, by the Dowd research group, have already produced yields of apogossypolone as high 68%. In addition, research is being conducted on methylated derivatives of apogossypol and apogossypolone.

An extensive number of crystal structures for gossypol have been reported showing varied structure types [123-135, 140]. The gossypol molecule is very versatile in how it packs into the crystalline state due primarily to molecular flexibility both internally and externally with itself and other molecules. Additionally, gossypol has the capacity to rearrange itself, in the crystalline state, to accommodate guest molecules. This has lead to an extensive list of gossypol crystal structures that are not only inclusion complexes but polymorphs as well.

Gossypol's ability to form a varied array of inclusion complexes, in general, is not difficult to understand since it contains all the basic components that promote inclusion formation: axial symmetry, a globular irregular composition, hydrophobic and hydrophilic regions, and a restricted number of conformational degrees of freedom between its ring system and functional groups. Examination of the gossypol molecule reveals a C_2 symmetry element at the center of the internaphthyl bond. Furthermore,

rotation is restrictive at the aryl-aryl bond because of the isopropyl groups at 1, 1'- and 3, 3'- positions that give rise to severe steric hindrances.

Observations of intermolecular and intramolecular hydrogen bonding of gossypol in the crystalline solid state overlap with observations of chemical reactivity. The six alcohol and two aldehyde groups are primarily responsible for creating the majority of bonding interactions between adjacent gossypol and guest molecules within all gossypol crystal structures. The intramolecular hydrogen bonding between O3-H$^{\cdots}$ O-2 atoms and O7-H$^{\cdots}$ O6 atoms are quite strong, where the donor-acceptor distances between these bonds range from 242-250 pm. In comparison, the equivalent bond length in salicylaldehyde is approximately 261.2(5) pm [30]. The hydroxyl protons at the O3-H and O7-H positions are thus considered to be strong intramolecular bonds and are very inaccessible to intermolecular hydrogen bonding within crystals. The alcohol groups, O8-H and O4-H contribute to both intermolecular and intramolecular hydrogen bonding depending on the environment. In certain crystal structures, the O8-H atom forms hydrogen bonds with the O7 atom and the O4-H atom forms hydrogen bonds with the O3 atom. In other structures, the O8-H and O4-H atoms hydrogen bond to neighboring gossypol molecules and or guest molecules, forming an extensive hydrogen-bonding network. This particular ability for the O8-H and O4-H hydroxyl groups to impart either intermolecular or intramolecular hydrogen bonding is probably the primary reason why when both alcohol groups are substituted for methoxy groups, DMG is still able to form a variety of crystal structures.

Understanding how gossypol forms inclusion complexes and polymorphs is of central importance to understanding how dimethyl gossypol itself forms polymorphs and

inclusion complexes. Gdaniec [133] has classified gossypol crystal structures into 22 groups based on polymorphic and inclusion complex characteristics. In addition, the hydroxyl and aldehyde groups that form hydrogen bonds, which are related by symmetrical relationships within the gossypol molecule, are taken into account with the classification schema. The gossypol crystal structures which contain inclusion groups exhibit a great deal of diversity in space groups, crystal symmetry, and host-guest stoichiometry. When attempting to predict how gossypol will orient in a crystalline state, not only should the number of inclusion complexes be taken into account but also the coordination interactions, topology, and the thermodynamics of crystal formation should be considered as well. The number of polymorphs reported by Gdaniec exists as three forms **P1**, **P2**, and **P3** that are represented by the $P2_1/c$ or $C2/c$ space groups. Four additional polymorphs, **P4**-**P7** have been reported by Ibragimov and Talipov [139]. One of the polymorphic forms of gossypol, **P3**, results from the decomposition of the guest molecules from the type-XIII inclusion group. The ability for the host network to still remain intact, in **P3**, clearly demonstrates the strength and malleability of gossypol's extensive hydrogen network in forming lattice networks.

Research and detailed analysis conducted on the multitude of gossypol inclusion compounds has resulted in a basic understanding of the underlying factors affecting polymorph formation. Gossypol's ability to form a large variety of hydrogen bonding networks is paramount to its ability to form a plethora of crystal configurations. On the other hand, when examining the geometric topology of gossypol's intermolecular hydrogen bonding network, it should be remembered that the hydrogen bonds are not strong. The main contributing factor for the lattice stability is the effect of not just one

hydrogen bond but the additive contribution of many hydrogen bonds existing on multicentric naphthalene rings whose functional groups have a certain degree of angular flexibility. These factors when combined lead to a host lattice that can accommodate changes without the need for large changes in thermodynamic energy.

While DMG is reduced in the number of hydrogen bonds it can form, it still contains a number of chemical characteristics that allow it to form a variety of crystal structures. The ability for DMG to readily crystallize has been known for some time. However, with the loss of two hydroxyl groups that are necessary in forming many gossypol inclusion complexes, it was not known whether DMG could incorporate guest molecules. The following thesis presents new data that validates the inclusion ability of DMG. Moreover, two polymorphs of DMG have been experimentally verified and support the recognition that the DMG molecule is also capable of forming a variety of crystal structures.

Methods

Isolated dimethyl gossypol (DMG)-acetic acid (1:1) (4-5 mg) was dissolved in various solvents including diethyl ether, pentan-2-one, acetone, and cyclohexanone in micro centrifuge tubes. The volumes for all four solvents consisted of approximately 200 µL. A volume of 1-1.5 mL of petroleum ether (PE) was slowly pipetted into each micro centrifuge tube to create a supersaturated state. Care was taken not to mix the solvents with the petroleum ether. Then, the samples were placed in the dark at room temperature (~20°C) for extended periods of time. The two DMG polymorphs (**P1** and **P2**) were generated from diethyl ether and acetone (and other conditions). Consideration was given to the possibility that the polymorphs were sensitive to temperature during crystallization formation, however this was not explicitly researched. The DMG-water (1:1) (**S1**) solvate was obtained from both the pentan-2-one/PE and chloroform/PE mixtures. The crystallization process required several months for the chloroform. Apparently, the long time for crystallization formation allowed for water vapor to slowly diffuse into the micro centrifuge tube. It should be noted that the pentan-2-one solution used was acknowledged to contain trace amounts of water (less than 1%). In addition, difficulty was experienced in reproducibly growing crystals of this form, which is believed due to its formation requiring a very narrow range of conditions dealing primarily with temperature and water concentration. Consequently, repeated attempts to recrystallize this structure from water-saturated chloroform or penta-2-one invariably resulted in the formation of one of the non-solvated polymorphs. The DMG-cyclohexanone crystal (**S2**) was acquired from the cyclohexanone/PE solution.

Once adequately sized crystals were formed in the micro centrifuge tubes, they were carefully removed from the mother liquor and examined under a polarizing microscope. All of the crystal specimens were examined to determine if they extinguished polarized light at 90 degrees, contained micro fractures, and whether or not they contained satellite crystals. The crystals were then attached to a thin glass fiber mounted on a goniometer head.

Diffraction data was collected with a Bruker single crystal x-ray diffractometer fitted with a graphite monochromator. The detector consisted of a SMART 1K CCD detector (Table 1). Diffraction data was collected at low-temperature (180-200 K) using a nitrogen flow cryostream generated by boiling liquid nitrogen. The diffraction data for **S1** was collected at room temperature because of frosting problems due to high humidity and mechanical problems with the cryostream. A minimum of two full sets of psi and omega scans were collected for each crystal structure. Bruker SMART and SAINT software was utilized to acquire and integrate the peak intensities, and SHELX NT was used for the structure solution and refinement. All four sets of structural data were solved by direct methods and refined by least squares of all observed reflections. All non-hydrogen atoms were modeled with anisotropic thermal parameters. In general, hydrogen atoms were found in difference maps and were refined isotropically. However, several CH_3 and methylene hydrogen atoms, especially those associated with disordered groups, were placed at their theoretical calculated positions to improve geometries. In addition, the cyclohexanone molecules in **S2** were also found to be disordered. The solid state structure of cyclohexanone is a "chair" form; this particular molecule was refined as two essentially "inverted" chair conformations. The methylene hydrogen atoms were also

placed at calculated positions to improve their spatial geometries. Both DELU and SIMU restraints were utilized for certain structures to improve their thermal distributions defined by the 3×3 thermal tensor.

Carbon, hydrogen, and oxygen atoms were labeled according to previous gossypol structures studies in the literature. Three of the four structures contain no internal symmetry between each naphthalene ring system for DMG. The carbon atoms pertaining to the naphthalene rings are labeled 1-10 and 11-20. The five carbon atoms of substituents on the first naphthalene ring are numbered 21-25, and the five carbon atoms of substituents on the second ring system are assigned numbers 26-30. There exists a non-crystallographic pseudo 2-fold axis between the internapthyl bond between C2- and C12- atoms. The methoxy methyl groups are labeled C31 and C32. The oxygen atoms on the first naphthalene ring system are labeled O1-O4, and the oxygen atoms on the second naphthalene ring system are assigned labels O5-O8. The isopropyl groups are assigned numbers via the first and second ring. For instance, the carbon on the first ring is assigned C23 and the corresponding carbon on the second ring has a label increased by 10 units C33. Labeling of solvent structures begins with C40 and O9, and the minor component of cyclohexanone begins with C50.

The DMG molecule crystallizing in the *C*2/c space group (**P2**) has an asymmetric unit containing one half of the DMG molecule. The labeling of carbon atoms consisting of the naphthalene ring system begins with C1-C10. The carbon atoms pertaining to the periphery of the naphthalene ring are labeled C21-C25, and oxygen atoms are assigned O1-O4. The methoxy carbon group is assigned C31.

Results

Molecular conformation

The four crystal structures contain DMG in of the aldehyde tautomeric form. All gossypol and DMG structures, so far, have reported the aldehyde form in the crystalline solid state (Figure 2).

Least-squares planes were determined for each unique naphthalene ring in each crystal structure. Individual carbon positions generally don't deviate significantly from the best-fit plane. However, variations of carbons positions from the planes were more prominent in both polymorphs than in the solvate structures. For example, the root-mean-squared deviation for ring 2 in **P1** is 0.108 Å and the root-mean-squared deviation for **S2** is 0.048 Å. The naphthalene planes within each molecule are oriented in an approximately perpendicular fashion with dihedral angles ranging from 84.3 to 104.0 degrees. Also the naphthalene rings are oriented so there is both hydrophobic and hydrophilic overlap between the rings of adjacent molecules.

The DMG molecules exhibit intramolecular hydrogen bonding similar to gossypol. The hydroxyl hydrogen atom at carbon position 7 hydrogen bonds with the carbonyl oxygen atom at carbon position 6, and hydrogen bonding occurs between the hydroxyl hydrogen at carbon position 3 and the oxygen from the aldehyde group at carbon position 8. In all four DMG crystal motifs, the methoxy groups are oriented by

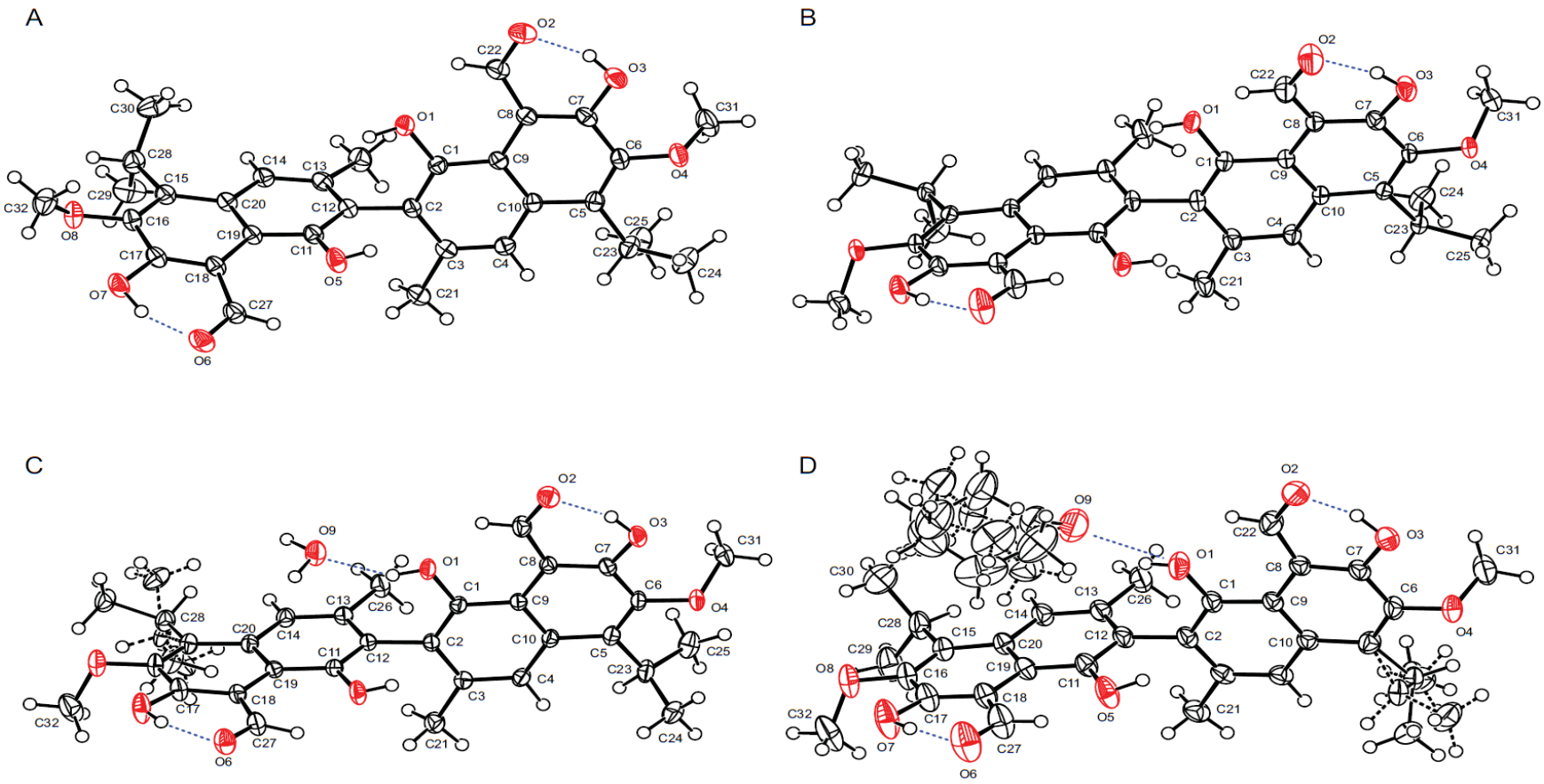

Figure 2. 6,6’-dimethoxygossypol (DMG) and solvate crystal structures. **A.** DMG [**P1**] **B.** DMG [**P2**] **C.** DMG-H_2O (1:1) [**S1**] **D**. DMG-cyclohexanone (1:1) [**S2**].

more than 80 degrees away from the naphthalene planes which is also observed in other DMG crystals. The DMG molecule contains four hydroxyl groups that may participate in hydrogen bonding, as opposed to the six hydroxyl groups in gossypol, and they are usually positioned within the plane of the naphthalene framework.

The isopropyl groups for gossypol and DMG have similar spatial orientations. While there are differences in the spatial displacement of the isopropyl groups for all four DMG crystal structures, in general, they extend outward and away from the central naphthalene structures. The methyl groups position themselves in a similar spatial arrangement which has also been observed in gossypol crystal structures. The isopropyl groups do exhibit crowding of certain hydrogen atoms. The orientation of the H23 atom, from the isopropyl group, comes in close contact with the H4 atom within the naphthalene ring limiting the degrees of angular orientation from the naphthalene plane. The isopropyl group's sigma bond between the C15 atom and C28 atom, however, allows for a degree of rotation of the isopropyl group that alleviates unfavorable steric interactions.

Differences between the orientations of both isopropyl groups were observed for all DMG crystal structures except **P2**. Internal symmetry present in **P2** resulted in both isopropyl groups having identical spatial geometries. Since the other crystal structures don't possess internal symmetry, as in **P2,** the isopropyl group orientations are observed to be different for each half of the molecule (figure 2). **P1** has an isopropyl group oriented away and outward from the central naphthalene ring while the other isopropyl group is more oriented towards the center of the naphthalene ring. The solvated structures contain less internal symmetry than the polymorphs primarily due to the presence of

guest molecules. The **S1** structure contains an isopropyl group oriented away and outward from the center of the molecule like most DMG and gossypol isopropyl groups (figures 2 & 3). The second isopropyl group for **S1** exists in a disordered state, with both an outward and inward position. The refined occupancy for the preferred position is 83.8(4) % for the outward orientation and 16.2% for the inward orientation. The **S2** structure contains one isopropyl group positioned outward and away from the central structure. Similarly, the **S2** structure contains the second isopropyl in a disordered state in both an inward and outward state (figure 3). In contrast, to **S1**, the **S2** second isopropyl methyl groups favor the inward orientation at a refined occupancy of 67.5(6) %. The torsion angles for the outward orientation for the isopropyl groups are 8 and 34 degrees for **S1** and **S2** respectively.

For **S2**, the cyclohexanone solvate is disordered and refinement yields occupancies of 22.2(4) % and 77.8(4) % for the two chair conformers. The hexagonal aliphatic ring system is assigned Cremer-Pople puckering parameters (q = 0.524 Å, O = 9.4°, ψ = 161° for the major component; q = 0.527 Å, O = 6.3°, ψ = 136° for the minor component). The major and minor ring systems of cyclohexanone have similar Cremer-Pople puckering parameters of pure crystalline cyclohexanone (q = 0.536 Å, O = 8.2°, ψ = 170°) [8].

Crystal packing

DMG exhibits packing arrangements and internal symmetry similar to gossypol. The packing and molecular associations for gossypol crystal structures are extensive. Review papers by Ibragimov [139] and Gdaniec [133] have explored the different

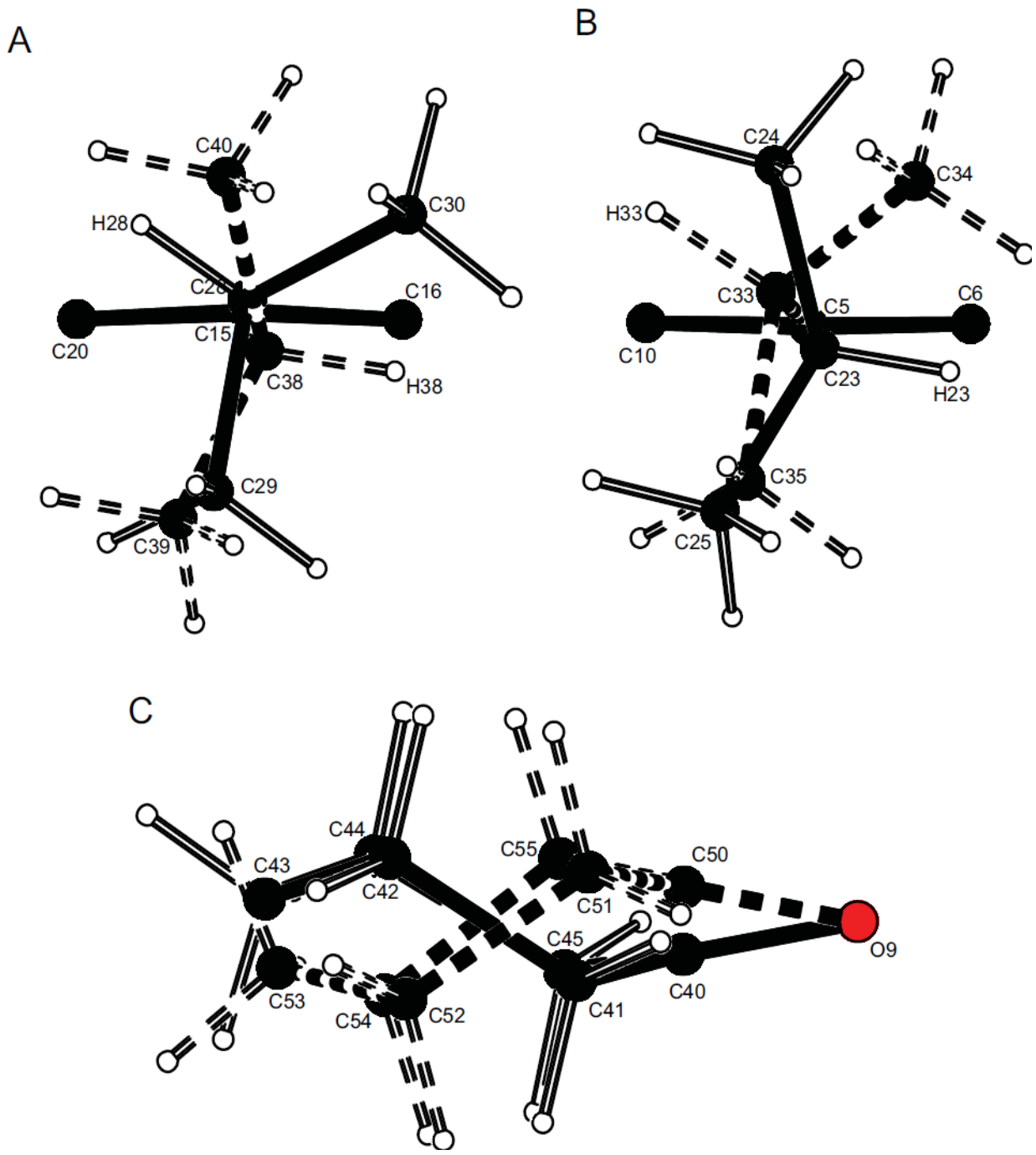

Figure 3. Crystal disorder within 6, 6'-dimethoxygossypol (DMG) and solvates. **A** Isopropyl group in DMG:H_2O (1:1) [**S1**].**B** Isopropyl group of DMG:cyclohexanone (1:1) [**S2**]. **C** cyclohexanone solvate in [**S2]**.

packing motifs, and these can be extended to DMG. Although DMG contains substituted methoxy groups in place of alcohol groups, it doesn't appear to severely limit the range intramolecular bonding motifs that are found similarly in gossypol. DMG is still able to retain many of its centrosymmetric intermolecular associations. Gossypol commonly forms centrosymmetric dimer bundles that are fortified by hydrogen bonding between O5-H atoms and O3 atoms. Furthermore, hydrogen bonding between O4-H atoms and O5 atom, along with hydrophobic stacking between the naphthalene rings further stabilizes dimer orientation. **S1**, **S2**, and **P1** (figure 4a) have intermolecular hydrogen bonding between O5-H and O3 atoms. In contrast, the centrosymmetric dimer associations in **P2** are shifted, where the O1-H hydroxyl hydrogen atom hydrogen bonds to the O4 methoxy oxygen atom (figure 4b). **S2** contains a greater degree of overlap between adjacent naphthalene rings. Additionally, the non-stacked naphthalene rings have a greater degree of overlap than what is observed in DMG dimmer orientations.

In **S2**, the centrosymmetric packing association between dimers is similar to the packing of gossypol-cyclohexanone (1:1) solvate crystal structures. Gossypol, however, is able to form columns from its centrosymmetric assemblies that arise from the intermolecular hydrogen bonding between O4-H and O8 atoms which is not present in DMG. The carbonyl oxygen atom, present in cyclohexanone, hydrogen bonds to the DMG O1 hydroxyl group. This solvate association is also present in the gossypol-cyclohexanone (1:1) complex.

For **S1**, the DMG molecules organize themselves in the same fashion as in the DMG-acetic acid (1:1) crystal structure [140]. The packing motif for **S1** is similar to the structural arrangement for Type-II triclinic inclusion compounds described by Gdaniec et

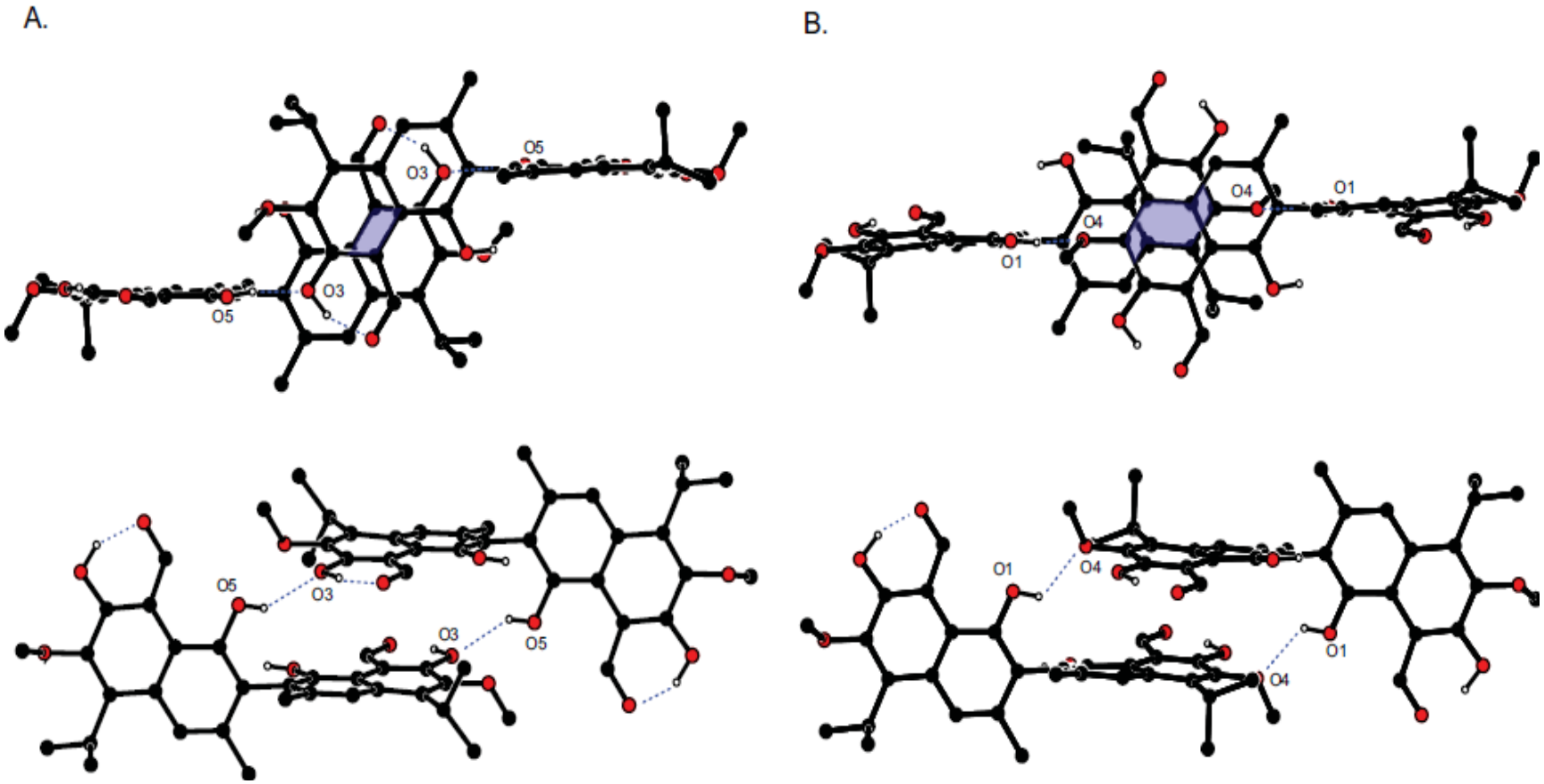

Figure 4. 6,6’-dimethoxygossypol (DMG) centrosymmetric dimer associations in the crystalline state, and atomic labeling of hydrogen (white) and oxygen atoms (red) that are in involved in hydrogen bonding between dimers. **A**. Top and side views of DMG dimer in **P1**. **B**. Top and side views of DMG dimer in **P2**. Shaded areas represent ring over lap from the top view perspective. Note that **P2** structure has greater ring overlap than the **P1** structure.

al. In **S1**, the guest water molecule interacts via hydrogen bonding donating to adjacent DMG molecules via the O6 carbonyl oxygen atom. Water guest molecules also hydrogen bond to the O7 hydroxyl oxygen atom, and the inclusion compounds are locked to the DMG dimers by a hydrogen bond existing between the O1-H… O9 atoms. The water molecules also form hydrogen bonds with the O6 carbonyl oxygen atom and O7 hydroxyl atoms that results with the water molecule interacting with adjacent DMG dimmers, forming large arrays of infinite columns (figures 5 & 6). The formation of these columns creates layers of repeating DMG units. The layers themselves are oriented where the polar groups point inward, thus creating hydrophobic surfaces on opposing sides of the layers. Overall, **S1** has varying levels of structure. The primary structure involves interactions between adjacent DMG dimers that help in the assembly of infinite DMG columns forming a secondary structure. A tertiary structure is created by columns forming stacked layers.

The **P1** structure exists in a structural geometry reminiscent of the known P1 polymorph for gossypol. While centrosymmetric DMG dimers are formed, they don't form secondary structures like repeating columns. Dimers are formed by hydrogen bonds between the O1 hydroxyl hydrogen atom and the O6 carbonyl oxygen atom located on the adjacent DMG molecule. The orientation of these dimers is perpendicular to dimer associations between the assemblies containing the O1 hydroxyl group. Extended pairs due to these interactions lock the dimer assemblies into serpentine chains that form a zigzag pattern (figure 7). The chains are stabilized further by hydrophobic interactions that aid in stacking between naphthalene rings that don't encompass the O5-H… O3-hydrogen bonds.

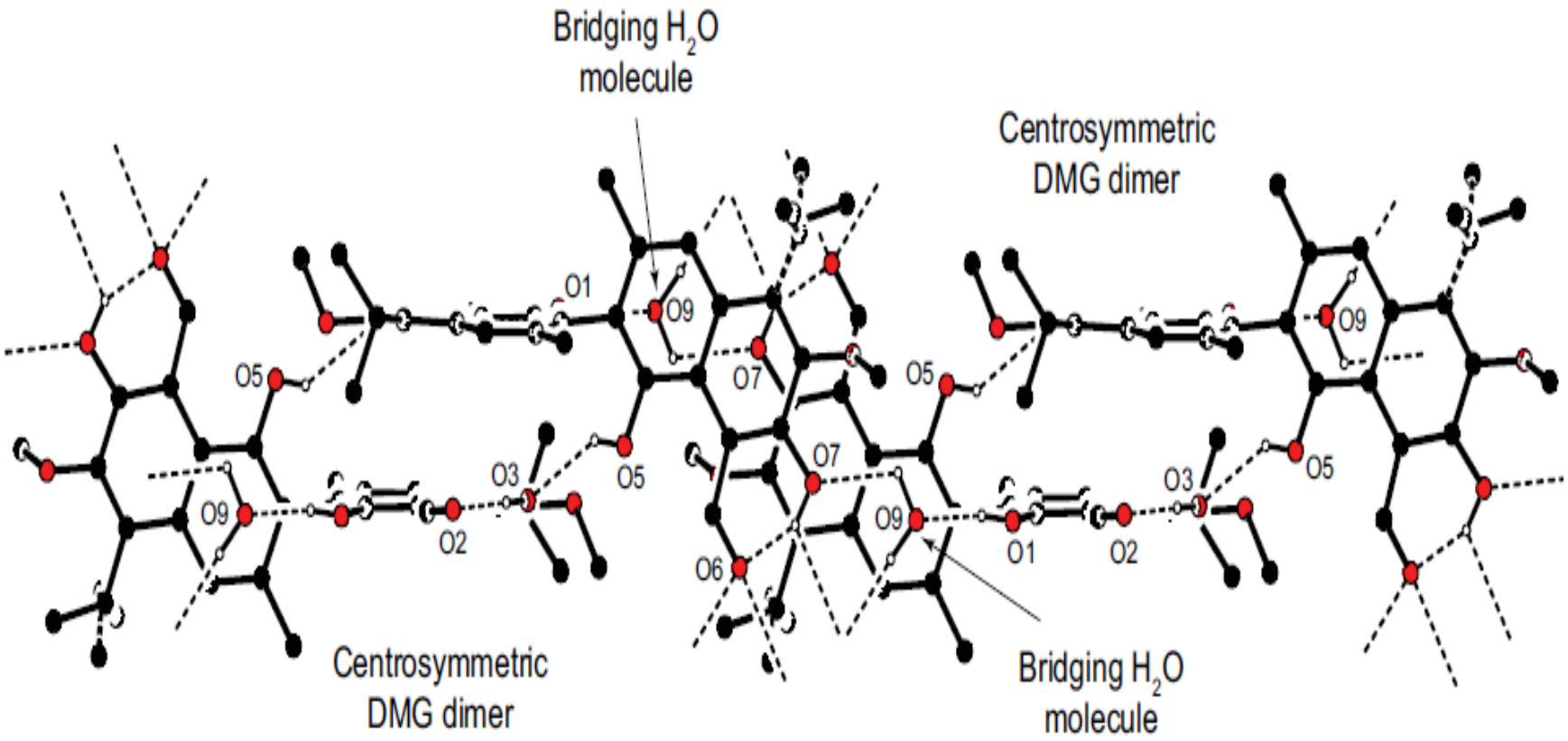

Figure 5. **S1** column structures arising from 6,6'-dimethoxygossypol (DMG) dimers bridged by water molecules. Water molecules interact to the DMG dimers by an O1-H$^{\cdots}$ O9 hydrogen bond. The water molecules also participate in hydrogen bonding to the O7 hydroxyl oxygen atom and the O6 carbonyl oxygen atom.

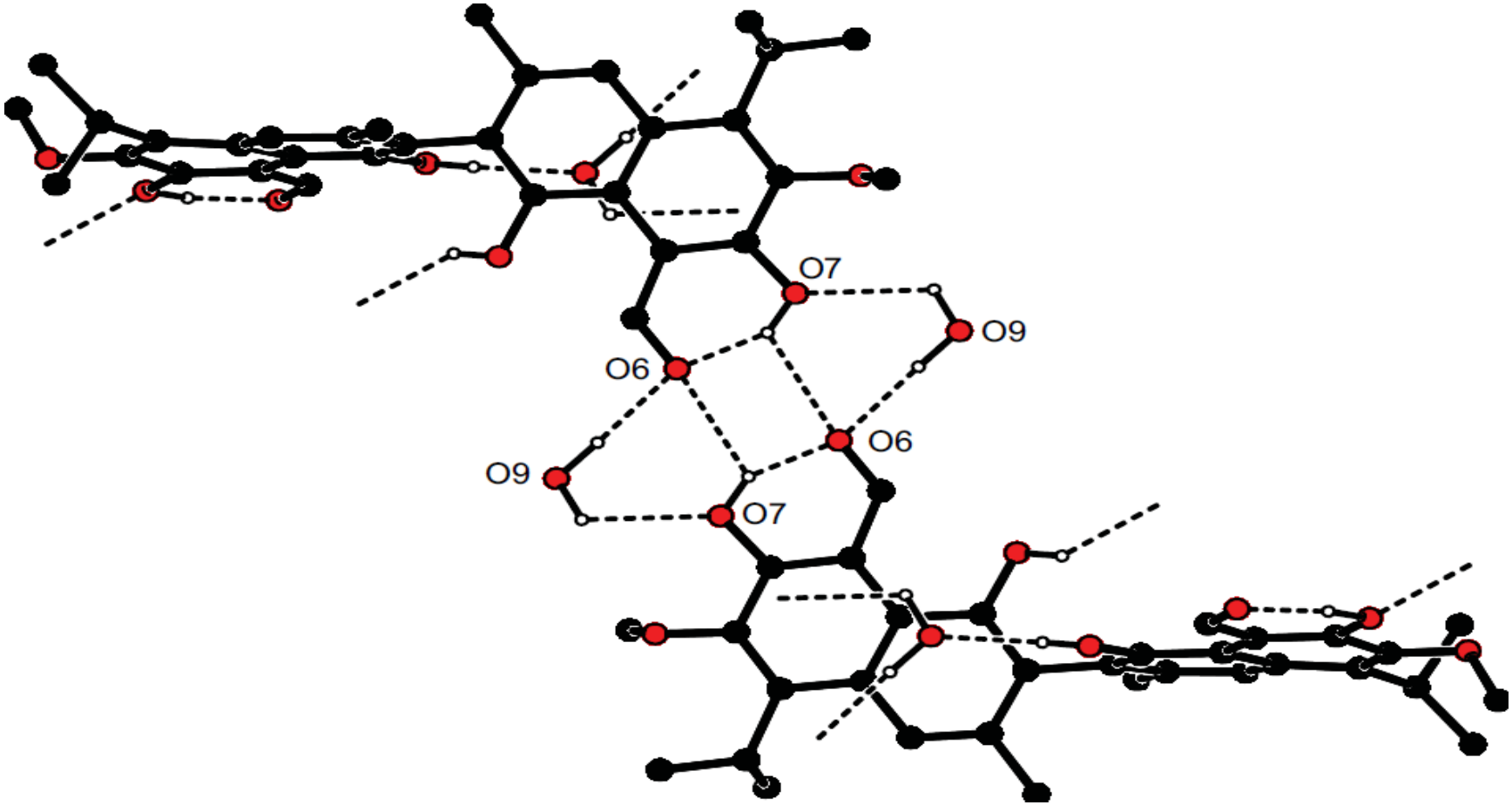

Figure 6. Crystalline hydrogen bond associations of 6,6'-dimethoxygossypol (DMG) and neighboring water molecules. The water molecule acts as bridging molecule between centrosymmetric dimers that form columns in DMG:water (1:1) [**S1**].

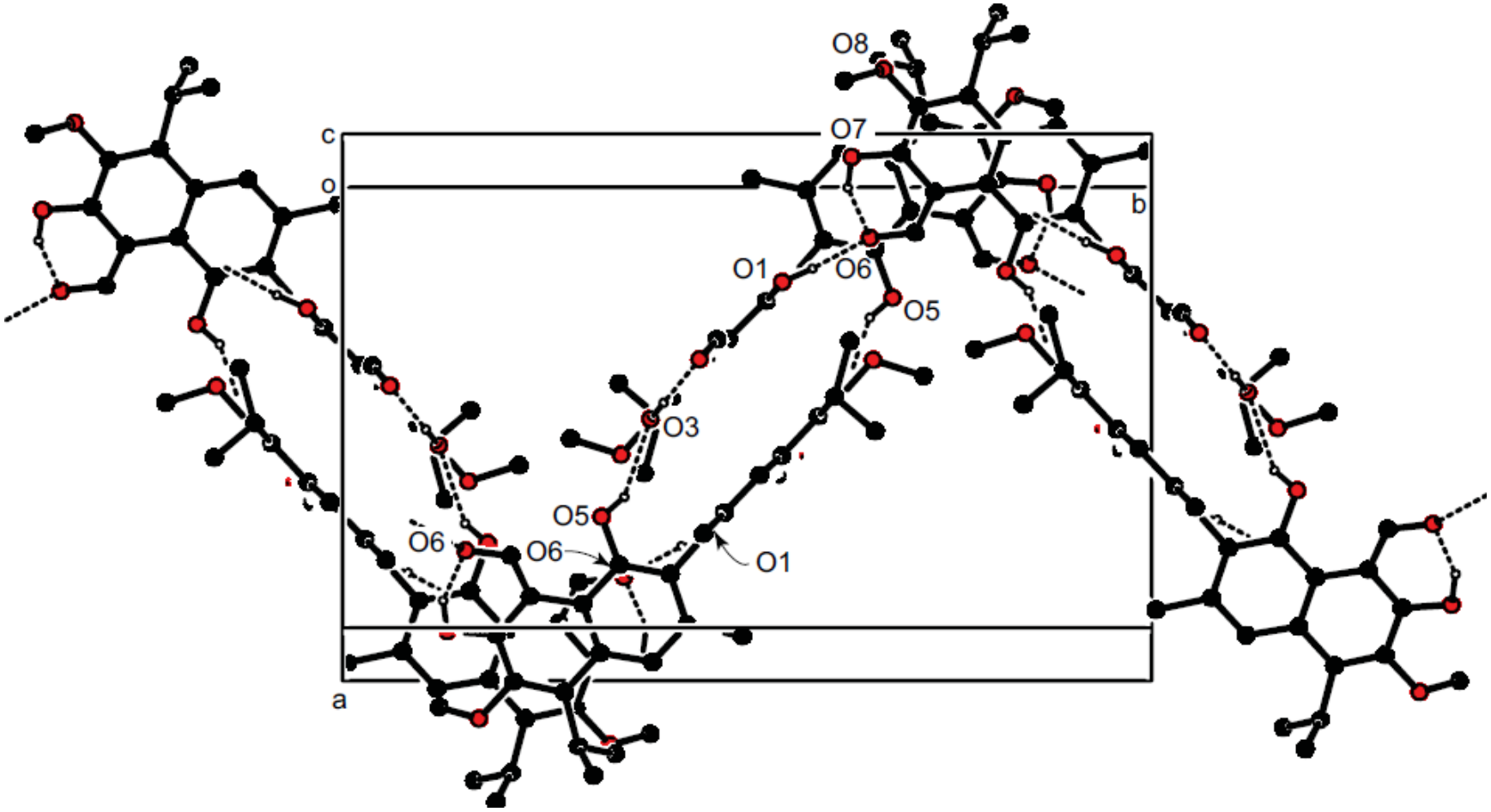

Figure 7. 6',6- Dimethoxygossypol (DMG) centrosymmetric dimer associations in **P1**. Hydrogen bonds are formed between the O1 hydroxyl hydrogen atom and the O6 carbonyl oxygen atom on the adjacent dimer. Overlap of the naphthalene rings contributes to hydrophobic staking, further stabilizing dimer associations. Dimer associations form into serpentine assemblies.

P2 contains a packing motif that has not been observed in previous gossypol complexes. The DMG molecules assemble in pairs of enantiomeric dimers forming centrosymmetric dimers. Furthermore, every DMG molecule forms a similar centrosymmetric arrangement with a second adjoining DMG molecule, resulting in a symmetrical column structure. The primary difference between **P2** and **P1** is that **P2** contains a shift in the hydrogen bonding and a greater degree of intercalation between the naphthalene ring pairs of the centrosymmetric dimer, which presumably confers closer packing of the DMG columns (figure 8). According to the guidelines provided by Gdaniec [133], **P2** is similar to the Type-5a gossypol clathrate group(s). Gossypol crystal structures exhibiting Type-5 structures have been found with various hydrogen bonding patterns, and a variety of gossypol to solvent ratios. This suggests that gossypol Type-5 structures have versatility in their method of packing. However, one important factor differentiates DMG from gossypol: induced de-solvation of Type-5 gossypol frameworks results in the destruction of the crystal form, while the DMG lattice is fixed without the presence of a guest molecule.

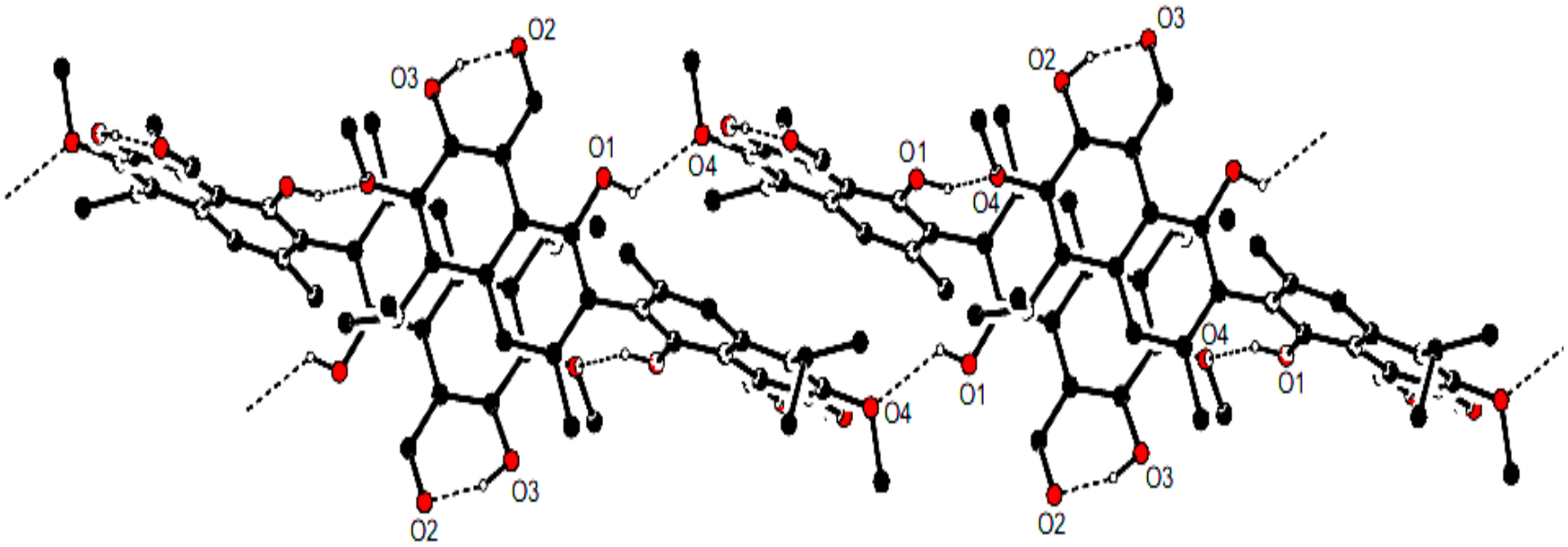

Figure 8. Column formation in **P2** by overlapping centrosymmetric 6,6'-dimethoxygossypol (DMG) dimers. Dimers are associated by hydrogen bonds between the O1-H hydroxyl hydrogen atom donating to the O4 methoxy oxygen atom.

Discussion

The number of solvates that gossypol can form is extensive, which includes organic molecules, such as esters, alcohols, nitriles, carboxylic acids, nitro compounds, ketones, ethers, and a multitude of aromatic compounds. Gossypol also forms solvates with compounds that are chlorinated or brominated. Despite gossypol's ability to form an array of solvates its chemical and crystalline structure does limit the possibilities of guest molecules. Gossypol's central framework consist of two planer naphthalene rings that are interconnected by a bridged bond that allows for each ring to be oriented with an interplaner angle of approximately 70-110°. The limited rotational range of gossypol's naphthalene planes restricts its ability to achieve optimal packing. Gossypol's alcohol and aldehyde groups provide numerous hydrogen bond donor and acceptor groups that provide for an assortment of possibilities in intermolecular hydrogen bonding. The geometric orientations of the hydrogen bonds, to a certain degree, are flexible and further add to the possible crystalline states that gossypol has the potential to accommodate. Moreover, gossypol's polar functional groups residing on approximately one half of each naphthalene ring and aliphatic groups which reside on the other half of the rings create hydrophilic and hydrophobic domains on the molecule itself. In many instances, the assembly of these hydrophobic and hydrophilic regions creates a unique way neighboring gossypol molecules position themselves creating channels and/or cavities of alternating degrees of hydrophobicity or hydrophilicity. The above-mentioned features provide the basis for how gossypol molecules can accommodate various categories of guest molecules based on charge, topology, and size. As a consequence, gossypol has the ability to make a diverse arrangement of packing motifs in the crystalline solid state. The

number of possible polymorphic and inclusion complexes appears to be only limited by the amount of time and energy one is willing to invest in growing crystals. The McCrone statement holds steadfast for gossypol that "the number of forms known for a given compound is proportional to the time and energy spent in research on that compound." DMG contains two methyl groups in place of the hydrogen atoms at the 6 and 6' hydroxyl positions. Methylation decreases the number of alcohol groups that are able to contribute to intramolecular and intermolecular bonding. Since the methoxy groups orient themselves out of the extended naphthalene plane, it allows for the possibility of adjacent DMG molecules to pack in a more compact geometry without solvent. For that reason, one would assume that DMG molecules packing would be more restrictive and diminish its ability to form diverse packing motifs when compared to gossypol crystals. Furthermore, these restrictions would decrease the number of solvates that DMG could accommodate into its crystal lattice. Literature reports and our data supports the idea that DMG is limited in its ability to form solvates, since no solvates are formed with pentan-2-one, diethyl ether, chloroform, and acetone under experimental conditions that gossypol readily forms solvates. Despite the methoxy groups that differentiate DMG from gossypol, DMG still retains a majority of the bonding characteristics of gossypol including the presence of hydrophobic and hydrophilic groups existing on the opposing planes of the naphthalene rings, the perpendicular orientation of the naphthalene rings, the planer geometry of the naphthalene rings, and the presence of aldehyde groups. The preservation of these key features in DMG, infers that it should still have the capacity to form solvates, and the observation that DMG has formed solvates with cyclohexanone, water, and acetic acid confirm this assumption.

The known gossypol solvates serve as a starting point for predicting possible DMG crystals. Prior to our investigations, while acetone and cyclohexanone inclusion complexes had been observed for gossypol, only the cyclohexanone solvate had been successfully crystallized for DMG. According to Gdaniec et al. [133], cyclohexanone and acetone solvates, in gossypol, are classified as Type-1 packing arrangements where the O8-H hydroxyl hydrogen atom donates to the O4 oxygen atom. Type-1 compounds are further defined by having triclinic space groups and a 1:1 host-guest stoichiometric ratio. The intermolecular interaction appears to be weak for the gossypol-cyclohexanone (1:1) solvate which has a long donor-acceptor oxygen distance of 3.75 Angstroms. This particular weak interaction most likely occurs in order for the gossypol lattice to coordinate the large globular cyclohexanone molecule. In comparison, the DMG and cyclohexanone solvate form a similar intermolecular arrangement even with the substituted methyl groups in place of the hydroxyl groups. This demonstrates that the absence of these hydroxyl groups in not essential to the packing motif. Nevertheless, DMG does not form the same packing arrangement with acetone which leads to the conclusion that DMG will not consistently form similar packing arrangements similar to gossypol. DMG's inability not to form solvates with acetone may be assumed to be primarily due to the loss of hydroxyl groups. Either the hydroxyl groups provide for a host lattice structure that thermodynamically favors incorporation of acetone or the hydroxyl groups participate on intermolecular hydrogen bonding. The above enforces the suggestion that DMG is more predisposed to forming non-solvated crystalline structures than gossypol in identical chemical environments.

The DMG solvates of acetic acid and water, according to Gdaniec et al. [133], are representative of gossypol Type-2 triclinic inclusion compounds. In Type-2 gossypol complexes, the O8 and O4 hydroxyl groups do not interface with adjacent gossypol molecules but with guest molecules, where the guest molecule is coordinated as a hydrogen bond acceptor and hydrogen bond donor. The guest molecules representative in Type-2 structures includes protic molecules, such as alcohols like methanol and ethanol and aliphatic acids, and aprotic molecules. As in many gossypol solvates, there exists flexibility in which hydroxyl groups make themselves available for hydrogen and the methoxy groups adopt angular arrangements to accommodate the guest molecules. These observations indicate that DMG's additional methyl groups do not hinder the packing arrangement of Type-2 solvate formation. Consequently, with this particular type of packing motif, the guest molecules act as a bridging structure that links centrosymmetric gossypol dimers into networks of columns and layers. The formation of multiple DMG structures with this organization affirms that the loss of the guest-host intermolecular interaction does not destabilize the packing order.

Gossypol readily forms solvates with pentan-2-one in the crystalline state; however DMG in similar conditions does not readily form solvates. According to Gdaniec et al. [133], gossypol solvated with pentan-2-one corresponds to Type-X (monoclinic; *C*2/c or $P2_1/n$; host-guest ratio 2:1) compounds. The host molecules formed are inter linked between the O4-H and O8-H atoms which account for four hydrogen bonds that generally give rise to layer-type assemblies. In addition, the O1-H hydroxyl atom is aligned to the aldehyde O-6 atom on the adjacent gossypol molecule and is related by a twofold axis. The gossypol naphthalene structures (C1 – C10) are oriented in

a manner that forms symmetrical cavities where guest molecules are inserted in the pockets. The host layers form alternating R and S enantiomeric assemblies of gossypol molecules. Consequently, the gossypol molecules do not form centrosymmetric dimers and the guest molecules interface with the O1-H hydroxyl group forming coordination-assisted clathrates. The gossypol O1-H hydroxyl atoms position themselves on one wall of the cavity, and donate to the guest molecule's carbonyl oxygen atom. Since intermolecular bonding between the O4-H and O8-H hydroxyl groups for Type-X compounds serve as the central backbone for this particular motif, methylation of the hydroxyl groups eradicates the hydrogen bonding between sub-assemblies. Although DMG still retains the ability to form bonds at the O1-H and the aldehyde O-6 atom it still not sufficient to stabilize this particular packing arrangement. These observations lead to the conclusion that Type-X solvates are not possible with DMG.

Conclusion

The structures of four new crystalline forms of DMG have been determined [8]. These structures provide evidence that while DMG is limited in its intramolecular bonding with other molecules, when compared to gossypol, it still retains the ability to incorporate guest molecules into the crystalline state. Like gossypol, DMG contains a number of functional groups, internal symmetry, and possible alternate tautomeric states. In addition, DMG retains a great deal of the rich chemistry present in gossypol. Much of gossypol's complex chemical behavior has been elucidated by x-ray diffraction studies, and while DMG possesses many of structural components of gossypol, the presence of the methyl groups or absence of the alcohol group provides a significant change in both the chemical and physical behavior of DMG. Thus, crystallographic analysis of DMG is needed to understand how substitution of the hydroxyl groups with methyl groups changes the overall chemistry of the molecule.

The DMG molecular arrangement for **P1** has been reported by Gdaniec et al. [133] and is a common polymorph. This particular crystal structure provides data supporting the ability of DMG to form dimers and extended column like structures. The methylation present in DMG doesn't structurally alter the naphthalene rings, allowing hydrophobic effects from the ring system to still play an integral component in crystal packing.

The **P2** packing arrangement introduces a lattice composition that has not been observed in gossypol. It's interesting to note that while DMG has a reduction in the possible positions for allowing hydrogen bonding and contains bulkier methyl subunits in

place of alcohol groups, the **P2** structure appears to pack in a denser fashion that has not been observed in gossypol. Like the **P1** crystal, **P2** inherently displays a high degree of symmetry within its unit cell where each DMG molecules forms two centrosymmetric units with neighboring molecules. The packing arrangement for **P2** is similar to Type-5a gossypol clathrates where the major differences correspond to the greater overlap in the naphthalene rings pairs of the centrosymmetric dimers. Type-5a gossypol clathrates exhibit variability in hydrogen bonding, making DMG a likely candidate for this particular packing scheme.

The packing arrangement for **S1**, as noted, introduces a DMG-clathrate organization that has not been seen in gossypol but has similarities to DMG-acetic acid. **S1**, according to Gdaniec et al. [133], infers Type-2 structure designation where host-guest complexes are classified as coordination-assisted clathrates. While this packing arrangement is unique to DMG and not found for gossypol, **S1** is still defined by a triclinic crystal system where the host aggregates, to a certain extent, can adjust to the requirements of the guest molecule as in gossypol. This supports further evidence that DMG, like gossypol, may be flexible in forming other clathrates. An assortment of Type-2 gossypol complexes that have been observed forming clathrates with protic molecules, aliphatic acid homologs, and DMSO. The Type-2 structure suggests DMG may still retain some of the ability to form guest with molecules of the same chemical nature. The DMG-cyclohexanone crystal system **S2** provides supporting evidence for the existence of intermolecular bonding similar to gossypol Type-1 systems. While both gossypol and DMG form clathrates with cyclohexanone, DMG is not able to form bonds between the O8-H hydroxyl hydrogen atoms to an O4 atom at an adjacent gossypol

molecule as observed in Type-1 gossypol systems. This observation suggests that the hydrogen bonding present in gossypol is not as critical for this particular molecular architecture. Such information on the intermolecular interactions would not be recognized without the determination of the **S2** structure.

While the number of publications for gossypol far exceeds that of DMG, the numbers of crystal structures for DMG will more than likely increase in the future. Few structural studies of gossypol derivatives, other than amino based gossypol derivatives, are currently present in the literature and many gossypol derivatives are being investigated. For instance gossypolone, a well known derivative of gossypol that has proven to be a central in many paths for synthesizing other gossypol derivatives, has only one known structure determination. However because of active research with gossypol and its derivatives; x-ray structure analysis is providing indispensable amounts of information on the molecular behavior of these biological active molecules. Currently, at the fore front of this research are apogossypol, apogossypol derivatives, apogossypolone, and apogossypolone derivatives. While the biochemistry and physiology of apogossypol and its derivative are moderately understood, there have yet to be any crystal structures reported for these molecules. In the last 30 years, enzymology has greatly advanced because of molecular structural studies and studies of how enzymes interact with their molecular counterparts. Thus, x-ray structural analysis of apogossypol and its derivative coupled with modeling and activity studies are crucial in providing a better understanding the biochemical mechanisms of these molecules in anticancer activity and other pharmacological effects.

Future research endeavors will include not only attempts in generating new crystal forms of gossypol and gossypol derivatives, but will also include detailed charge density studies based on accurate high-resolution crystallographic data sets. Charge density studies yield three dimensional maps of the valance electron distribution that compromise the most chemically active regions of atoms and molecules [136]. The electron maps are calculated by taking a large number of intensity observations from the crystals being studied. Once the data is acquired, it's processed and refined using various statistical programs and imaging software.

The core principle of charge density maps is to build a quantum mechanically derived electron distribution centered on the precise atomic positional framework of the solved x-ray data. Quantum mechanics provides the central mathematical model for the charge density within the crystal. The electron distribution within the molecule is described by a "multipole" model that has the same spherical harmonic angular functions as wavefunctions that are solutions to the three dimensional Schrödinger equation. The electron mappings provide a highly detailed analytical description of the electronic distribution within atoms and molecules (figure 9). Using the Atoms in Molecules Theory [141], properties can be extracted from the multipole maps which include the curvature of the charge distribution, critical points, maximum, minimum, saddle, deformation density, electrostatic potential, and a number of other properties.

References

1.) Dowd, M. K.; Pelitire, S. M. Isolation of 6-Methoxy Gossypol and 6, 6'-Dimethoxy Gossypol from *Gossypium barbadense* Sea Island Cotton, *J. Agric. Food Chem.* **2006**, *54*, 3265- 3270.

2.) Howell, C.R.; Hanson L. E.; Stipanovic R. D.; Puckhaber L. S. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with Trichoderma virens. *Phytopathology* **2000**, *90*, 248- 252.

3.) Hoeffer A. P.; Agarwal A.; Meltzer P.; Naqvi R.; Matlin S. A. Antifertility, spermicidal, and ultrastructural effects of gossypol and derivatives administered orally and by intratesticular injections. *Contraception* **1988**, *37*, 301.

4.) Flack, M. R.; Pyle, R. G.; Mullen, N. M.; Lorenzo, B.; Wu, Y. W.; Knazek, R. A.; Nisula, B. C.; Reidenberg, M. M. Oral gossypol treatment of metastatic adrenal cancer. *J. Clin. Endocrinol. Metabol.* **1993**, *76*, 1019-1024.

5.) Coyle, T.; Levante, S.; Shetler, M.; Winfield J. In vitro and in vivo cytotoxicity of gossypol against central nervous system tumor cell lines. *J. Nuero-Oncol*. **1994**, *19*, 25-35.

6.) Bottger, G. E.; Sheehan, E. T.; Lukefahr, M. J. Relation of gossypol content of cotton plants to insect resistance. *J. Econ. Entomol.* **1964**, *57*, 283.

7.) Dowd, M. K.; Stevens, E. D. The gossypol-cyclododecanone (1/2) inclusion complex. *Acta Cryst., Sect. C: Cryst. Struct. Commun.* **2003**, *59*, 397- 399.

8.) Zelaya, C. A.; Dowd, M. K.; Stevens, E. D. 6, 6'-Dimethoxygossypol: molecular structure, crystal polymorphism, and solvate formation. *Struct. Chem*. **2010**, *21*, 113-122.

9.) Marchlewski, L. Gossypol: Ein Bestandtheil de Baumwollsamen, *J. Prakt. Chem.* **1899**, *60*, 84-94.

10.) Longmore, J. Cotton Seed Oil: Its Coloring Matter and Mucilage, and Description of a New Method of Recovering the Loss Occurring in the Refining Process. *J. Chem. Ind.* (London) **1886**, *5*, 200-206.

11.) Miller, R. F.; Adams, R. The Structure of Gossypol 4. Anhydrogossypol and Its Derivatives. *Ibid*. **1937**, *59*, 1736-1738.

12.) Adams, R.; Morris, R. C.; Kirkpatrick, E. C. Structure of Gossypol IX. Oxidiation and Degradation of Gossypol Hexamethyl Ether; Gossic Acid. *Ibid.* **1938**, *60*, 2170-2174.

13.) Adams, R.; Friedman, B. S.; Pierce, C. C.; Morris, R. C.; Kirkpatrick, E. C.; Structures of Gossypol VI. Addition Products with Butadienes. *Ibid*. **1938**, *60*, 2160-2162.

14.) Adams, R.; Kirkpatrick, E. C. Structure of Gossypol. XI. Absorption Spectra of Gossypol, Its Derivatives and of Certain Dinaphthalene Compounds. *Idib*. **1938**, *60*, 2180-2184.

15.) Campbell, K. N.; Morris, R. C.; Adams, R. The Structures of Gossypol. I. *J. Am. Chem. Soc*. **1937**, *59*, 1723-1728.

16.) Adams, R.; Geissman, T. A. Structures of Gossypol. VII. Gossypol Dimethyl Ether, *Ibid*. **1938**, *60*, 2163-2166

17.) Adams, R.; Butterbaugh, D. J. Structure of Gossypol. X. Appogossypol and Its Degradation Products. *Ibid* **1938**, *60*, 2174-2180.

18.) Adams, R.; Morris, R. C.; Butterbaugh, D. J.; Kirkpatrick, E. C. Structure of Gossypol. XIV. Apogossypolic Acid. *Ibid*. **1938**, *60*, 2191-2193.

19.) Adams, R.; Baker, B. R. Structure of Gossypol. XXI. Synthesis of 1, 2-Dimethoxy-3-isopropyl-4-benzoic Acid and of Apogossypolic Acid. *Ibid.* **1939**, *61*, 1138-1142.

20.) Adams, R.; Geissman, T. A.; Morris, R. C. Structures of Gossypol XVI. Reduction Products of Gossypolone Tetramethyl Ether and Gossypolonic Acid Tetramethyl Ether. *Ibid*. **1938**, *60*, 2967-2970.

21.) Adams, R.; Hunt, M. Structure of Gossypol. XIX. Synthesis of 1, 2-Dihydroxy-3-isopropyl-6-benzoic Acid. *Ibid*. **1939**, *61*, 1132-1133.

22.) Adams, R.; Hunt, M.; Morris, R. C.; Structure of Gossypol. XVII. Synthesis of 1,2-Dimethoxy-3-isopropyl-5-aminobenzene, a Degradation Product of Gossypol. *Ibid*. **1938**, *60*, 2972-2974.

23.) Adams, R.; Geissman, T. A.; Dial, W. R.; Fitzpatrick, J. T. Structure of Gossypol, XXVI. Gossypolic Acid. *Ibid*. **1941**, *63*, 2439-2441.

24.) Adams, R.; Geissman, T. A. Structure of Gossypol, XXIII. Attempts to Prepare Desapogossypolone Tetramethyl Ether. Condensation of Hexadiene-2, 4 with Dibenzoylethylene. *Ibid*. **1939**, *61*, 2083-2089.

25.) Adams, R.; Baker, B. R. Structure of Gossypol, XXV. Synthesis of Desapogossypolone Tetramethyl Ether. *Ibid*. **1941**, *63*, 535-537

26.) Edwards Jr., J.D.; Cashaw, J. L. Studies in the Naphthalene Series. III. Synthesis of Apogossypol Hexamethyl Ether. *Ibid*. **1957**, *79*, 2283-2285.

27.) Boatner, C. H. *Pigments of Cottonseed, in Cottonseed and Cottonseed Products, Their Chemistry and Chemical Technology*, Bailey, A. E. Eds.; Interscience: New York, 1948; pp 213- 363.

28.) Edwards, J. D. Total Synthesis of Gossypol. *J. Am. Chem. Soc*. **1958**, *80*, 3798-3799.

29.) Edwards, J. D.; Cashew, J. L. Synthesis of Apogossypol Hexamethyl Ether. *Ibid*. **1956**, *78*, 3224- 3225.

30.) Kenar, J. A. Reaction Chemistry of Gossypol and Its Derivatives. *JAOCS*. **2006**, *83*, 269- 296.

31.) Withers, W. A.; Carruth, F. E. Gossypol a Toxic Substance in Cottonseed. A preliminary Note. *Science*, **1915**, *41*, 324.

32.) Withers, W. A.; Carruth, F. E. Gossypol a Toxic Substance in Cottonseed Meal. *J. Agric. Res.*, **1915**, 5, 261- 288.

33.) National Coordinating Group on Male Infertility Agents. Gossypol-a new ant fertility agent for males. *China Med. J. (New Series)* **1978**, 417.

34.) Waller , D. P.; Zanevald, L. J. D.; Farnsworth, N. R. Gossypol: Pharmacology and Current Status as a Male Contraceptive. *Econ. Med. Plant Res*. **1985**, *1*, 87-112

35.) Segal, S. J. *Gossypol, A Potential Contraceptive for Men*, Plenum Press: New York, 1985.

36.) Qian, S. Z.; Wang, Z. G. Gossypol: A Potential Antifertility Agent for Males. *Annu. Rev. Pharmacol. Toxicol.* **1984**, *24*, 329-360.

37.) Sang, G. W.; Lorenzo, B.; Reidenberg, M. M. Inhibitory Effects of Gossypol on Corticosteriod 11-β-Hydroxysteriod Dehydrogenase from Guinea Pig Kidney: A Possible Mechanism Hypokalemia. *J. Steroid Biochem. Mol. Biol*. **1991**, *30*, 169-176.

38.) Waites, G. M.; Wang, C.; Griffin, P. D. Gossypol: Reasons for its Failures to be Accepted as Safe, Reversible Male Antifertility Drug. *Int J Androl*. **1998**, *21*(5), 8-12

39.) Coutinho, E. M. Gossypol: A Contraception for Men. *Contraception* **2002**, *65*, 259-263.

40.) Reidenberg, M. M.; Gu, Z. P.; Lorenzo, B. J. Differences in serum potassium concentrations in normal men in different geographic locations. *Clinical. Chem*. **1993**, *39*, 72-75.

41.) Zhan, Y.; Jia, G. A Novel Method of Apogossypolone and its Antitumor Activity. *Letters in Drug Design and Discovery* [Online] **2009**, *6*, 129-132 http://www.ingentaconnect.com/content/ben/lddd/2009/00000006/00000002/007aj

42.) Meng, Y.; Tang, W.; Dai, Y.; Wu, X.; Liu, M.; Ji, Q.; Ji, M.; Pienta, K.; Lawrence, T.; Xu, L. Natural BH3 mimetic (-)- Gossypol Chemosensitizes Human Prostate Cancer via Bxl-xl Inhibition Accompanied by Increase of Puma and Noxa. *Mol. Cancer Ther*. **2008**, *7*(7), 2192-2202.

43.) Arnold, A.; Aboukameel, A.; Chen, J.; Yang, D.; Wang, S.; Al-Katib, A.; Mohammad, R. M. Preclinical studies of Apogossypolone: a new nonpeptidic pan small-molecule inhibitor of Bcl-2, Bcl-X_L and Mcl-1 proteins in Follicular Small Cleaved Cell Lymphoma model. *Molecular Cancer* [Online] **2008**, *7*, 1-10 http://www.molecular-cancer.com/content/7/1/20/abstract

44.) Abdullaev, N. D.; Tsychenko, A. A.; Nazarova, I. P.; Ul'chenko, N. T.; Yagudaev, M. R.; Glushenkov, A. I. H and C NMR Spectra of Transformation Products of Gossypol in Solutions. *Chem. Nat. Compd*. **1990**, *26*, 129-138.

45.) Vander Jagt, D. L.; Deck, L. M.; Royer, R. E. Gossypol: Prototype of Inhibitors Targeted to Dinucleotide Folds. *Curr. Med. Chem*. **2000**, *7*, 479-498.

46.) Gdaniec, M.; Ibragimov, B. T.; Talipov, S.A.; *Gossypol*. *Comprehensive Supramolecular Chemistry*, Vol. 6, *Solid-State Supramolecular Chemistry: Crystal Engineering*, edited by D. D. MacNicol, F. Toda & R. Bishop, 117-146. Oxford: Pergamon Press **1996**, 6, 117-146.

47.) Stipanovic, R. D.; Lopez, J. D.; Dowd, M. K.; Puckhaber, L. S.; Duke, S. E. Effect of Racemic and (+)- and (−)-Gossypol on the Survival and Development of *Helicoverpa zea* Larvae *J. Chem. Ecol*. **2006**, *32*, 959–968.

48.) Zhu, G. D.; Chen, D. H.; Huang, J. H.; Chi, C. S. Regioselective Bromination and Fluorination of Apogossypol Hexamethyl. *J. Org. Chem*. **1992**, *57*, 2316-2320.

49.) Datta, S. C.; Murti, V. V. S.; Seshadri, T. R. Isolation and Study of (+)-Gossypol from Thespesia populnea. *Indian J. Chem. Sect. B* **1972**, *10*, 263-266.

50.) Seshadri, T. R.; Sharma, N. N. Isolation of the Hexamethyl Ether of the Dilactol Form of Racemic Gossypol. *Indian J. Chem. Sect. B* **1975**, *13*, 865-866.

51.) Seshadri, T. R.; Sharma, N. N. Further Study of the Three Forms of (+)-Gossypol Hexamethyl Ether. *Ibid*. **1975**, *13*, 866-868.

52.) O'Connor, R. T.; Von der Haar, P.; DuPre, E. F.; Brown, L. E.; Pominski, C. H. The Infrared Spectra of Gossypol. *J. Am. Chem. Soc*. **1954**, *76*, 2368-2373.

53.) Wichmann, K.; Krusius, T.; Sinervirta, R.; Puranen, J.; Janne, J. Studies on Structure-Activity Relationship of Gossypol, Gossypol Ethers, and Three Naphthaldehydes in the Inhibition of Spermatozol Metabolism. *Contracepton* **1967**, *33*, 519-528.

54.) Baram, N. I.; Ismailov, A. I. Biological Activity of Gossypol and Its Derivatives. *Chem. Nat. Compd*. **1994**, *29*, 275-287.

55.) Carruth, F. E. Contribution to the Chemistry of Gossypol, the Toxic Principle of Cottonseed. *Ibid*. **1918**, *40*, 647-663.

56.) Clark, E.P. Studies on Gossypol. I. The Preparation, Purification, and Some of the Properties of Gossypol, the Toxic Principle of Cottonseed. *J. Biol. Chem*. **1927**, *75*, 725-739.

57.) Miller, R. F.; Butterbaugh, D. J.; Adams, R. Structure of Gossypol. II. Acylation. *J. Am. Chem. Soc.* **1937**, *59*, 1729-1731.

58.) Correa, O. G.; Cappi, H. M.; Salem, M.; Staffa, C. New Gossypol Derivatives. *J. Am. Oil Chem. Soc*. **1966**, 43, 678-680.

59.) Clark, E. P. Studies on Gossypol III. The Oxidation of Gossypol. *J. Biol. Chem*. **1928**, *77*, 81-87.

60.) Dao, V.-T.; Dowd, M. K.; Gaspar, C.; Martin, M.-T.; Hemez, J.; Laprevote, O.; Mayer, M.; Michelot, R. J. New Thioderivatives of Gossypol and Gossypolone, as Prodrugs of Cytotoxic Agents. *Bioorg. Med. Chem*. **2003**, *11*, 2001-2006.

61.) Hass, R. H.; Shirley, D. A. The Oxidation of Gossypol. II. Formation of Gossypolone with Ferric Chloride. *J. Org. Chem*. **1965**, *30*, 4111-4113.

62.) Scheiffele, E. W.; Shirley, D. A. The Oxidation of Gossypol. I. Early Stages in the Reaction of Gossypol and Oxygen. *J. Org. Chem*. **1965**, *29*, 3617-3620.

63.) Dao, V.-T.; Dowd, M. K.; Martin, M.-T.; Gaspard, C.; Mayer, M.; Mechelot, R. J. Cytotoxicity of enantiomers of gossypol Schiff's bases and optical stability of gossypolone. *Eur. J. Med. Chem*. **2004**, *39*, 619-624.

64.) Carruth, F. E. Contribution to the Chemistry of Gossypol, the Toxic Principle of Cottonseed. *Ibid*. **1918**, *40*, 647-663.

65.) Clark, E. P. Studies on Gossypol V. The Action of Chromic Acid upon Some Gossypol Derivatives. *J. Am.Chem. Soc*. **1929**, *51*, 1475-1478.

66.) Wei, J.; Rega, M. F.; Kitada, S.; Yuan, H.; Zhai, D.; Risbood, P.; Seltzman, H. H.; Twine, C. E.; Reed, J. C.; Pellecchia, M. Synthesis and Evaluation of Apogossypol Atropisomers as Potential Bcl-xL antagonists. *Cancer Letters* **2009**, *273*, 107-113.

67.) Zhan, Y.; Jia, G. A Novel Synthesis Method of Apogossypolone and its Antitumor Activity. Lett. Drug De. Discovery [Online] **2009**, *6*, 129-133 http://www.ingentaconnect.com/content/ben/lddd/2009/00000006/00000002/007aj

68.) Jia, L.; Coward, L. C.; Kerstner-Wood, C. D.; Cork, R. L.; German, G. S.; Noker, P. E.; Kitada, S.; Pellechia, M.; Reed, J. C. Comparison of pharmacokinetic and metabolic profiling among gossypol, apogossypol and apogossypol hexaacetate. *Cancer Chemother. Pharmacol.* **2008**, *61*, 63-73.

69.) Carruth, F. E. Methods for Approximating the Relative Toxicity of Cottonseed Products. *J. Biol. Chem.* **1917**, *32*, 87-90.

70.) Clark, E. P. Studies on Gossypol. II. Concerning the Nature of Carruth's D Gossypol. *Ibid*. **1928**, *76*, 229-335.

71.) Abou-Donia, M. B. Physiological Effects and Metabolism of Gossypol. *Residue Rev.* **1976**, *61*, 125-160.

72.) Dao, V. T.; Gaspard, C.; Mayer, M.; Werner, G. H.; Nguyen, S. N.; Michelot, R. J. Synthesis and cytotoxicity of gossypol related compounds. *Eur. J. Med. Chem*. **2000**, *35*, 805-813.

73.) Royer, R. E.; Mills, R. G.; Deck, L. M.; Mertz, G. J.; Vander-Jagt, D. L. Inhibition of Human Immunodeficiency Virus Type I Replication by Derivatives of Gossypol. *Pharmacol. Res*. **1991**, *24*, 407-412.

74.) Lin, T. S.; Schinazi, R. F.; Zhu, J.; Birks, E.; Carbone, R.; Si, Y.; Wu, K.; Huang, L.; Prusoff, W. H. Anti-Hiv Activity and Cellular Pharmacology of Various Analogs of Gossypol. *Biochem. Pharmacol*. **1993**, *46*, 251-255.

75.) Bejcar, G.; Przybylski, P.; Brzeinski, B. NMR, FT-IR as Well as PM5 Semiempirical Studies of New Hydrazone of Gossypol with 3-Oxa-n-Butylhydrazine. *J. Mol. Struct*. **2005**, *734*, 45-49.

76.) Ziyaev, Kh. L.; Kamaev, F. G.; Baram, N. I.; Biktimirov, L.; Ismailov, A. I. New Gossypol Imines. *Chem. Nat. Compd*. **1997**, *33*, 545-547.

77.) Matlin, S. A.; Roshdy, S.; Cass, G. B.; Freitas, C. G.; Longo, R. L.; Malvestiti, I. Structual Investigations of Gossypol Schiff's Bases. *J. Brazil. Chem. Soc*. **1990**, *1*, 128-133.

78.) Baram, N. I.; Kamaev, F. G.; Ziyaev, Kh. L.; Biktimirov, L.; Ismailov, A. I.; Nazarov, G. B.; Ibragimov, B. T. Structure of Gossypol Arylimines. *Ibid.* **1988**, *24*, 550-553.

79.) Przybylski, P.; Schilf, W.; Kamienski, B.; Brzezinski, B.; Bartl, F. C-13, N-15, CP-MAS, FT-IR and PM5 Studies of Some Schiff Bases of Gossypol in Solid. *Ibid.* **2005**, *748*, 111-117.

80.) Przybylski, P.; Schilf, W.; Brezinski, B. C-13, N-15, NMR and CP-MAS as Well as FT-IR and PM5 Studies of Schiff Bases of Gossypol with L-Phenylalanine Methyl Ester in Solution and Solid. *Ibid*. **2005**, *734*, 123-128.

81.) Przybylski, P.; Jasinski, K.; Brezinski, B.; Bartl, F. Spectroscopic Studies and PM5 Semiempirical Calclulations of New Schiff Bases of Gossypol with Amino Deriviatives of Crown Ethers. *Ibid.* **2002**, *611*, 193-201.

82.) Przybylski, P.; Schilf, W.; Brzezinski, B. C-13, N-15 NMR and CP-MAS as Well as FT-IR and PM5 Studies of Schiff Base of gossypol with l-Phenylalainine Methyl Ester in Solution and Solid. *Ibid*. **2005**, *734*, 123-128.

83.) Przybylski, P.; Wlodarz, M.; Schroeder, G.; Pankiewicz, R.; Brzezinski, B.; Bartl, F. ESI MS and PM5 Semiemperical Studies of Gossypol Base with (R)-Tetrahydrofurfuryl-amine Complexes and Monovalent Cations. *Ibid*. **2005**, *693*, 95-102.

84.) Przybyski, P.; Woldarz, M.; Brzezinski, B.; Bartl, F. Spectroscopic Studies and PM5 Semiempirical Calclulations of Tautomeric Forms of Gossypol Schiff Bases with (R)-Tetrahydrofurfurylamine. *Ibid*. **2004**, *691*, 227-234.

85.) Przybylski, P.; Ratajczak-Sitarz, M.; Katrusiak, A.; Schilf, W.; Wojciechowski, G.; Brzezinski, B. Crystal Structures of Schiff Base Derivative of Gossypol with 3, 6, 9,-Trioxa-decylamine. *Ibid*. **2003**, *655*, 293-300.

86.) Przybylski, P.; Schroeder, G.; Pankiewicz, R.; Brzezinski, B.; Bartl, F. Complexes of Schiff Base of Gossypol with n-Butylamine and Some Monovalent or Bivalent Cations Studied by ESI MS, NMR, FT-IR as Well as PM5 Semiemperical Methods. *Ibid*. **2003**, *658*, 193-205.

87.) Przybylski, P.; Brzezinski, B. The Complexes Between Schiff Base of Gossypol with L-Phenylalanine Methyl Ester and Some Monovalent Cations Studied by HNMR, ESI MS, FT-IR as Well as PM5 Semi-empirical Methods. *J. Mol. Struct*. **2003**, *654*, 167-176.

88.) Pryzbylski, P.; Schroeder, G.; Brzezinski, B. The Schiff Base of Gossypol with 2-(Aminomethyl)-18-crown-6 Complexes and H, Li, Na, K, Rb, Cs Cations Studied by ESI MS, HNMR, FT-IR and PM5 Semiempirical Methods. *Ibid*. **2004**, *669*, 65-77.

89.) Przybylski, P.; Bejcar, G.; Schroeder, G.; Brzezinski, B. Complexes of Schiff Base of Gossypol with 5-Hydroxy-3-oxapentylamine and Some Monovalent Cations Studied by ESI MS as Well as PM5 Semiempirical Methods. *Ibid*. **2003**, *654*, 245-252.

90.) Przybylski, P.; Brzezinski, B. Spectroscopic studies and PM5 semiempirical calculations of new Schiff bases of gossypol with polyoxaalkylamines. *Biopolymers* **2002**, *67*, 61-69.

91.) Lyman, C. M.; Cronin, J. T.; Trant, M. M.; Odell, G. V. Metabolism of Gossypol in the Chick. *J. Am. Oil Chem. Soc*. **1969**, *46*, 100-104.

92.) Talipov, S.A.; Manakov, A.; Ibragimov, B. T.; Lipkwoski, J.; Tilijakov, Z. G. Sorption of Ammonia, Methylamine, and Methanol by the P3 Poloymorph of Gossypol. Synthesis of Unsymmetrical Monoamine Derivatives of Gossypol by a Solid State Reaction. *J. Inclusion Phen. Macrocyclic Chem.* **1997**, *29*, 33-39.

93.) Baram, N. I.; Ziyaev, Kh. L.; Ismailov, A. I.; Ziyamov, D.; Mangutova, Y. S. New Azoderivatives of Gossypol. *Chem. Nat. Compd*. **2000,** *36*, 185-188 & 541-542.

94.) Nazarova, I. P.; Glushenkova, A. I.; Markman, A. L. Some Products from the Combining of Gossypol with Diazotized Amines. *Khim. Prir. Soedin*. **1976**, *5*, 607-609.

95.) Bekarek, V.; Rothschein, K.; Vetesnik, P.; Vecera, M.; Estimation of Azo-Hydroazo Tautomeric Equilibrium in Orthohydroxy-azocompounds by NMR. *Tetrahedron Lett*. **1968**, *9*, 3711-3713.

96.) Kaul, B. L.; Nair, P. M.; Rama Rao, A. V.; Venkataraman, K. NMR Spectra of Azophenol and Quinone Hydrazones. *Tetrahedron Lett*. **1966**, *7*, 3897-3903.

97.) Rezhepov, K. Zh.; Ziyaev, Kh. L.; Baram, N. I.; Kamaev, F. G.; Levkovich, M. G.; Saiitkulov, A. M.; Ismailov, A. I. Azo-derivitives of Gossypol and Its Imines. *Khim. Prir. Soedin.* **2003**, *4*, 289-291.

98.) Fischer, P. B.; Kaul, B. L.; Zollinger, H. Untersuchungen uber die Struktur von Formazanen I N-H-Kopplung des Chelatwasserstoffatoms. *Helv. Chim. Acta* **1968**, *51*, 1449-1451.

99.) Zhu, G. D.; Chen, D. H.; Huang, J. H.; Chi, C. S. Regioselective Bromination and Flourination of Appogossyopol Hexamethyl Ether. *J. Org. Chem*. **1992**, *57*, 2316-2320.

100.) Royer, R. E., Deck, L. M.; Campos, N. M.; Hunsaker, L. A.; Vander-Jagt, D. L. Biologically Active Derivatives of Gossypol: Synthesis and Antimalarial Activities of Peri-acylated Gossylic Nitriles. *J. Med. Chem*. **1986**, *29*, 1799-1801.

101.) Vander Jagt, D. L.; Deck, L. M.; Royer, R. E. Gossypol: Prototype of Inhibitors Targeted to Dinucleotide Folds. *Curr. Med. Chem*. **2000**, *7*, 479-498.

102.) Adams, R., Geissman, T. A.; Edwards, J. D. Gossypol a Pigment of Cottonseed. *Chem. Rev*. **1960**, *60*, 555-574.

103.) Markman, A. L.; Rzhekhin, V. P. Gossypol and Its Derivatives. Israel Programs for Scientific Translations: Jerusalem 1965, 148.

104.) Ramaswamy, H. N.; O'Connor, R. T. Physical and Chemical Properties of Selected Metal Complexes of Gossypol. *J. Am. Oil Chem. Soc*. **1968**, *45*, 841-844.

105.) Berardi, L. C.; Goldblatt, L. A. *Gossypol in Toxic Constituents of Plant Foodstuffs*; 2nd ed.; Liener, I. E. ed.; Academic Press: New York, 1980; p 184-237.

106.) Ramaswamy, H. N., O'Conner, R. T. Metal Complexes of Gossypol. *J. Agric. Food Chem.* **1969,** *17*, 1406-1408.

107.) Przybylski, P.; Schroeder, G.; Brzezinski, B. Complexes of Schiff Base of Gossypol with 5-Hydroxy-3-oxapentylamine and Ca, Ba, or Pb Cations Studied by NMR, FT-IR, ESI MS, as Well as PM5 Semiempirical Methods. *Ibid*. **2003**, *658*, 115-124.

108.) Brzezinski, B.; Marciniak, B.; Paszyc, S.; Zundel, G. The Tautomerization of Gossypol as a Function of the Presence of Ni, Cu, Zn Cations. *Ibid.* **1992**, *268*, 61-66.

109.) Brzezinski, B.; Rozwadowski, J.; Marciniak, B.; Paszyc, S. Spectroscopic Study of Gossypol-Lanthanide Cation Complexes in Acetonitrile Solution. *J. Mol. Struct*. **1997**, 435, 275-279.

110.) Brezinski, B.; Paszyc, S.; Zundel, G. The Structure of Gossypol as a Function of the Presence of $HAuCl_4$ and of Be Ions. *Ibid*. **1991**, *246*, 45-51.

111.) Zaidi, R., S. M. Hadi. Strand Scission in DNA by Gossypol and Cu (II): Role of Cu(I) and Oxygen-Free Radicals. *J. Biochem. Toxicol*. **1992**, *7*, 213-217.

112.) Zaidi, R.; Hadi, S. M. Complexes Involving Gossypol, DNA and Cu (II). *Biochem. Int*. **1992**, *28*, 1135-1143.

113.) Laatsch, H. Dimeric Naphthaquinones. X. A Convienent Synthesis of Deapogossypolone Tetraethyl Ether. *Anorg. Chem. Org. Chem*. **1984**, *38B(2)*, 244-247.

114.) Edwards, J. D.; Cashaw, J. L. The Synthesis of 1, 2- Dimethoxy-3-isopropylbenzene. *J. Org. Chem*. **1955**, *20*, 847-849.

115.) Manmade, A.; Herlihy, P.; Quick, J.; Duffley, R. P.; Burgos, M.; Hoffer, A. P. Gossypol. Synthesis and in vitro Spermicidal Activity of Isomeric Hemigossypol Derivatives. *Experienta* **1983**, *39*, 1276-1278.

116.) Ognyanov, V. I.; Petrov, O. S.; Tiholov, E. P.; Mollov, N. M. Synthesis of Gossypol Analogues. *Helv. Chim. Acta* **1989**, *72*, 353-360.

117.) Venuti, M. C., Efficient Synthesis of the Gossypol Binaphthyl Backbone. *Ibid*. **1981**, *46*, 3124-3127.

118.) Yu, Y.; Deck, J. A.; Hunsaker, L. A.; Decker, L. M.; Royer, R. E.; Goldberg, E.; Vander-Jagt, D. L. Selective Active Site Inhibitors of Human Lactate Dehydrogenases A4, B4, and C4. *Biochem. Pharmacol*. **2001**, *62*, 81-89.

119.) Meltzer, P. C.; Bickford, P. H.; Lambert, G. J. A Regioselective Route to Gossypol Analogs: The Synthesis of Gossypol and 5, 5' –Didesisopropyl-5,5'-diethyl Gossypol. *Ibid*. **1985**, *50*, 3121-3124.

120.) Meyers, A. I.; Willemsen, J. J. The Synthesis of (S)-(+)-Gossypol. *Tetrahedron Lett.* **1998**, *54*, 10493-10511.

121.) Meyers, A. I.; Willemsen, J. J. An Asymmetric Synthesis of (+)-Apogossypol Hexamethyl Ether. *Tetrahedron Lett*. **1996**, *37*, 791-792.

122.) Meyers, A. I.; Willemsen, J. J. The Synthesis of (S)-(+)-Gossypol via an Asymmetric Ullmann Coupling. *J. Chem. Soc., Chem. Commun*. **1997**, *16*, 1573-1574.

123.) Weber, E.; Josel, H. P. A Propasol for the Classifcation and Nomenclature for Host-Guest-Type Compounds. *J. Inclusion Phenom*. **1983**, *1*, 79-84.

124.) Ibragimov, B. T.; Talipov, S. A.; Aripov, T. F.; Sadykov, A. S. Inclusion Complexes of the Natural Product Gossypol. Crystal Structure of the 2:1 Complex of Gossypol with *m*-Xylene. *J. Inclusion Phenom. Mol. Recognit. Chem*. **1990**, *8*, 323-333.

125.) Green, B. S.; Knossow, M. Lamellar Twinning Explains the Nearly Racemic Composition of Chiral, Single Crystals of Hexahelicene. *Science* **1981**, *214*, 795.

126.) Talipov, S. A.; Ibragimov, B. T.; Nazarov, G. B.; Aripov, T. F.; Sadykov, A. S. An X-ray Structural Investigiation of Gossypol and Its Derivatives. V. Crystal Structures of the Ligroin Modification of Gossypol. Chem. *Abstr*. **1986**, *104*, 139.

127.) Ibragimov, B. T.; Nazarov, G. B.; Talipov, S. A. X-ray Structural Investigation of Gossypol and Its Derivatives. VIII. A New Class of Inclusion Compounds Based on Dianilinegossypol. *Chem. Nat. Compd*. **1988**, *24*, 565-567.

128.) Gdaniec, M.; Ibragimov, B. T.; Dadabaev, B. N. Lattice inclusion compounds of gossypol. Structure of the 2:3 gossypol-benzaldehyde coordinatoclathrate. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1991**, *47*, 573-577.

129.) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer: Berlin, 1991; pp 24.

130.) Gdaniec, M.; Czajka, H. Inclusion Compounds of Gossypol. Structure of the Gossypol-n-Valeric Acid (1/2) Coordinato-clathrate. *J. Inclusion Phenom. Mol. Recognit. Chem*. **1995**, *22*, 187.

131.) Gdaniec, M. Lattice inclusion compounds of gossypol. Structure of the 1:2 Gossypol/Salicylaldehyde Coordination Clathrate. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1991**, *47*, 1499-1503.

132.) Dowd, M.; Stevens, E. *The gossypol-cyclododecanone (1/2) inclusion complex. Acta Crystallogr., Sect. C: Cryst. Struct. Commun*. **2003**, *59*, 397-399.

133.) Gdaniec, M.; Ibragimov, B. T.; Talipov, S. A. In *Gossypol: Solid-state supramolecular chemistry: crystal engineering*, Vol. 6, MacNicol, D.D.; Bishop, T. F., Eds.; Pregamon Press: Oxford, 1996; pp 117-146.

134.) Dowd, M.; Stevens, E. Inclusion complexes of gossypol with 2-pentanone, 3-pentanone and 2-hexanone. *J. Inclusion Phenom. & Macrocyclic Chem*. **2005**, *51*, 65-71.

135.) Dowd, M.; Stevens, E. The (-)-gossypol-2, 4-pentanedione (1:2) inclusion complex. *J. Chem. Crystallogr*. **2004**, *34(8)*, 559-564.

136.) Coppens, P. *X-Ray Charge Densities and Chemical Bonding, (International Union of Crystallographers Text on Crystallography)* USA ed.; Oxford University Press: USA, 1997; pp 1-14.

137.) Vermel, E. M.; Kruglyak, S. A. Anitcancer Activity of Some Alkaloids. *Vopr. Onkol*. **1962**, *8*, 9-17.

138.) Sampath, D. S.; Balaram, P. Resolution of racemic gossypol and interaction of enantiomers with serum albumins and model peptides. *BBA*, **1992**, *882*, 183-186.

139.) Ibragimov, B. T.; Talipov, S. A. Supramolecular Association of Gossypol in the Crystalline State. *J. Struc. Chem*., **1999**, *40(5)*, 686-703.

140.) Dowd, M. K.; Stevens, E. D. Crystal and Molecular Structure of 6,6'-Dimethoxy gossypol:Acetic acid (1:1). *J. Chem. Crystallogr*., **2007**, *37*, 765-770.

141.) Bader, R. F. W. *Atoms in Molecules: A Quantum Theory (International Monograph in Chemistry)*; Oxford University Press: USA, 1994; pp 1-97.

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