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**DYNAMIC REACTIVITY OF BIOMOLECULES; A
SYNERGY BETWEEN THEORY AND EXPERIMENT**

HUYEN TRANG VU

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2018

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SYNERGY BETWEEN THEORY AND EXPERIMENT**

HUYEN TRANG VU

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2018

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

24/08/2018

Date

A handwritten signature in black ink, reading "TRAN G." in a stylized, cursive-like font.

Huyen Trang Vu

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

.. 24 August 2018.
Date

A/P Andrew Clive Grimsdale

Authorship Attribution Statement

This thesis contains material from a paper published in a following peer-reviewed journal where I was the first author.

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The contributions of the co-authors are as follows:

- Dr Gregory Adam Chass provided guidance for the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by Dr Kun Viviana Tian and reviewed by F. V. Song and Prof. Haibin Su.
- I co-designed the study with Dr Gregory Adam Chass and performed all the theoretical work at the School of Materials Science and Engineering and A*STAR Computational Resource Centre.
- All calculations, including molecular modelling, was conducted by me in the facility for analysis, characterization, testing and simulation.

..10 November 2019. .
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Huyen Trang Vu

Abstract

Elevated levels of oxidative stress caused by reactive oxygen species (ROSs) are linked to a variety of degenerative diseases including cancer, cardiovascular disease, dementia and ageing in general. Reactive species damages vital biological components in the body causing these ageing diseases. Antioxidants alleviate oxidative stress. Theaflavin is a natural polyphenolic antioxidant found in black tea; the most consumed manufactured drink in the world is tea. Its antioxidant properties established at the physiological scale, yet atomistic understanding is lacking, and thus, the potential for optimisation too. Thereby, a comprehensive conformational study of the antioxidant tea theaflavin by high-level computation density functional theory (DFT) was initiated. Exploration of the structural properties of neutral theaflavin using the theoretical methodology Becke-3-Lee Yang Parr (B3LYP) method together with the basis set 6-31G(d,p), in the gas and aqueous phase, for a full systematic conformational analysis. The conformational search yielded 153 distinct local energy minima conformers which suggest that theaflavin is a flexible antioxidant. The benzotropolone moiety (a characteristic of black tea) is the backbone to the energetic stability of theaflavin, alongside the extensive network of intramolecular interactions formed. The theoretical vibrational spectra in the fingerprint region were analysed for the comparison with an inelastic neutron scattering (INS) spectra, towards synergy between experiment and theory. INS spectroscopy has access to the region below 1000 cm^{-1} and is sensitive to hydrogen bond motions, enabling the characterisation of low energy vibrational modes of theaflavin conformers pre-radical capture. The antioxidant activity of theaflavin is associated with the stability of the antioxidant formed, post radical capture, as to prevent further damage to biological systems. The nature of this pronounced stabilisation at the atomic scale is unknown. To aid in its characterisation, a comprehensive conformational study of the antioxidant radical tea theaflavin by high-level computation DFT was performed. Conformational analysis of the antioxidant radical structures of theaflavin were generated by the radical scavenging mechanisms: hydrogen atom abstraction and hydrogen atom addition.

The most stable antioxidant radical H-abstraction was either at the 2-H site in the C ring or the b-OH hydroxyl site in the B ring, in gas and in a solvent. H-addition to the e-C and c-C sites in the B' ring yielded the most energetically stable antioxidant radical.

Lay Summary

In this study, a rational structure-activity relationship database was designed for antioxidant tea theaflavin. Oxidative stress (OS) is a common term used to describe the unfavourable balance of reactive species to antioxidants in the body. Reactive oxygen species are produced as an inevitable by-product of normal metabolic processes (~5% of inhaled oxygen) and from numerous environmental sources, namely radiation, smoking, and diet. The unfavourable balance of reactive species to antioxidants in the body is what causes oxidative stress. Reactive species (RS) are free radicals, highly reactive open-shell intermediates containing an unpaired electron. Free radicals are unstable, and thus very reactive, and have a tendency to react with compounds in close proximity to obtain paired electrons in its atomic orbital for stabilisation. Consequently, readily attacking biological systems such as DNA, lipids and protein. Therefore, elevated levels of oxidative stress have been linked to a variety of degenerative diseases. Oxidative stress may be alleviated by antioxidants - biologically active molecules that function to protect cellular damage from free radicals. Fruit, vegetables, wine and tea are rich sources of antioxidants. The consumption of a diet abundant in antioxidants, therefore, promotes the alleviation of reactive species by reducing oxidative stress in the body, in turn, diffusing the progressive deterioration of vital biomolecules from oxidative damage. The antioxidant's ability to scavenge and deactivate reactive species is correlated to the stability of the antioxidant formed. The antioxidant radical must remain stable, post-radical capture, to prevent further damage to the biological systems. Thus, the antioxidant's stability plays an essential role in the antioxidant's activity. Theaflavin is a natural polyphenolic antioxidant found in black tea and has displayed a numerous variety of health properties at the physiological scale, yet atomistic understanding is lacking, and thus, the potential for optimisation too. Therefore, a comprehensive conformational study by theoretical computation was carried out for both neutral antioxidant theaflavin and antioxidant radical theaflavin. The conformational search yielded 153 distinct local energy minima conformers. All conformers of theaflavin displayed an extensive network of intramolecular

interactions, eliciting a stabilising effect on the antioxidant. The intramolecular interactions could be formed due to the large and flexible nature of theaflavin's structure. A general trend was established between the conformer's stability and the structural features (hydroxyl conformation arrangement and planarity) of the benzotropolone moiety characteristic to black tea. Thus, highlighting the impacting factor, the benzotropolone has on the antioxidant's activity of theaflavin. The preferred radical scavenging activity by hydrogen atom abstraction and hydrogen atom addition was either in the benzotropolone moiety or in close proximity as the extensive π electronic structure, in this region, stabilised the antioxidant radical tea theaflavin.

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Abbreviations

a	Anti
Å	Angstrom
ALC-μSR	Avoided Level Crossing Muon Spin Resonance Spectroscopy
AO	Antioxidant
B. C.	Before Christ
B3LYP	Becke-3-Lee Yang Parr
CAT	Catalase
CTC	Crush tea curl
DFT	Density Functional Theory
DNA	Deoxyribonucleic Acid
e ⁺	Positron, positive electron
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
FAOstat	Food and Agriculture Organization statistic
FRTA	Free radical theory of ageing
H	Enthalpy/ Hydrogen
H·	Hydrogen radical
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
H-Abstraction	Hydrogen atom abstraction
H-Addition	Hydrogen atom addition
HAT	Hydrogen atom transfer
HF	Hartree-Fock
ILL	Institute Laue Langevin
INS	Inelastic Neutron Scattering

IR	Infrared spectroscopy
J-PARC	Japan-Proton Accelerator Research Complex
mtDNA	Mitochondrial deoxyribonucleic acid
n	Neutron
ns	Nano second, 10^{-9} s
O ₂	Oxygen molecule
O ₂ ^{-·}	Superoxide anion
OS	Oxidative stress
p	Proton
P _μ	Muon linear momentum
PCM	Polarised continuum model
PPO	Polyphenol oxidase
PSI	Paul Scherrer Institut
R	Restricted
R [·]	Radical
RAL	Rutherford Appleton Laboratory
ROS	Reactive oxygen specie
RS	Reactive specie
s	Syn
S	Entropy
SAR	Structure activity relationship
S _μ	Muon spin
SCRF	Self-consistent reaction field
SOD	Superoxide dismutase
TF	Theaflavin
TF-3, 3'-diG	Theaflavin-3, 3'-digallate
TF-3'-monoG	Theaflavin-3'-monogallate
TF-3-monoG	Theaflavin-3-monogallate
U	Unrestricted
UK	United Kingdom
UV	Ultra violet

WHO	World Health Organization
+	Gauche +
-	Gauche -
μ	Muon
μ^+	Positive muon
μ^+e^-	Muonium
$\cdot\text{OH}$	Hydroxyl radical
σ	Neutron cross section
$\bar{\nu}_\mu$	Muon neutrino
Δ	Delta/ Denotes change/ In-plane deformation of skeleton atoms
ν	Stretching
ν_a	Asymmetric stretching
ν_s	Symmetric stretching
ν_e	Electron neutrino
γ	Out-of-plane deformation
δ	In-plane deformation

Chapter 1

Introduction and Rationale

A brief statement regarding the research motivation and its significance were presented. The motivation and significance inspired: 1. the problem statement - 'this thesis aids in the rational design of a structure-activity relationship of the antioxidant tea theaflavin, a molecule that provides a large variety of biological functions due to their ability to scavenge reactive species, thus alleviating oxidative stress.' and 2. the thesis topic - 'Dynamic Reactivity of Biomolecules; A Synergy Between Theory and Experiment'. The aims and objectives are specified to guide the research towards solving the problem statement. A preview of the succeeding chapters are offered and concludes with the findings from this research.

1.1 Problem Statement

1.1.1 Research Motivation & Significance

Degenerative diseases are the leading causes of death worldwide. According to the World Health Organization (WHO), 17.5 million out of an estimated 56 million people died due to cardiovascular-related diseases, a staggering 8.2 million cancer-related deaths and 1.5 million caused by diabetes, in 2012.^{1, 2} The foundation of degenerative diseases are the result of impaired functions, in living organisms. These diseases often arise due to progressive deterioration of cells over time as a result of ageing and are influenced by the environment, lifestyle choices, reactive oxygen species (ROS) and ineffective defence mechanisms.³ In that particular are referred to as ageing-associated diseases.

Polyphenols have been shown to inhibit deleterious ageing diseases by having antioxidant properties which function to protect cellular damage to biological systems from free radicals. Fruit, vegetables, wine and tea, are rich sources of antioxidants. The consumption of a diet abundant in antioxidants, therefore, promotes the alleviation of reactive species by reducing oxidative stress in the body, in turn, diffusing the progressive deterioration of vital biomolecules from oxidative damage. Antioxidants reduce oxidative stress as they facilitate the stabilising of reactive species by donating or accepting the electrons to or from the radical, resulting in a more stable radical; an antioxidant radical as the alternative.^{4, 5}

1.1.2 Tea

Consumption of tea traces back to ~ 2700 B.C.; it is one of the oldest beverages in the world and still in demand today (Figure 1.1).⁶ Tea as a refreshment is the most consumed manufactured drink worldwide. In addition to a pleasantly aromatic and tasty thirst quencher, the tea plant (*Camellia sinensis*) was traditionally used in

China and India for their medicinal properties including detoxification, teeth strengthening, laxative, calming effects and longevity.⁷ The health benefits of green tea have been far more exhausted than black tea. However, the popularity of black tea and its health-promoting properties has gained interests, in recent years. Predominantly, black tea and its health benefits associated with antioxidant activity,⁸ bone health,⁹ antimicrobial,¹⁰ antivirals,¹¹ oral health,^{12, 13} neuroprotective,¹⁴ cardiovascular diseases,¹⁵ and cancer.^{8, 13, 16} Due to the ever-growing scientific pharmacological and physiological evidence of tea highlighting their antioxidant and anti-ageing properties, growing consumer interest provoked application of tea extracts in various food products,¹⁷⁻²⁰ cosmetics^{21, 22} and medicinal ointments.²³ Furthermore, tea has been made known to assist in the inhibition of cancer, heart disease and dementia, amongst other ageing-related diseases.^{6, 8, 14, 21, 24-28} These ailments are the leading causes of death worldwide and are evidenced as being triggered by reactive oxygen species (ROSs); radical-containing reactive metabolic intermediates.³ Traditional and modern medicinal properties associated with tea relates the polyphenolic antioxidant content as the main contributor to the various health benefits.

Bio-active compounds in tea show promising radical scavenging ability. The main antioxidant constituents of green tea are polyphenolic catechins, which have a common backbone structure consisting of two benzene rings connected via a dihydropyran heterocycle. Most varieties of teas start out as green tea. The manufacturing of black tea is a result of an additional fermentation step, crafting even more complex antioxidants.²⁹ Tea fermentation is an enzymatic process, wherein two catechin molecules bind together to form theaflavin, with one of the 6-membered aromatic B-rings converting to a stable 7-membered pseudo-aromatic benzotropolone (Figure 1.1).^{29, 30} Fermentation and ageing processes produces theaflavin molecules which are substantially larger catechin molecules, qualitatively described as two catechin molecules joined together. The transformation yields theaflavin and its derivatives, broad and flexible structures of high stability. It is the polyphenol theaflavin that provides the characteristic colours

and fragrances associated with black teas; ageing increases theaflavin concentration, and thus, the quality and price of this commodity.²⁹ From a molecular perspective, theaflavin's unique structure and B and B' - ring, in particular, are thought to underlie its superior ability to neutralise ROSs through free-radical scavenging.

Figure 1.1 Fresh powdered Pu'Er tea and its constituent black tea antioxidant theaflavin (1) with IUPAC ring-nomenclature.

1.2 Aims and Objectives

Constructed by the motivation of the increasing ageing-related diseases and its deleterious impact on people, the community and economy, and its prospective reduction by the promising radical scavenger tea theaflavin, I at this moment present the scope of this thesis - Dynamic Reactivity of Biomolecules; A Synergy Between Theory and Experiment. The current work explores the molecular structure of theaflavin in connection to its antioxidant properties. Therefore, the aim of this thesis is to investigate the structural features of the antioxidant tea that

results in their antioxidant activity. *This thesis aids in the rational design of a structure-activity relationship of the antioxidant tea theaflavin, a molecule that provides a large variety of biological functions due to their ability to scavenge reactive species, thus, alleviating oxidative stress.*

The objectives of this thesis are as follows:

- Determine the conformational preference of antioxidant tea theaflavin.

To determine the conformational preference of theaflavin a thorough conformational study of all possible conformers will be modelled, computed and analysed. The stability of the successfully computed conformers will be characterised by collecting and analysing the energy and structural data. The antioxidant will be investigated in gas and in aqueous solvent, to study the dynamics of the conformer to the changing environment.

- Determine the antioxidant radical site of activity.

All possible sites of H-addition and H-abstraction in theaflavin will be modelled, computed and analysed. The energetics of the antioxidant radical tea theaflavin generated by the radical scavenging mechanism hydrogen atom abstraction (HAB) and hydrogen atom addition (HAD) will be investigated towards a comprehensive conformational study of the antioxidant radical tea theaflavin. The antioxidant radical will be investigated in gas and in aqueous solvent, to study the dynamics of the conformer to the changing environment.

- The synergy between theory and experiment.

To formulate a synergy between theory and experiment, a theoretical database must be formed in preparation for the comparison and analysis with the experimental data. To build the theoretical database the fingerprint region of the theoretical vibrational spectra will be explored by determination of the peaks detected in the low vibrational modes, analysis of the different vibrational modes and investigating

the source resulting in the peaks to shift. Therefore, a thorough analysis of the fingerprint region on the vibrational spectra will be performed.

1.3 Dissertation Overview

The contents of the chapters address the following:

Chapter 1: Introduction and Rationale

This chapter provides a brief summary of the current health-related issues and a possible means to assist in its reduction. The problem statement, aims, and objectives of the thesis were discussed.

Chapter 2: Literature Review

To help understand the material in this study, a literature review of the material tea and ageing process was formed. Specifically, the chapter provided insight into the bio-material production, structure and properties, and developed a timeline of the different theories of ageing.

Chapter 3: Methodology

The theory of computational chemistry and inelastic neutron scattering were discussed. The specific details of the theoretical methods employed in the study of the antioxidant theaflavin pre-radical scavenging and post-radical scavenging, in both gas and aqueous solvent, were described.

Chapter 4: Antioxidant Tea theaflavin

In this chapter, the conformational analysis of tea theaflavin was initiated. The scope for the thorough analysis of theaflavin neutral conformers, pre-radical capture, were determined. The structure and energies of the refined shortlist of conformers, in both gas and aqueous solvent, were explored.

Chapter 5: Antioxidant Radical Tea Theaflavin

In this chapter, the conformational analysis of antioxidant radical tea theaflavin was initiated. To determine the preferred antioxidant activity vicinity in theaflavin, analysis of the antioxidant radicals energetics, - post radical capture, in both gas and aqueous solvent, were analysed.

Chapter 6: Inelastic Neutron Scattering

In preparation for the synergy between theory and experiment, the data produced through theoretical calculations were explicitly refined to be comparable to the results obtained by experimental means. INS characterisation technique has access to low vibrational modes (fingerprint region). Thus, analysis of the theoretical vibrational spectra in the range 0 – 1000 cm⁻¹ of selected antioxidant tea conformers were explored.

Chapter 7: Impact, Implications and Future Work

To finalise the thesis, the results obtained in the study were referred back to the problem statement for coherence. In the end, the chapter paved the way towards future studies to build on the structure-activity relationship database initiated by this study. *Comprehensive conformational analysis of the dynamic reactivity of bio-molecule tea theaflavin.*

1.4 Findings and Outcomes/ Originality

The research in this study led to the following outcomes:

- Antioxidant tea theaflavin has 153 distinct local energy minima conformers.
- The conformers of theaflavin are stabilised by the benzotropolone moiety's planarity and hydrogen bonding motifs, an extensive network of intramolecular interactions, and the formation of meta-stable rings.
- Rings A, A', C, C', its functional group substituents and ψ_2 dihedral angle (containing the C-C linker d-C-2-C) affected the energy of the conformers to a

lesser degree. Thus, they are potential sites for systematic research and development (R & D) for innovative drugs.

- The preferred radical scavenging was in the vicinity of the benzotropolone moiety - having the superior ability to neutralise reactive species,
 - proficient facilitator to electron-spin stabilisation.
- The fingerprint region provided information on hydrogen bonding interactions in the system. Hydroxyl functional groups participating in hydrogen bonding interactions formed peaks at higher frequencies as compared to the groups that did not. The stronger the hydrogen bond, the higher the frequency in the out-of-plane deformation (γ) torsion vibrational peaks detected.

The antioxidant theaflavin has a large and flexible structure with a distinct benzotropolone moiety characteristic to black tea. Theaflavin adopted numerous stable conformers, within a small relative energy range. Thus, indicating that theaflavin likely exists as a complex conformational mixture; attributable to the backbone flexibility and allowing for widely differing skeletal structures. Therefore, the health benefits of theaflavin could commence from its conformational flexibility, as each conformational isomer possessed different structural properties; thus, they can impart differing chemical and biological functions.

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Chapter 2

Literature Review

A comprehensive literature review to gain insight into the commodity, tea and its importance. Delving into the most common types of teas, the difference in the tea types, namely, the bio-active compounds and properties of the tea, to understand the material in the study. The different theories of ageing were also delved into. The background on the different theories of ageing and its timeline are provided, as well as, the effects of ageing and how ageing may be alleviated are presented.

2.1 Tea

The tea plant *Camellia sinensis* is the manufactured drink most consumed in the world. Consumption of tea traces back to approximately 2700 B.C.; it is one of the oldest beverage in the world and is still in demand.¹ The tea industry impacts the economy significantly, highlighted by the continuous growth in the world's tea production. Tea production reached almost 5.3 million tonnes, in 2013. The leading countries in tea production are China and India; China surpassing India as the leading tea production country, from 2005 (Figure 2.1).² The ability to convey the atomistic structure of tea in relation to their health properties will increase the demand for this commodity, thus, having a substantial impact economically.

Figure 2.1 World production of tea from 1961 to 2013. The leading tea producing countries are China and India. Reproduced from FAOSTAT.²

2.1.1 Tea Production

Based on the processing technique applied, different types of teas are produced (Figure 2.2). The most common commercial types of teas available in the market are green, oolong and black tea. Each tea master will have a unique production technique, creating distinctive flavours in the various teas.

Figure 2.2 The manufacturing process for various types of tea: green tea, oolong tea and black tea. To produce uniform sized tea leafs, the production method crush tea curl (CTC) is employed. Reproduced from Hara.¹

Green tea

Fresh green tea leaves are the foundation of all the types of tea. A preparation method for creating green tea is initiated by steaming then drying off the freshly plucked tea leaves; caution is taken to prevent any bruising to the leaves. Steaming and drying prevent enzymatic reactions to occur by denaturing the enzymes.^{1,3} This stops oxidation and preserves the original catechin content from the tea plant, retaining the freshness and essence of green tea. The following rolling step determines the shape of the final tea leaves.

Oolong tea

The tea variety oolong is a partially fermented tea, thus, capturing characteristics of both green and black tea. Therefore, the catechin content in oolongs is lower than that of green tea; also, the theaflavin content less than black tea.¹ Solar withering and indoor withering reduces the moisture content and promotes oxidation of the leaves.^{1,3} The degree of oxidation produces different styles of oolong tea as the

polyphenol content. Thus, producing a range of distinctive flavours and colours from green to red.

Black tea

Unlike its given name, black tea when brewed is copper brownish in colour. The distinct colour is produced by the oxidation process, involving the enzyme polyphenol oxidase (PPO). The manufacturing of black tea begins with withering followed by rolling and fermenting. The rolling process is the focal stage as it entails the mixing of the enzyme and catechins located in different structural components of the leaf. Next, the bruised leaves are left to oxidise completely. Over time the colour of the leaves changes from green to coppery red, then dried.^{1,3} Black tea is commonly referred to as fully oxidised tea.

2.1.2 Tea Structure

(2)	(-)-epicatechin (EC)	$R_1 = H$
(3)	(-)-epicatechin gallate (ECG)	$R_1 = G$
(4)	(-)-epigallocatechin (EGC)	$R_1 = H$
(5)	(-)-epigallocatechin gallate (EGCG)	$R_1 = G$

Figure 2.3 The four abundant catechins found in green tea: (-)-epicatechin (EC, 2), (-)-epicatechin gallate (ECG, 3), (-)-epigallocatechin (EGC, 4), and (-)-epigallocatechin gallate (EGCG, 5).

Green tea

Tea contains a naturally occurring group of polyphenols called flavonoids. The main antioxidant constituent of green tea is polyphenolic flavanol catechins, which have a common backbone structure consisting of two phenyl rings connected via a heterocyclic ring (Figure 2.3 - rings A, C, B, respectively). The most abundant catechins in green tea are (–)-epicatechin (EC) (**2**), (–)-epicatechin gallate (ECG) (**3**), (–)-epigallocatechin (EGC) (**4**) and (–)-epigallocatechin gallate (EGCG) (**5**) making ~20-30% of the dry leaf matter (Figure 2.3).^{3, 4} Due to the absence of the oxidation process, the catechin content is retained, producing the distinctive taste, flavours, scent, and colour associated with green teas.

Figure 2.4 Formation of theaflavin (**1**) facilitated by polyphenol oxidase (PPO) enzyme from (–)-epicatechin (EC, **2**) and (–)-epigallocatechin (EGC, **4**). Benzotropolone moiety highlighted in red.³

Black tea

All teas start-out as green teas. To produce black tea, withered leaves are subjected to additional steps to promote oxidation.³ Tea oxidation is an enzymatic process, wherein two catechin molecules bind together to form theaflavin. The process converts one of the six-membered aromatic B-rings to a stable seven-membered pseudo-aromatic benzotropolone ring (B') (Figure 2.4).^{3, 4} Oxidation and ageing produce theaflavin molecules which are essentially larger catechin molecules. Thus, theaflavin and its derivatives can qualitatively be described as 'two catechin

molecules joined together'. The reactions transform 'simple' catechins to very large and flexible structures of high stability (Figure 2.5) for example, theaflavin (**1**) from (-)-epicatechin (EC, **2**) and (-)-epigallocatechin (EGC, **4**) and theaflavin-3-monogallate (TF-3-monoG) (**6**) from (-)-epicatechin (EC, **2**) and + (-)-epigallocatechin gallate (EGCG, **5**).⁴ The oxidation process which results in theaflavin and its derivatives contributes to the characteristic colour and fragrance associated with black tea.³

(1)	Theaflavin	$R_1 = H, R_2 = H$
(6)	Theaflavin 3-monogallate	$R_1 = H, R_2 = G$
(7)	Theaflavin 3'-monogallate	$R_1 = G, R_2 = H$
(8)	Theaflavin 3,3'-digallate	$R_1 = G, R_2 = G$

Figure 2.5 The different theaflavin constituents found in black tea where the benzotropolone moiety is highlighted in red. Theaflavin (TF) (**1**), theaflavin-3-monogallate (TF-3-monoG) (**6**), theaflavin-3'-monogallate (TF-3'-monoG) (**7**), and theaflavin-3, 3'-digallate (TF-3, 3'-diG) (**8**).

2.1.3 Tea Properties

Traditionally, in China and India, the tea beverage was thought to have medicinal properties and were used for numerous health-related properties. The assumed health-related properties included detoxification, teeth strengthening, laxative, calming effects and longevity, in addition to a pleasantly aromatic and tasty thirst

quencher.⁵ The modern society, based on numerous studies, support the alleged medicinal properties from the ancient traditions, relating the polyphenolic content in tea as the main contributor to the various health benefits associated with tea.

Green tea catechins have been reported to elicit a variety of health-related beneficial effects including, antioxidant properties: lipid peroxidation and free radical generation,⁶⁻⁸ cardiovascular disease: hypertension and coronary heart disease,^{1, 9} oral health,¹⁰ antimicrobial,^{11, 12} neuroprotective,¹³ obesity,¹⁴ and various cancers.^{8, 15-18} The health-promoting properties of green tea has been far more exhausted than black tea. However, the popularity of black tea and its health-promoting properties has gained interests in recent years. Predominantly, black tea and its health benefits associated with antioxidant activity,⁸ bone health,¹⁹ antimicrobial,¹² antivirals,²⁰ oral health,^{21, 22} neuroprotective,¹³ cardiovascular diseases: hypertension,²³ and cancer.^{8, 22, 24} Due to the ever-growing scientific pharmacological evidence of tea, highlighting their antioxidant and anti-ageing properties, growing consumer interest provoked the application of tea extracts in various food products,²⁵⁻²⁸ cosmetics^{18, 29} and medicinal ointments.³⁰

Tea antioxidants come under the umbrella of polyphenolic antioxidants called flavonoids. Flavonoids are a group of antioxidants that have a basic backbone structure of two phenyl rings and a heterocyclic ring.³¹ This group of antioxidants is vast, and its structure varies in the type, number, and position of their electron donating group (-OH, -OMe, OCH etc.). The molecular properties of various flavonoids have been studied by DFT.³²⁻³⁶ The different structural features in the flavonoids altered the extent of π -electron delocalisation and the number of intramolecular hydrogen bond formation, therefore, affected the antioxidants radical scavenging ability. The study revealed, flavonoids with the following structural characteristics had higher antioxidant activity:

- Hydroxyl groups at the C3 and C4 in the ortho-position.
- A pyran ring with a double bond at C2 and C3, a ketone at C4 and a hydroxyl group at C3.

- A pyran ring with a ketone group at C4, a hydroxyl at C3 and an A ring with a hydroxyl group at C5.

Antioxidant flavonoids containing these structural characteristics, mentioned above, are more stabilised as they have numerous intramolecular hydrogen bonding formations and an extensive π -electron delocalisation network.

2.1.4 Components Affecting Tea

Antioxidant stability plays a significant role in their protective health properties. The antioxidant must remain stable after donation or acceptance of radicals to prevent further damage to biological systems. The antioxidants in tea are the polyphenols which are susceptible to change in concentration by various sources. Thus, altering the health benefits of tea.

In the previous section (2.1.1), the formation of different tea types by tea production, post tea plucking, was introduced. Alteration of the components in tea is not limited to the production process but varies from the environment of the tea plant all the way through to the storage of the tea leaves.

The tea crop requires a hot and moist climate; such climates are constrained to tropical and sub-tropical regions.³⁷ Thus, tea cultivation is limited to specific regions geographically, as it has rather specific climate requirements to grow. The locality of tea plantation causes variation in the flavonoid content of the tea plant, due to environmental factors (sunlight, altitude and rainfall).

Other aspects of tea cultivation include both harvesting time of year and 'body' part of the plant. The harvesting season alters the polyphenolic content of the tea plant. The concentration of catechins is reduced in spring yet greater during summer and autumn. This is attributable to greater sun exposure during the latter months.^{1, 38} Depending on the type of tea the farmers intend to produce, a particular location of the tea plant is plucked. Generally, for green tea cultivars, the young leaves and tip

are selected. Catechin formation is prolific in these fragments, to protect the leaves from excess sun exposure.^{1, 4}

Biotic stress caused by insects is another aspect that alters the composition of tea. When the tea plant is under attack by insects, the tea plant makes adjustments to cope with the stress applied. Elevated levels of specific endogenous enzymes such as the activity of polyphenol oxidase (PPO) and epicatechin (EC) content were present during insect attacks.³⁹ The increased epicatechin (EC) and PPO contents appear to be the tea plant's defence mechanism.

The catechin and theaflavin content partially determine the richness (quality and price) of the various tea types. Maintaining a high concentration of these polyphenols will have a definite beneficial effect on the tea plants properties too. The exposure of the tea crop to environmental factors (sun, altitude, biotic stress) altered various metabolic and catechin production rate, to protect the tea plant from harm.

2.2 Ageing

Various hypotheses emerged in the mid-1900s regarding the roots of ageing (Figure 2.6). One of the first theorised was in 1956; the free radical theory of ageing (FRTA), conceptualised by Harman.⁴⁰ This theory states that ageing and its association with diseases are induced as a result of oxidative damage to biological constituents by reactions with ROSs. ROSs are highly reactive species produced as an inevitable by-product of standard metabolic processes (~5% of inhaled oxygen) and from numerous environmental sources namely, radiation, smoking, and diet. In 1972, Harman modified the FRTA to the mitochondrial theory of ageing.⁴¹ The revised theory emphasised mitochondrial decay as the source of ageing and diseases. This impairment resulted in reduced energy production while enhancing levels of ROSs, and consequently, increasing mitochondrial DNA (mtDNA) mutations. Johan Bjorksten proposed the cross-linking theory of ageing, in 1942.⁴² This theory

states that increased cross-linking of macromolecules influence the ageing process. Cross-linking alters the structure of macromolecules, and thus, may initiate their malfunction. The immunological theory of ageing was presented by Walford, in 1964.⁴³ Walford hypothesised that with age, our line of defence against foreign invaders; the immune system, deteriorates. Due to these changes, the capability to evade diseases declines as does the ability to distinguish self from foreign materials resulting in further damage. The Hayflick limit theory of ageing was developed, in 1961, by Leonard Hayflick, after discovering the human fibroblast ability to divide is finite.⁴⁴ The Hayflick limit theory suggests that the lifespan of cells could increase using decreasing functional failures and improve cellular repair systems.⁴⁵ In 1959, the somatic mutation theory of ageing was put forth. Szilard hypothesised that ageing is due to the alteration and mismatching of genetic information, caused by the accumulation of DNA damage.⁴⁶ Ultimately, the accumulation of DNA damage resulted in the deterioration and malfunction of cells.

Theories of Ageing

Figure 2.6 A timeline of the different theories of ageing.

These theories are by no means exclusive. They all propose the role of ageing as the root determinant of degenerate disease, stemmed from dysfunctional biomolecules. Amongst the various theories put forth, the most popular is the free radical theory of ageing (FRTA). The role of free radicals is essential to bodily functions: maintenance of homoeostasis and cell signalling.⁴⁷ However, an abundance of free radicals causes the body to undergo oxidative stress. Oxidative stress (OS) is a common term used to describe the unfavourable balance of reactive species to antioxidants in the body (Figure 2.7).⁴⁸ Elevated levels of reactive species result in oxidative stress to the body. Free radicals are highly reactive open-shell intermediates (containing an unpaired electron). They are unstable, and thus, very reactive, and tend to react with compounds in close proximity to obtain paired electrons in its atomic orbital for stabilisation, therefore, readily attacking biological systems such as DNA, lipids and protein.⁴⁷⁻⁴⁹ Oxidative stress is believed to induce 'electronic diseases' like cancer, diabetes and ageing.⁵⁰⁻⁵²

Figure 2.7 An illustration of high (right) and low levels (left) of oxidative stress, above. RSs are readily attacking biological components for stabilisation. An increase in antioxidants (free radical scavengers) stabilises the RS, restoring the body's electronic balance by terminating the chain reaction, below.

ROSs may be formed in-vivo in any locality where O_2 is present, as a by-product of normal metabolism (Eq. 1-4) and exogenously via radiation (Eq. 5). The resulting ROSs are superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydrogen radical ($H^{\cdot}$) and hydroxyl radical ($\cdot OH$). The hydroxyl radical is a very reactive radical known to react with many biological molecules in its vicinity.⁵³ Enzymatic antioxidants are the internal defence system, functioning to protect cells from oxidative stress. The system includes superoxide dismutase (SOD) and

catalase (CAT). SOD functions to catalyse the dismutation of superoxide anion ($O_2^{\cdot-}$) radical to hydrogen peroxide (H_2O_2) and oxygen (O_2) while CAT catalyses the decomposition of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). The combination SOD and CAT moderates the formation of the highly reactive hydroxyl radical ($\cdot OH$).

Theoretically, the imbalance can be restored by endogenous enzymes. However, the environment and lifestyle factors make this virtually impossible without the introduction of neutralising agents such as antioxidants.

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Chapter 3

Methodology

Chapter 3 provides a brief introduction to computational chemistry. Highlighting the inner workings of the theoretical methodology and basis set, geometry optimisations and frequency calculations applied in the computations, for the conformational analysis of the antioxidant tea theaflavin, and the radical scavenging mechanism of the antioxidant radical tea theaflavin. In addition, the theory of the experimental methodology of inelastic neutron scattering was described.

3.1 Theoretical Methodology

3.1.1 Computational Chemistry

Computational modelling has become a viable approach to studying molecules and material systems at the atomic scale. This field has gained popularity in recent years earning Nobel Prizes, in 1998 and 2013, by Kohn and Pople, and Levitt, Karplus and Warshel, respectively. Through their contribution, characterising dynamics, energetics and structural properties of complicated systems have become more efficient and accurate.

Computational methods incorporate the fundamental laws of physics/ chemistry via two primary methods: molecular mechanics and quantum mechanics: *ab initio* and density functional theory.¹ The two methods apply different physical/chemical approximations to the computations, thus, garnering different properties to the system investigated. Increasing mathematical approximations yields more reliable results, consequently, this increases computation time and power. Nevertheless, computational modelling is generally cheaper and utilises a reduced amount of resources, compared to experimental techniques.

Computational chemistry is a great tool to analyse open shell structures. There is a void in uncovering general trends of open-shell systems, as they are commonly short-lived, hard to obtain and observe experimentally. Open-shell systems include vital antioxidant bio-molecules. Computational methods can aid in the translation of structure, property and activity phenomenon, to bio-molecular properties, and subsequently, to medicine and the society.

3.1.1.1 *Quantum Mechanic Based Theoretical Methodology*

The laws of quantum mechanics are applied to methods including ab-initio and DFT. These methods use different mathematical approximations to solve a variety of properties of systems.

Ab-initio computations use only theoretical approximations based on the quantum laws of physics and no experimental data. The calculations are time-consuming and computationally expensive but produce accurate results. The Hartree-Fock Self-Consistent Field (HF) is an ab-initio method. The HF method uses the least mathematical approximations resulting in the least accurate ab-initio method.¹ Thus, the calculations performed are economical, reliable and increases the quantity of result.^{1, 2} Limitations of the Hartree-Fock theory includes the absence of electron correlation effects on the system.

Density functional theory (DFT) methods are similar to ab initio methods; based on theoretical approximations and no experimental data.³ DFT methods include the effect of electron correlation; thus, acknowledges the different response close proximity electrons will have in the system investigated. Methods including electron correlation account for the instantaneous interactions of electrons. DFT avoids the expense of the more traditional methods; deriving the energy directly from the electron probability density, rather than the molecular wavefunction. Thus, DFT calculations require less computational resources than the average ab initio method.³

3.1.1.2 *Basis Set*

Mathematical descriptions called basis sets are used to represent the molecular orbitals (MO) of a system. A basis set functions to confine the electrons of a system to a region of space.¹ A smaller basis set restricts the locality of the electrons, thereby, reducing the accuracy of the orbitals in a system. A larger basis set applies fewer restrictions to the electron space region, subsequently, increasing the system

orbital accuracy. Smaller basis sets are cost-efficient while larger basis sets are more accurate.

3.1.1.3 *Geometry Optimisation*

Geometry optimisation is the process of minimising the energy of a system through an iterative manipulation of its atoms towards identifying their most stable configuration. The constructed model system is calculated until the gradient of energy reaches zero (stationary point) and approaches convergence. The calculation is terminated when the net energy of the system is minimised by the determination of optimised arrangements of each atom.

3.1.1.4 *Frequency Calculation*

Frequency calculations follow geometry optimisations as it requires optimised structures to operate. Calculations produced provides information on the systems vibrational frequencies, thermodynamic properties and characterisation of stationary points.¹ Relative contributions to the theoretical results will be analysed and compared with experimental data to determine theoretical-experimental synergy.

3.1.1.5 *Closed / Open Shell System*

Closed and open-shell systems are commonly referred to as restricted (R) and unrestricted (U), respectively. The terminology is in regards to the electron spin of the system where closed shell (R) systems have an even number of electrons, thereby, having a total electron spin of zero (electron spin = 0) and open-shell systems have an odd integer number of electrons (electron spin >0).¹ Therefore, closed shell systems are restricted (R) to occupying each orbital in pairs of opposite electron spin. Open-shell systems, however, are unrestricted to occupying each orbital in pairs; opposite electron spin residing in separate orbitals (Figure 3.1).

Figure 3.1 Illustration of electrons occupying restricted and unrestricted systems. An even number of electron in a system occupying each orbital in pairs (left) and an odd number of electrons in a system occupying separate orbitals according to opposite spins (right). α = alpha and β = beta.

3.1.2 Gas Phase Calculation

The programme package Gaussian 09⁴ was used to model, compute (quantum chemical calculations) and analyse all computations of theaflavin. Theaflavin can adopt 512 possible stable conformers; 2⁹ where the number two represents either the conformation anti or syn and the number nine was deduced from the number of free hydroxyls –O-H rotamers in theaflavin (Figure 3.2 and Appendix Table A 1). This defines the scope for computations of theaflavin to characterise all probable stable sets of conformers.

All 512 possible stable conformers were geometrically optimised, initially with the RHF method⁵ and 3-21G split-valence Pople basis set⁶⁻⁸, in the gas phase. All calculations were run in the gas phase to characterise the properties of isolated theaflavin. The relatively low method and basis set employed were to reduce both time and cost. The preliminary goal was to generate a quantitative refined shortlist of neutral conformers. The shortlist would later be computed at a more expensive and accurate (higher) level of theory.

The generated stable and non-degenerate optimised structures, using the RHF/3-21G, were subsequently re-optimised utilising the method, DFT B3LYP,^{3, 9-11} and basis set, 6-31G(d,p), level of theory. These computations were run to increase the accuracy and reliability of the results. All calculations were subjected to frequency calculations to ensure they were indeed at the minima, i.e. no negative frequency.

Figure 3.2 All possible rotamer sites of theaflavin highlighted in blue ($\zeta 1 - \zeta 9$, hydroxyl side chains) and red ($\phi 1$ and $\psi 2$, main backbone dihedral angles), *left*. The possible space conformations of theaflavin's side chains, *right*.

3.1.3 Solvent Calculation

To mimic physiological conditions, solvent calculations were employed. The shortlisted list of optimised calculations ran at the DFT B3LYP, in the gas phase, were re-ran in aqueous solvent conditions (dielectric constant (ϵ) = 78.355), using the self-consistent reaction field polarised continuum model (SCRF PCM).¹² The data generated will be used to observe theaflavin's structural dynamics and stability to the changing environment. Also, the effects of the environment to theaflavin's electronic properties, reactivity and functionality, at the atomic level. The pre-computed optimised geometry was used to increase computational efficacy.

3.1.4 Scavenging Activity

The free radical scavenging mechanism hydrogen atom abstraction and hydrogen atom addition mechanism were explored. A test set of 12 neutral conformers were used to investigate theaflavin's free radical scavenging activity. The test set selected contained the ten most stable neutral conformers (lowest energy), the least stable neutral conformer (highest energy) and the medium energy conformer, in the gas phase. The same selected test set was investigated in the solvent water, to understand how solvent effects the scavenging activity.

3.1.4.1 Hydrogen Atom Abstraction

Figure 3.3 17 possible hydrogen atom abstraction sites of theaflavin highlighted in the picture (top); nine hydroxyl side chains abstraction sites (bottom left) and eight hydrogen atom abstraction sites (bottom right).

The antioxidant theaflavin contains multiple locations for possible hydrogen atom abstraction. Specifically, 17 probable H-abstraction sites; nine hydroxyl side chains and eight H-atom, as seen in Figure 3.3. The relative energies and geometrical parameters of the traditionally accepted mechanism, hydrogen atom abstraction, (Figure 3.4) were computed at the unrestricted Becke-3-Lee-Yang-Parr (UB3LYP) method and 6-31G(d,p) basis set, for the test set of 12, at all possible 17 hydrogen atom abstraction sites. The calculations were run in various media including the gas-phase and implicit solvent (H_2O).

Figure 3.4 Hydrogen atom abstraction mechanism at one possible theaflavin site.

3.1.4.2 Hydrogen Atom Addition

Figure 3.5 Seven possible hydrogen atom addition sites of theaflavin highlighted.

Theaflavin has seven possible hydrogen atom addition sites, highlighted in Figure 3.5. The novel mechanism H-addition, displayed in Figure 3.6, was explored using the UB3LYP method and accompanied by the 6-31G basis set, for all potential H-addition sites. The relative energies and geometrical parameters for the H-addition mechanism were investigated in both the gas-phase and the aqueous solvent media, for the 12 neutral conformer test set.

Figure 3.6 Hydrogen atom addition mechanism at one possible theaflavin site.

3.2 Experimental Methodology

3.2.1 Theory of Inelastic Neutron Scattering (INS)

3.2.1.1 Neutron

An atom consists of three types of subatomic particles; protons, electrons and neutrons. The neutron is located in the nucleus of an atom together with the proton. Neutrons have a net charge of zero and a mass of 1.675×10^{-27} kg (slightly larger than a proton, and substantially, larger than an electron).¹³ The number of neutrons in a given element can vary, therefore, giving rise to isotopes, although the number of protons is unchanged. Thus, isotopes of a particular element will have the same atomic number (no. of protons) but different mass numbers (no. of protons + neutrons).

The characteristics of the neutron give it advantages as an experimental spectroscopy technique. Neutrons are scattered by the nuclear forces of an atom, enabling them to penetrate deeply into the system investigated and close to the nuclei, unlike photons or electrons.¹⁴ This nuclear interaction enables elements with low masses to be measured where scattering from electrons are unsuccessful.

3.2.1.2 *Inelastic Neutron Scattering (INS) Spectroscopy*

INS spectroscopy is an experimental technique similar to Infrared (IR) and Raman spectroscopy. These spectroscopy techniques are useful for investigating the molecular vibrations of materials. The significant advantages of INS compared to IR or Raman spectroscopy are:

- INS is not subjected to the optical selection rules.

Unlike IR and Raman, all vibrations are active in the INS spectra due to the absence of the selection rules. INS spectra analysis can, thus, be directly compared with high-level theoretical calculations. This is a valuable characteristic in modern science, by aiding experimental - theoretical synergy.¹⁴

- INS covers the entire scope of the molecular vibration spectra 0 - 4000 cm^{-1} .¹⁵
- At the lower energy range, IR and Raman spectroscopy are inadequate. This region is readily accessible with INS spectroscopy making it a great tool to characterise low energy collective modes.^{14, 15}

Despite the numerous advantages of INS, the disadvantages of INS relative to IR and Raman are:

- INS requires quite a large sample,
- INS is a more expensive spectroscopy technique,
- INS is not readily available to users,
- INS is more time-consuming.^{14, 15}

INS has been employed to study dietary phenolic acids: hydroxycinnamic derivatives.¹⁶ This study by Marques et al.¹⁶ was employed to determine the conformational preference and stability of the antioxidant cinnamic acid and its derivatives. The authors were able to link theoretical and experimental work

regarding the stability of the antioxidant through intramolecular and intermolecular hydrogen type interactions shown at the low-frequency region of the INS spectrum.

The conformation of bio-molecules plays a vital role in their stability. Access to the low energy collective modes can help characterise the conformational preference. Therefore, through both theory and experimental techniques, can substantiate the importance of the role of conformation in relation to their antioxidant potential.

Neutron Production

Neutron production is an expensive process making the overall experiment costly and not easily accessible. The production requires a nuclear process to free bound neutrons from the nuclei, either by fission or spallation. Neutron production by fission is used at the Institute Laue-Langevin (ILL) in Grenoble, France and involves the bombardment of a heavy metal, usually Uranium, in a nuclear reactor. The free neutrons can trigger more fissions, and thus, produces more neutrons resulting in a nuclear chain reaction. Neutron source via spallation is employed at the ISIS facility, in Rutherford Appleton Laboratory (RAL), Oxfordshire, United Kingdom (UK). Neutron production at a neutron spallation source requires a collision between a proton from a high energy particles accelerator and a heavy metal target (uranium, tantalum or mercury). The collision yields highly excited nuclei which relax by ejection of neutrons. The latter process of neutron production is more costly but gains an advantage in having a higher neutron flux and to the source being pulsed.

The principle of the Neutron Scattering - TOSCA Spectrometer

Figure 3.7 An illustration of the neutron time of flight instrument. t_1 = time taken for a neutron to travel from moderator to sample, t_2 = time taken for a neutron to travel from sample to detector, l_1 = incident flight path length from moderator to sample and l_2 = final flight path from sample to the detector.

TOSCA is a time of flight spectrometer (Figure 3.7) at the ISIS pulsed spallation neutron source and used for energy analysis of materials.¹⁷ The source of neutrons, on TOSCA, emerges in the direction of the white beam after high energy neutrons are suppressed in the water moderator. The neutron then passes through a chopper which operates to remove high energy neutrons (unmoderated neutrons) and slow neutrons. A small number of neutrons are in-elastically scattered by the sample, and those that are backscattered through an angle of 45° or 135° reach a graphite crystal analyser.¹⁷

Bragg's Law

$$\lambda = 2d\sin\theta \quad \text{Eq. 3.1}$$

Where λ = wavelength (Å), d = inter-planar spacing (Å) and θ = scattering angle ($^\circ$).

According to Bragg's law, since both d and θ are constant, only a fixed wavelength of neutrons will be Bragg scattered from the graphite crystal into the detector.¹⁴ The scattered neutrons with fixed energy are then passed through the beryllium filter and detected. Both the graphite crystal and beryllium filter function as a narrow band-pass filter.¹⁷

In the INS experiment, the neutron scattering strength (σ) is measured as a function of the change in neutron wave vector (Q) and energy (E).¹⁴ During the INS process, the neutron changes in both energy and momentum. The TOSCA experiment is designed to have one Q value for each energy.

Kinetic Energy (E)

$$E = \frac{1}{2}mv^2 \quad \text{Eq. 3.2}$$

Where m = mass of neutron and v = velocity of the neutron.

Energy Transfer

$$\Delta E = E_i - E_f \quad \text{Eq. 3.3}$$

Where E_i = incident neutron energy and E_f = final neutron energy.

Velocity (v)

$$v = \sqrt{\frac{2E}{m}} \quad \text{Eq. 3.4}$$

Where E = kinetic energy and m = mass of the neutron.

The final energy (E_f) of the neutron is known, but the initial energy (E_i) is unknown but can be determined by their time of flight (TOF).

TOF

$$T = t_1 + t_2 = \frac{l_1}{v_i} + \frac{l_2}{v_2} = l_1/\sqrt{\frac{2E_1}{m}} + l_2/\sqrt{\frac{2E_2}{m}} \quad \text{Eq. 3.5}$$

Where T = total time, t_1 = time taken for a neutron to travel from moderator to sample, t_2 = time taken for a neutron to travel from sample to detector, l_1 = incident flight path length from moderator to sample and l_2 = final flight path from sample to the detector.

As the mass of the neutron (m), incident flight path (l_1), final flight path (l_2) and final energy (E_f) are known, the initial energy (E_i) can be derived (Figure 3.7).

The Molecular Vibration Transition Intensity

$$I_i \propto Q^2 U_i^2 \exp(-Q^2 U_{\text{Total}}^2) \sigma \quad \text{Eq. 3.6}$$

Where Q = momentum transferred during the neutron scattering ($\text{\AA}^{-1}$), U_i = scattering atom displacement, U_{Total} = mean square displacement of molecule and σ = neutron cross section.

The observed intensity (I) is proportional to the magnitude of both the scattering atom displacement (U_i) and momentum transferred during the neutron scattering (Q). The exponential term is called the Debye-Waller factor. The molecular vibration transition intensity increases at low momentum values, influenced by the pre-exponential factors, however, decreases at high momentum values due to the exponential factors.

The neutron scattering strength is called the neutron cross section (σ); it describes the probability of interaction between the neutron and the sample. The neutron cross section value of each element is distinctive and can vary between different isotopes of the same element. For instance, the cross section value of hydrogen is more significant than its isotope deuterium and all the other elements; ~ 80 barns ($=10^{-28} \text{ m}^2$) and ~ 7 barns, respectively.¹⁴ The unique nature of the large interaction of neutron scattering from the hydrogen nuclei allows systems rich in hydrogen atoms to be straightforwardly investigated. The information produced can be used to determine the vibrations and positioning of the hydrogen atoms, and is thus, suitable for advanced materials, biological systems and organic compounds.

INS will be employed to characterise the low-energy collective modes and rotational- vibrational couplings of neutral theaflavin. The dynamic-structure factors for the systems pre-radical capture will be verified with the higher-level theoretical determinations.

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Chapter 4

Antioxidant Tea Theaflavin

The conformational analysis of tea theaflavin was initiated by the modelling of all possible conformers. The conformers were computed and analysed to obtain a refined shortlist. The energetics of the conformers in the refined shortlist were investigated, in the gas and solvent phase. The energetic stability of the conformers, concerning the hydroxyl groups in the benzotropolone moiety; a characteristic of black tea, were explored. Furthermore, to study the contributing factors associated with conformation preference and energetics, in detail, a test set of 12, consisting of the lowermost ten, mid and highest energy conformers were selected. To retain consistency, the same conformers selected in the test set of the gas phase were used to investigate the solvent effects.

4.1 Theoretical Methodology – Theaflavin Gas Phase

Theaflavin was computationally characterised towards resolving the topologically probable set of stable conformers (Figure 3.2, Appendix Table A 1). The dihedral angle of the hydroxyl group side chains labelled $\zeta 1$, $\zeta 2$, $\zeta 3$, $\zeta 4$, $\zeta 5$, $\zeta 6$, $\zeta 7$, $\zeta 8$, and $\zeta 9$ were initially modelled to either syn (s) = 0° or anti (a) = 180° . Thus, adopting 512 (2^9) possible conformers (Figure 4.1). The main dihedral angles ($\phi 1$ and $\psi 2$), between rings B' - C' and B - C, respectively, were modelled at anti (a) = 180° .

Note

- The initial modelling of all 512 possible conformers of theaflavin is tabulated in the Appendix Table A 1.
- Atoms 4'a-C, 5'-C, 5'-O-H represent $\zeta 1$, atoms 8'-C, 7'-C, 7'-O-H represent $\zeta 2$, atoms 2'-C, 3'-C, 3'-O-H represent $\zeta 3$, atoms g-C, h-C, h-O-H represent $\zeta 4$, atoms j-C, i-C, i-O-H represent $\zeta 5$, atoms a-C, b-C, b-O-H represent $\zeta 6$, atoms 2-C, 3-C, 3-O-H represent $\zeta 7$, atoms 4a-C, 5-C, 5-O-H represent $\zeta 8$ and atoms 8-C, 7-C, 7-O-H represent $\zeta 9$. The free rotamer $\phi 1$ corresponds to atom 2'-C and f-C and $\psi 2$ corresponds to 2-C and d-C (Figure 4.1).

Figure 4.1 All possible free rotamers of theaflavin. The free hydroxyl rotamers are highlighted in blue ($\zeta 1$ - $\zeta 9$) and the free C-C rotamers are highlighted in red ($\phi 1$ and $\psi 2$). The atoms corresponding to the dihedral $\zeta 1$ - $\zeta 9$ have blue bonds.

4.1.1 Structure and Energetic of Neutral Conformers

Figure 4.2 Relative energies of the 512 possible neutral theaflavin conformers at the HF/3-21G level of theory, in the gas phase. All optimised conformers (middle), non-degenerate (left) and degenerate (right) conformers out of the 512 are highlighted with its relative energy in kJ mol⁻¹.

The conformation of bio-molecules plays an essential role in their stability.¹ Although only a few conformers may exist in-vitro or in-vivo, when approaching other bio-molecules differing conformers may become active. Therefore, a database of all potential conformers permits quick and efficient characterisation of mechanisms in different environments.²

Conformational analysis of all 512 possible conformers of theaflavin was investigated at the RHF method and 3-21G basis set, in the gas phase.³⁻⁶ Results were obtained for all 512 conformers then grouped according to non-degenerate and degenerate conformations (Figure 4.2). After a thorough analysis of the energetics of each optimised conformer, 153 out of the total 512 conformers were shown to be non-degenerate while the remaining 359 conformers were degenerate. Thus, the latter conformers were not investigated further. The relative energies of

the 153 non-degenerate conformers of theaflavin ranged from 0.00 to 112.56 kJ mol⁻¹. Conformation analysis of theaflavin at the lower level of theory (RHF/3-21G)³⁻⁶ was employed to define the scope of probable conformers. The refined list will then be computed at a more expensive and accurate (higher) level of theory, thereby, reducing time and cost.

The generated refined shortlist of neutral conformers were subsequently re-optimised using the method DFT B3LYP and basis set 6-31G(d,p) level of theory, in the gas phase. All 153 conformers were successfully optimised at the higher level of theory and had relative energies ranging from 0.00 to 133.79 kJ mol⁻¹ (Figure 4.3). To study the contributing factors associated with conformation and energy, a test set of 12 containing the lowermost ten, intermediate, and highest energy conformers were explored (Figure 4.3).

Note

- The tabulated data of the 153 optimised conformers with their corresponding dihedral angles and energies are in the appendix Table A 2.

In the following results, the optimised dihedral angles (side groups ($\zeta 1 - \zeta 9$) and main $\phi 1$ and $\psi 2$) within the range of 0.0° to $\pm 30.0^\circ$ will be marked *s* (syn), -30.0° to -150.0° - (gauche-), $\pm 150.0^\circ$ to $\pm 180.0^\circ$ - *a* (anti), and 30.0° to 150.0° - (gauche+) (Figure 4.4).

Figure 4.3 Relative energies of the 153 neutral theaflavin conformers at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The test set of 12 conformers of theaflavin relative energies in kJ mol⁻¹ are highlighted and grouped.

Figure 4.4 An illustration of idealised dihedral angles. Gauche- (-) = -30° to -150° , gauche+ (+) = 30° to 150° , anti (a) = $\pm 150^\circ$ to $\pm 180^\circ$ and syn (s) = 0° to $\pm 30^\circ$. Reproduced from Chass et al.⁷

Table 4.1 Relative energies of the test set of 12 neutral theaflavin conformers at the B3LYP/6-31G(d,p) level of theory, in the gas phase. $\zeta_1 - \zeta_9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+, and R. ΔE = Relative Energy in kJ mol^{-1} .

Test Set B3LYP/6-31G(d,p) (Gas)		
Neutral Conformer		Relative Energy
Compound	Optimised ($\zeta_1 - \zeta_9$)	R. ΔE (kJ mol^{-1})
1a	as-ass-as	<u>0.00</u>
1b	sa-ass-as	<u>1.80</u>
1c	as-ass-aa	<u>6.68</u>
1d	as-ass-sa	<u>9.98</u>
1e	aa-ass-ss	<u>10.07</u>
1f	asaass-as	<u>10.08</u>
1g	aaaass-as	<u>10.91</u>
1h	aa-ass-sa	<u>11.14</u>
1i	aa-ass-as	<u>11.68</u>
1j	sa-ass-sa	<u>12.99</u>
1k	saaasa-ss	<u>73.91</u>
1l	sa-saa-ss	<u>133.79</u>

The ten lowest energy neutral conformers obtained after optimisation are the most thermodynamically stable. Within the lowermost ten energetically stable conformer, the most stable was compound **1a**, adopting the conformation as-ass-as (

Table 4.1 and Figure 4.5). The second most energetically stable conformer, compound **1b**, adopted sa-ass-as, followed by, as-ass-aa (**1c**), as-ass-sa (**1d**), aa-ass-ss (**1e**), asaass-as (**1f**), aaaass-as (**1g**), aa-ass-sa (**1h**), aa-ass-as (**1i**) and sa-ass-sa (**1j**) (

Table 4.1). Within a relative energy difference of $12.99 \text{ kJ mol}^{-1}$, theaflavin was able to adopt ten differing stable conformers (compound **1a** – **1j**), with minimal

energy exerted. The intermediate neutral energy conformer was compound **1k**, with the conformation arrangement saaasa-ss and relative energy of 73.91 kJ mol⁻¹ (

Table 4.1 and Figure 4.5). The most thermodynamically unstable conformer was compound **1l**, adopting the conformation sa-saa-ss with a relative energy of 133.79 kJ mol⁻¹ (

Table 4.1 and Figure 4.5). The hydroxyl side chain groups ζ4 – ζ6 associated with rings B and B' are identical in the lowermost ten stable conformers of theaflavin (**1a – 1j**), while distinct for the intermediate (**1k**) and highest energy conformer (**1l**) (

Table 4.1 and Figure 4.5). The conformation distinction in ζ4 – ζ6, in the test set, indicates that the overall stability of the molecule is influenced by the hydroxyl functional groups, in rings B and B'. The numerous conformational changes with minimal energy exerted illustrate that theaflavin may exist as a complex conformational mixture. The health properties of theaflavin could be attributed to its conformational flexibility, as each conformational isomer possess different structural properties, therefore can impart differing chemical and biological functions.

Figure 4.5 The optimised molecular structures of theaflavin's lowest (**1a**), intermediate (**1k**) and highest energy (**1l**) conformers with the space conformation adopted at the B3LYP/6-31G(d,p) level of theory, in the gas phase. $\zeta 1 - \zeta 9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche- and + = gauche+.

4.1.2 Side Chains

The hydroxyl side chains of flavonoids are thought to be a decisive factor for effective radical scavenging. Numerous authors have highlighted the importance of the hydroxyl groups on the B ring of flavonoids for antioxidant activity.^{1, 8-11} In theaflavin, the B ring is part of the benzotropolone moiety. The properties of the B and B' ring was thus investigated to understand its effect on the stability of theaflavin.

The hydroxyl side chain groups in rings B and B' ($\zeta 4 - \zeta 6$) were grouped according to the different conformation adopted, in the 153 optimised structures (Figure 4.3). The following combinations for the conformation of rings B and B' were obtained:

a,s,s, s,s,s, a,s,a, s,a,s, s,s,a and s,a,a (Figure 4.6). In ascending energetic order, the side chain conformation adopted for $\zeta 4 - \zeta 6$, in the B and B' ring, were: a,s,s < s,s,s < a,s,a < s,a,s < s,s,a < s,a,a. The more energetically stable conformers preferred the a,s,s conformation at $\zeta 4 - \zeta 6$ while the following s,s,s, a,s,a, s,a,s, s,s,a and s,a,a conformations were adopted in the lesser stable conformers. There is a general trend, in relation to theaflavin's conformer stability and the adopted conformation of the $\zeta 4 - \zeta 6$ side chains, in rings B and B' (Figure 4.6). The results underlie the structural importance of the benzotropolone moiety content found in black tea.¹²

The energetics of theaflavin conformers are influenced by the conformation adopted in the hydroxyl groups of rings B and B' (Figure 4.6). Specifically, the intramolecular hydrogen bond formations involving the side chain groups ($\zeta 4 - \zeta 6$).¹³ Illustrated, in Figure 4.7, are the various conformations adopted in the side chains of rings B and B'. The a,s,s arrangement had the maximum available amount of hydrogen bond formation, followed by the s,s,s, a,s,a and s,a,s conformations. The arrangement of the s,s,s, a,s,a and s,a,s formed two intramolecular hydrogen bonds, and thus, the difference in the energy of the conformers are a result of both the conformation arrangement of the hydroxyl side chains and the amount of hydrogen bond formation.

Figure 4.6 Energetics of neutral theaflavin in relation to the configuration adopted in the hydroxyl side chains of rings B and B' ($\zeta 4 - \zeta 6$) at the B3LYP/6-31G(d,p) level of theory, in the gas phase.

The strength of the intramolecular hydrogen bond varied. The least energy consuming hydrogen bond was formed between $\zeta 4$ and $\zeta 5$ then the ketone and $\zeta 6$, in ring B. In contrast, the most energy consuming hydrogen bond was between the ketone and the hydroxyl group $\zeta 5$. Subsequently, the order of the hydrogen bond formation affecting the overall energy least was $\zeta 4$ and $\zeta 5 < \text{ketone and } \zeta 6 < \text{ketone and } \zeta 5$. This was emphasised in conformers with the s,s,a and s,a,a conformation arrangements, as both had one hydrogen bond, but the latter was more unstable since the hydrogen bond present was the weaker $\zeta 4$ and $\zeta 5$ interaction.

The most energetically stable conformers had the conformation a,s,s, in the B and B' rings. Adopting a,s,s, in $\zeta 4 - \zeta 6$, was the optimum conformation arrangement

for the maximum number of hydrogen bonds. On the contrary, conformers with the arrangement s,a,a in $\zeta 4 - \zeta 6$ had the fewest number and weakest hydrogen bond. Moreover, those conformers contained the maximum steric hindrance, consequently, being the least stable conformers of theaflavin.

Figure 4.7 A skeleton representation of theaflavin illustrating intramolecular hydrogen bonding and steric hindrance of the B and B' rings hydroxyl side chains ($\zeta 4 - \zeta 6$) at the B3LYP/6-31G(d,p) level of theory, in the gas phase. a = anti and s = syn.

4.2 Theoretical Methodology – Theaflavin Solvent Phase

4.2.1 Structure and Energetic of Neutral Conformers

To observe the structural dynamics of theaflavin, and the relative stability of the molecule to the changing environment, all 153 optimised conformers, from the gas phase, were successfully re-optimised, in an aqueous solvent (Figure 4.8). The relative change in energy for the neutral conformers in aqueous solvent ranged from 0.00 kJ mol⁻¹ to 103.54 kJ mol⁻¹. The labelling of the conformers and corresponding colour coordination of the test set, from the gas phase, will be applied with the addition of the suffix –H₂O, in the solvent, to retain consistency (

Table 4.1).

Note

- The tabulated data of the 153 optimised conformers of theaflavin in an aqueous solvent with their corresponding dihedral angles and energies are in the appendix Table A 3.

The test set used in the gas phase was followed through using the same test set in an aqueous solvent. The most energetically stable conformer in the gas phase remained the same in the aqueous solvent too: compound **1a** with the conformation as-ass-as and compound **1a-H₂O** with the conformation as-ass-as, respectively (Figure 4.5). Out of the test set of 12, the second most energetically stable conformer in solvent was as-ass-aa (**1c-H₂O**), followed by sa-ass-as (**1b-H₂O**), asaass-as (**1f-H₂O**), aaaass-as (**1g-H₂O**), as-ass-sa (**1d-H₂O**), aa-ass-sa (**1h-H₂O**), aa-ass-as (**1i-H₂O**), aa-ass-ss (**1e-H₂O**), sa-ass-sa (**1j-H₂O**), saaasa-ss (**1k-H₂O**) and sa-saa-ss (**1l-H₂O**), in ascending order (Table 4.2). The conformation arrangement for each of the test set conformers persisted during the change in environment from gas to aqueous solvent (

Table 4.1 and Table 4.2, respectively). Thus, the change in the environment did not influence the structural dynamics of the conformers.

Figure 4.8 Relative energies of the 153 neutral theaflavin conformers at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. The test set of 12 conformers of theaflavin relative energies in kJ mol⁻¹ are highlighted and grouped.

The change of environment influenced the energetics of the conformers. All conformers were more energetically stable in the solvent than in gas (1a-H₂O, 1c-H₂O to 1l-H₂O), with the exception of conformer 1b-H₂O. The change in energy can be accounted for by the inclusion of aqueous solvent, therefore, stabilising the many hydroxyl side chain groups, resulting in a more energetically stable compound. The conformational changes in solvent required less energy in comparison to gas, illustrating a more complex conformational flexible mixture.

Table 4.2 Relative energies of the test set of 12 neutral theaflavin conformers at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. $\zeta_1 - \zeta_9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+ and R. ΔE = Relative Energy in kJ mol^{-1} .

Test Set B3LYP/6-31G(d,p) (Solvent)		
Neutral Conformer		Relative Energy
Compound	Optimised ($\zeta_1 - \zeta_9$)	R. ΔE (kJ mol^{-1})
1a-H₂O	as-ass-as	<u>0.00</u>
1c-H₂O	as-ass-aa	<u>0.12</u>
1b-H₂O	sa-ass-as	<u>2.63</u>
1f-H₂O	asaass-as	<u>3.59</u>
1g-H₂O	aaaass-as	<u>3.65</u>
1d-H₂O	as-ass-sa	<u>4.45</u>
1h-H₂O	aa-ass-sa	<u>5.26</u>
1i-H₂O	aa-ass-as	<u>5.95</u>
1e-H₂O	aa-ass-ss	<u>6.02</u>
1j-H₂O	sa-ass-sa	<u>8.61</u>
1k-H₂O	saaasa-ss	<u>50.19</u>
1l-H₂O	sa-saa-ss	<u>103.54</u>

4.2.2 Side Chains

To see the influence of the characteristic benzotropolone moiety on the overall stability of theaflavin, the hydroxyl side chains in rings B and B' ($\zeta_4 - \zeta_6$) were grouped according to the adopted configuration. This was done for all 153 optimised structures in an aqueous solvent (Figure 4.8). The different conformations for the hydroxyl side chains in the B and B' rings were acquired: a,s,s, s,s,s, a,s,a, s,s,a, s,a,s and s,a,a (Figure 4.9).

Figure 4.9 Energetics of neutral theaflavin in relation to the configuration adopted in the hydroxyl side chains of rings B and B' (ζ4 – ζ6) at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent.

The configuration obtained in the solvent was consistent with that obtained in gas. However, the s,s,a configuration in the solvent was more energetically stable than s,a,s, which was not the case in the gaseous environment. The most stable hydroxyl group configuration adopted in the solvent was, in the following order, a,s,s > s,s,s > a,s,a > s,s,a > s,a,s > s,a,a (Figure 4.9). The conformation vs energetic pattern was similar for the conformers in both gas and aqueous solvent (Figure 4.6). There is a trend between the stability of the overall molecule and the adopted configurations at the hydroxyl groups on the B and B' rings (Figure 4.9, Figure 4.3). Emphasising the fundamental importance of the benzotropolone moiety to the stability of tea theaflavin.

Figure 4.10 A skeleton representation of theaflavin illustrating intramolecular hydrogen bonding and steric hindrance of the B and B' rings hydroxyl side chains ($\zeta_4 - \zeta_6$) at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent.

The influence of the hydroxyl side chains ($\zeta_4 - \zeta_6$) in rings B' and B on the overall stability of theaflavin was highlighted, in Figure 4.9. The nature of the stability was associated with the amount of intramolecular hydrogen bond formed which altered due to the rotation of the hydroxyl side chain $\zeta_4 - \zeta_6$ (Figure 4.10). The conformation a,s,s yielded the maximum number of hydrogen bonds available between the hydroxyl groups $\zeta_4 - \zeta_6$, in the benzotropolone moiety. The second

highest number of intramolecular hydrogen bond attainable is two: conformation s,s,s, a,s,a and s,a,s, and lastly, the conformation s,s,a and s,a,a formed one hydrogen bond (Figure 4.10). Unexpectedly, adopting the s,s,a conformation was more stable than s,a,s, despite having more intramolecular hydrogen bonds. This was not true, in the gaseous environment (Figure 4.7). In solvent, the loss of the intramolecular hydrogen bonding concerning the ketone and $\zeta 5$ resulted in the least energetically stable conformations. Thus, the steric hindrance introduced was more dominant than the stability of the intramolecular hydrogen bond, unlike the gas phase. In the aqueous environment, the most energetically stable neutral conformations adopted a,s,s at the hydroxyl side chain groups in the B and B' rings. Contrastingly, the least energetically stable conformations were s,a,s and s,a,a (Figure 4.10), as a result of steric hindrance imparted by the absence of hydrogen bonding between ketone and $\zeta 5$.

4.3 Intramolecular Interaction

Polyphenolic antioxidants have characteristic structural features namely, benzene rings, hydroxyl groups or other electron donating groups attached to the benzene ring, C-C linkers and C=C double bonds.¹⁴ These characteristic functional groups stabilised the compound through π -electron delocalisation, intramolecular interactions and intermolecular interactions.^{14, 15}

4.3.1 Tea Theaflavin, Gas Phase

An analysis of the intramolecular interactions of the individual structures in the test set containing the 12 representatives (**1a** – **1k**) was carried out. This was to determine what effects the intramolecular interactions had on the energetic stability of theaflavin's conformers.¹⁴

Note

- The tabulated intramolecular OH/O interaction is found in the appendix Table A 4.

In Figure 4.11, the **1a** conformer exhibited various self-association interactions linking rings A to A'. The interactions involved hydrogen bonding (2.0 Å) between the 7-OH/7'OH group and CH/C (2.7 Å), while also forming CH/O (2.2 Å/2.1 Å) and H/H (1.9 Å) interactions connecting the C, B and B' ring. OH/O interactions were displayed between the 3-OH and 1-O (2.6 Å), and 3'OH to the 1'-O atom (2.2 Å). Structure **1b** displayed identical interactions to **1a**. However, the distance between the CH/C, CH/O and H/H contact increased by 0.1 Å. The greater distance resulted in weaker interactions. Thus, this small alteration probably contributed to the 1.80 kJ mol⁻¹ rise in free energy in **1b**, relative to the most stable conformer (**1a**) (

Table 4.1).

Similarly to conformers **1a** - **1b**, the structures **1c** - **1e** displayed hydrogen bonding interactions (OH/O) via the 3-OH/1-O and 3'OH/1'-O, in the C and C' ring. In structures **1c** - **1e**, the ψ_2 d-C-2-C backbone twisted, resulting in the A and C rings to be non-mirror images of rings A' and C'. The torsions decreased the number of spatial interactions concerning the A and A' rings and also, showed no hydrogen bond interactions between 7-OH/7'OH. However, the H/H interactions between the C and A'-ring were present. Unlike rings A and C, the A' and C 'rings remained relatively still, as the ϕ_1 dihedral angle was restricted. This was due to the formation of the g-CH/3'-O (2.2 Å) interaction which produced a pseudo-six-membered ring. The pseudo-six-membered ring was present in all 12 conformers (Figure 4.11).

Conformers **1f** and **1g** showed significant similarity to conformer **1a**, in relation to the spatial interactions. The differences between the conformers were the lack of a hydrogen bond interaction, in the C' ring (Figure 4.11). Thereby, contributing to the energy increase of ~10 – 11 kJ mol⁻¹ than the most stable conformer, **1a** (

Table 4.1). The higher energy seen in **1g** compared to **1f** may be attributed to the 0.1 Å increased distance of the CH/O and H/H intramolecular interaction.

The **1h** conformer resembles the conformer **1e**; the number and type of intramolecular interactions are alike. Both conformers have two CH/C interactions between the 4β-H to the 8'-C and the c-H to the 2'-C at 3.6 Å and 2.3 Å, respectively. Additionally, hydrogen bond interactions OH/O (2.4 Å and 2.2 Å) were displayed by the 3-OH/1-O and 3'-OH/1'-O in the C-ring and C'-ring, respectively. Two CH/O type interactions were present between the B-ring e-H and 1'O in the C'-ring (2.2 Å) and the g-H in the B'-ring with the 3'-O in the C'-ring. H/H interactions were in both structures involving the 3-H and 8'-H (2.8/2.9 Å) for **1e** and **1h**, respectively. The closer proximity of the 3-H and 8'-H interaction, in structure **1e**, may have contributed to the increased stability of the conformer, and thus, having the lower relative energy.

The conformers **1i**, **1h** and **1e**, have comparable spatial arrangements of the hydroxyl side chain groups and free energies: aa-ass-as (11.68 kJ mol⁻¹), aa-ass-sa (11.14 kJ mol⁻¹) and aa-ass-ss (10.07 kJ mol⁻¹), respectively. The main contrast of structure **1i** from **1h** and **1e** was the ψ torsion. The twisting of the ψ torsion resulted in different intramolecular interactions. In structure **1i**, an OH/C (2.4 Å) interaction was present between the 7-OH and 6'-C, a CH/O (2.1 Å) interaction between the e-H and 1-O, and a CH/C (2.4 Å) interaction involving the 8-CH and 8'-C.

The spatial configuration arrangement of conformers **1j** and **1b** are similar, sa-ass-sa and sa-ass-as, respectively. Structure's **1j** ψ torsion promoted distance between the A and C rings to the A' and C' rings, and thus, weakening the intramolecular interaction. The increased distance contributed to the 11.19 kJ mol⁻¹ higher free energy in conformer **1j**. Nevertheless, there were many intramolecular interactions in **1j** including: two CH/C interactions between the 4β-H and 8'-C (3.5 Å) and e-H and 2'-C (2.3 Å), three CH/O interactions involving the 4β-H and 7'-O (3.3 Å), e-H and 1'-O (2.2 Å) and g-H and 3'-O (2.3 Å), one H/H interaction (3-H and 8'-H)

and two OH/O interactions between the 3-OH/1-O in the C ring and 3'-OH/1'-O in the C' ring.

Structure **1k** corresponds to the intermediate energy conformer and **1l** to the highest energy conformer. Despite this, intramolecular interaction still occurred through rings A and C to the A' and C'. In conformer **1k**, an OH/C (2.4 Å), CH/C (3.1 Å) and OH/O (2.3 Å) interaction was present between the 7-OH and the 6'-C, the 8-CH and the 8'-C and the 3-OH to the 1-O oxygen atom in the C ring, respectively. Conformer **1l** had an OH/O interaction (2.2 Å) resulting from the 7-OH to the 7'-O, two H/H interactions involving the 5-OH and 4 α -H (2.0 Å), as well as, the e-H to the 2'-H (2.1 Å), and a OH/O interactions from the 3'-OH to the 1'-O oxygen atom. The 3-OH may have formed a possible OH/ π interaction with the B ring. Three CH/O interactions were displayed in conformer **1k** involving the c-H to the 3-O on the C ring (2.4 Å), e-H to the 1'-O (2.3 Å) and g-H to the 3'O (2.2 Å). Only two CH/O interactions were shown in structure **1l**; c-H to the 3-O on the C ring (2.4 Å) and g-H to the 3'-O (2.2 Å).

Theaflavin had an extensive network of intramolecular interactions including OH/O, OH/C, CH/C, CH/O and H/H, as shown by the test set in Figure 4.11. The abundance in both number and type of intramolecular interactions displayed are due to high backbone flexibility. The backbone flexibility permitted widely different skeletal structures to exist, and the intramolecular interactions contributed to the overall stability of tea theaflavin conformers.

Alteration of the intramolecular interaction concerning the A, C, A', C' rings and ψ dihedral angle does not affect the overall energetics greatly. The statement is highlighted for structures **1l** (highest energy conformer), and **1k** (mid energy conformer) as the energetics of these conformers are unfavourable, regardless of intramolecular interactions being present. Thus, rings A, C, A' and C', its corresponding hydroxyl side chains and ψ_2 dihedral angle have the freedom to be moulded and shaped, without significant energy shifts.

Figure 4.11 Intramolecular interactions of optimised theaflavin conformers **1a** – **1l** at the B3LYP/6-31G(d,p) level of theory, in the gas phase. Dashed lines show selected intramolecular interactions in Å. The carbon atom is represented by dark grey, oxygen by red and hydrogen light grey. $\zeta_1 - \zeta_9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+ and the relative energy in kJ mol⁻¹.

Broad fluctuations in the energy of theaflavin ascend from the intramolecular hydrogen bonding interactions, due to the conformation arrangement of the b-OH, h-OH and i-OH, in the benzotropolone moiety, as discussed in the previous section (Figure 4.6). The inflexibility of the ϕ_1 dihedral angle arose from the g-CH/3'-O (CH/O) interaction. The formation of the CH/O produced a pseudo-six-membered ring linking the C' and B' ring, thus, retarding the free rotation of the f-C-2'-C bond.

4.3.2 Tea Theaflavin, Solvent Phase

Theoretical calculations of theaflavin's conformational isomers, in the aqueous solvent, yielded many intramolecular interactions. The intramolecular interactions of the conformers (**1a-H₂O** – **1l-H₂O**) were analysed, to understand its contribution to the stability of theaflavin.

Note

- The tabulated intramolecular OH/O interaction is found in the appendix Table A 5.

In solvent, the conformer **1a-H₂O** displayed numerous self-association interactions (Figure 4.12). Rings A and A' were linked through the 8-CH/8'-C (2.7 Å) and 7-OH/7'O (2.0 Å) hydrogen bond interaction. The benzotropolone e-CH formed CH/O (2.2 Å) and H/H (1.9 Å) interactions with 1-O and 2'-CH, respectively. These interactions resulted in pseudo-five and six-membered rings (Figure 4.12). An additional six-membered pseudo ring was formed through a CH/O connection between the g-CH and 3'-O. Hydrogen bond interactions OH/O (2.6 Å and 2.3 Å) were shown by the 3-OH/1-O and 3'OH/1'-O, in the C and C' ring, respectively. The conformer **1b-H₂O** displayed the same amount and type of self-association interactions as conformer **1a-H₂O**. However, structure **1b-H₂O** is 2.63 kJ mol⁻¹ higher in energy (Table 4.2). The higher energy may be partially attributable to the H/H interaction, being 0.1 Å longer, and thus, producing slightly weaker interactions.

The type of self-association interactions shown in structures **1c-H₂O** - **1e-H₂O**, **1h-H₂O** and **1j-H₂O** are identical. Conformers' **1c-H₂O** - **1e-H₂O**, **1h-H₂O** and **1j-H₂O** had an H/H interaction, two of (CH/O and CH/C) and five OH/O H-bonds. Connecting the A and C rings to the A' and C' rings was the 3-CH/8'-CH interaction at 3.0 Å or 3.1 Å (Figure 4.12). This was different from the **1a-H₂O** and **1b-H₂O** conformers where the link involved the OH/O and CH/C interaction. Also, the rotation around the ψ_2 dihedral angle involving the d-C and 2-C bond twisted the A and C ring to be non-mirror images of the A' and C' ring, consequently, resulted in differing self-association interactions. The negligible energy difference concerning structures **1d-H₂O**, **1e-H₂O**, **1h-H₂O** and **1j-H₂O** may be accountable by the differences in the 3'-OH/1'-O and the H/H interaction and the conformation arrangements of the hydroxyl groups.

The type of interactions found in conformers **1f-H₂O** and **1g-H₂O** are similar to the conformers **1a-H₂O** and **1b-H₂O**, with the exception of the lacking 3'-OH/1'-O interaction, in the C' ring. This is due to the anti (a) orientation of the ζ_3 dihedral angle where the **1f-H₂O** and **1g-H₂O** ζ_3 conformation arrangement was anti (a) while syn (s) in both **1a-H₂O** and **1b-H₂O**. Consequently, the former conformers having a higher relative energy (Table 4.2). Structure **1i-H₂O** also demonstrated the same interaction as conformers' **1a-H₂O** and **1b-H₂O** but rather than an OH/O hydrogen bond interaction connecting the A and C rings to the A' and C' ring; the conformer **1i-H₂O** had the OH/C (2.6 Å) interaction (Figure 4.12).

Figure 4.12 Intramolecular interactions of optimised theaflavin conformers **1a-H₂O** - **1l-H₂O** at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. Dashed lines show selected intramolecular interactions in Å. The carbon atom is represented by dark grey, oxygen by red and hydrogen light grey. $\zeta 1 - \zeta 9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+ and the relative energy in kJ mol⁻¹.

Conformers **1k-H₂O** showed a vast amount of self-association interactions (Figure 4.12), despite having a relative energy of 50.19 kJ mol⁻¹ higher than the lowest energy conformer **1a-H₂O**. Unlike conformer **1k-H₂O**, the highest energy conformer **1l-H₂O** did not have as many self-association interactions. Nevertheless, both conformers had the g-CH/3'-O (2.2 Å) interaction which was present in all the conformers, in the test set. Linking rings A and C to A' and C', conformer **1k-H₂O** had the 7-OH/6'-C (2.5 Å) and 8-CH/8'a-C (2.9 Å) self-association interactions, while conformer **1l-H₂O** had the 7-OH/7'-O (2.2 Å) interaction.

All 12 conformers (**1a-H₂O** – **1l-H₂O**) displayed the g-CH/3'O (2.2 Å) interaction. This concoction resulted in a restricted 2'-C – f-C bond, thus, causing the ϕ_1 dihedral angle to remain relatively stagnant. The g-CH/3'O connection formed a pseudo-six-membered ring involving the atoms g-C, f-C, 2'-C, 3'-C, 3'-O--HC-g and was present, in both the gas and aqueous solvent test sets.

4.4 Planar/ Puckered Benzotropolone Moiety

4.4.1 Planarity

Polyphenolic compounds like theaflavin have aromatic ring systems which stabilise the molecule through extensive π -electron delocalisation. Aromatic systems are planar in nature and are very stable. The planar system increases the effectiveness of π -electron delocalisation, and thus, planar geometries increase the extent of π -electron delocalisation in a molecule, resulting in a stabilising effect.¹⁴

An analysis of the benzotropolone constituent of tea theaflavin, in the test set containing 12 representatives, were analysed in both gas and in an aqueous solvent. This was to determine the effects the planarity of the B and B' ring on the energetic stability of theaflavin's conformers.¹⁴

The benzotropolone moiety in theaflavin (B and B' ring) were planar, in the lowermost ten test set of neutral structures, in both gas and the aqueous solvent (**1a** - **1j** and **1a-H₂O** - **1j-H₂O**, in Figure 4.13 and Figure 4.14, respectively). The planarity of the B and B' ring stabilised the structure via conjugation, due to the overlapping of the p-orbitals. Subsequently, the planarity contributed to the formation of strong H-bonds, as the hydroxyl groups h-OH, i-OH and b-OH are coplanar to the benzotropolone portion. Thus, extending the area of the π -electron delocalisation.¹⁶

Lesser stable conformers namely, **1k**, **1l**, **1k-H₂O** and **1l-H₂O** revealed a loss of planarity as a result of a puckered B-ring (Figure 4.13 and Figure 4.14). The less stable conformers **1k**, **1l**, **1k-H₂O** and **1l-H₂O**, had the ζ_6 dihedral angle in the anti (a) orientation. The anti-configuration results in a loss of H-bond between b-OH/C=O, also causing steric hindrance by exposing the b-O to the C=O atom. To reduce the steric hindrance amid the a-C=O functional group and the b-O atom, the hydroxyl b-OH distorts out of plane (Figure 4.13 and Figure 4.14). Furthermore, the least energetically stable conformers **1l** and **1l-H₂O** had the dihedral angle ζ_5 in the anti (a) orientation resulting in repulsions from i-O and C=O, in addition to the b-O/C=O repulsion. The added repulsion resulted in the further distortion of the B' ring as to reduce the steric hindrance amid the a-C=O functional group and the two adjacent hydroxyls b-OH/ i-OH.

Figure 4.13 Perpendicular view of the planar B' ring of optimised theaflavin conformers **1a** - **1l** at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The dashed lines are on the planar B' ring. The carbon atom is represented by dark grey, oxygen by red and hydrogen light grey. $\zeta 1 - \zeta 9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+ and the A, C, B', B represent the rings in theaflavin.

The geometries of the test set, in both gas and the solvent, showed the orientation of the $\zeta 5$ and $\zeta 6$ dihedral angles affecting the planarity of the B' ring. Common to the higher energy conformers are the steric repulsion(s) induced by the anti (a) conformation, in either or both $\zeta 5$ and $\zeta 6$. The steric repulsion(s) caused the B' ring to strain. As a result, impairing the conjugated system. Consequently, the energetic stability of the molecule decreased, as illustrated by conformers **1k**, **1l**, **1k-H₂O** and **1l-H₂O** (Figure 4.13 and Figure 4.14). In the most stable configurational arrangements, the dihedral angles $\zeta 5$ and $\zeta 6$ were in the favourable orientation of syn (s). The syn (s) conformation in $\zeta 5$ and $\zeta 6$ optimised the planar geometry of the benzotropolone moiety which had a stabilising effect on the π -electron delocalisation, maximised the number of hydrogen bonding and being devoid of steric hindrance and loss of planarity. Therefore, the lack of ring puckering facilitated the overall increase in the energetic stability of the antioxidant tea theaflavin.

Figure 4.14 Perpendicular view of the planar B' ring of optimised theaflavin conformers **1a-H₂O** - **1l-H₂O** at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. The dashed lines are on the planar B' ring. The carbon atom is represented by dark grey, oxygen by red and hydrogen light grey. $\zeta 1 - \zeta 9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti, - = gauche-, + = gauche+ and the A, C, B', B represent the rings in theaflavin.

4.5 Conclusion

The conformational analysis of theaflavin was initiated by modelling all 512 possible conformers of theaflavin. The conformers were subsequently inputted for calculations then the data output analysed. After analysis, a refined shortlist was generated and to be investigated at a higher level of theory. Thus, the initial scope was reduced from 512 conformers to 153.

The 153 conformers were successfully optimised at a higher level of theory in both gas and an aqueous solvent. In the gas phase, the relative energies of the conformers ranged from 0.00 to 133.79 kJ mol⁻¹. The energy range in the solvent was smaller than in gas, ranging from 0.00 kJ mol⁻¹ to 103.54 kJ mol⁻¹. Within a relative energy of 12.99 kJ mol⁻¹, theaflavin could adopt ten stable conformational isomers, in the gas phase. In solvent, however, the conformers were able to adopt even more conformational isomers. This suggests that it is probable that theaflavin exists as a

complex conformation mixture and the health properties of theaflavin could be attributed to its conformational flexibility. Providing each conformational isomer possesses different structural properties, therefore, can impart differing chemical and biological functions.

The hydroxyls in the B ring of flavonoids have been associated as an essential component in eliciting antioxidant activity. In theaflavin, the B ring is part of the benzotropolone moiety; a characteristic of black tea. The properties of the B and B' ring was thus investigated to understand the effects on the stability of conformers in theaflavin. In the 153 conformers, the following conformations were adopted in the $\zeta 4$ - $\zeta 6$ of the benzotropolone moiety, a,s,s, s,s,s, a,s,a, s,a,s, s,s,a and s,a,a, in both gas and the aqueous solvent. In the gas phase, the conformers were more energetically stable adopting the s,a,s conformation than s,s,a. However, in the aqueous solvent, the conformers were more stable with the s,s,a conformation rather than s,a,s. The s,a,s conformation arrangement had two hydrogen bonds, but the stronger hydrogen bond i-OH/C=O was absent. In the s,s,a conformation, the stronger i-OH/C=O hydrogen bond was present, but the two weaker H-bond were absent. Thus, in the aqueous solvent, the effects of the steric hindrance introduced by the missing i-OH/C=O bond was more energetically costly than the stability of two weaker hydrogen bonds.

In both gas and the aqueous solvent, the most energetically stable conformers had the conformation arrangement a,s,s, in their B and B' rings, as this arrangement resulted in the maximum amount of hydrogen bond formations and minimum steric hindrance. On the contrary, conformers with the arrangement s,a,a in $\zeta 4$ - $\zeta 6$ had the fewest number and weakest hydrogen bond. Moreover, those conformers contained the maximum steric hindrance, and consequently, being the least stable conformers of theaflavin, in both the gas and the solvent environment. Therefore, the energetics of theaflavin conformers are influenced by the conformation adopted in the hydroxyl groups of rings B and B'. Specifically, the intramolecular hydrogen bond formations involving the $\zeta 4$ - $\zeta 6$ hydroxyl side chain groups.

A test set of 12 out of the 153 conformers of theaflavin were selected to study the contributing factors associated with conformation and energy in detail. The test set of 12 contained the lowermost ten, intermediate, and highest energy conformers, in the gas phase. For consistency, the same conformers were used as the test set, in the solvent. The change in environment from gas to solvent did not influence the structural dynamics of the conformers but did influence the energetics. With the exception of conformer **1b-H₂O**, all conformers were more energetically stable in the solvent than in gas. The change in energy can be accounted for by the inclusion of aqueous solvent, therefore, stabilising the many hydroxyl side chain groups, and thus, resulting in a more energetically stable conformer.

Intramolecular interactions helped in the stabilising of theaflavin's conformers, in both gas and the aqueous solvent. CH/O, CH/C, H/H, OH/O and OH/C type interactions were displayed and formed pseudo-five, six and eight-membered rings which created an extensive stabilising electronic network. Within a small energy difference, theaflavin could obtain vast amounts of variation in its ζ_1 - ζ_3 , ζ_7 - ζ_9 , and ψ_2 arrangements, without destabilising the structure considerably. The dihedral angle ϕ_1 was restricted due to the g-CH/3'-O (2.2 Å) interaction. The g-CH/3'-O interaction formed a pseudo-six-membered ring. The g-CH/3'-O probably aided in the stabilising effect of the conformers, as the interaction was unaltered in all test sets, in both gas and in the solvent environment. The energetics of the test set conformers were more stable in the solvent than in gas. It is likely that the hydroxyl side chains not participating in the intramolecular interactions were stabilised by the formation of hydrogen bonds by the aqueous solvent.

The planarity of the benzotropolone moiety stabilised the energetics of the conformers. In the ten most stable neutral structure, in gas and an aqueous solvent, the benzotropolone moiety was planar. The planarity stabilised the structure via conjugation due to the overlapping of the p-orbitals. In the mid and highest energy conformers of theaflavin, in both gas and in the solvent, showed puckering of the

benzotropolone ring system. The ring strain distorted the planarity of the system, to reduce the steric hindrance between the α -C=O (ketone) group and the hydroxyl h-O and i-O oxygen atom. And thus, consequently impairing the conjugated system. The loss of planarity in the benzotropolone fraction, as a result of ring strain, destabilised the structure and significantly increased the energetics of the conformer.

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Chapter 5

Antioxidant Radical Tea Theaflavin

An antioxidant's ability to scavenge and deactivate reactive species is correlated to the stability of the antioxidant radical formed. The antioxidant radical must remain stable post-radical capture as to prevent further damage to the biological system. The conformational analysis of the antioxidant radical tea theaflavin for the test set of 12 conformers were generated by the radical scavenging mechanism hydrogen atom abstraction (HAB) and hydrogen atom addition (HAD). The structures of the antioxidant radical tea theaflavin obtained were subsequently analysed, to determine the preferred radical scavenging vicinity, in the gas and the aqueous solvent environment.

5.1 Scavenging Activity Gas Phase

5.1.1 Hydrogen Atom Abstraction

To explore the mechanism, hydrogen atom abstraction (HAB), the optimised test set containing the 12 conformers from the gas phase were re-computed using the UB3LYP¹⁻³ method and 6-31G(d,p) basis set. In the mechanism hydrogen atom abstraction, the antioxidant radical scavenger are hydrogen donors.^{4,5} To determine the preferred radical scavenging site, all possible hydrogen donor sites were computed. The antioxidant theaflavin contains multiple possible hydrogen atom abstraction sites - O-H and - C-H.⁴⁻⁷ Specifically, 17 competing abstraction sites comprising of nine hydrogen atoms from hydroxyl side chains: 5'-OH, 7'-OH, 3'-OH, h-OH, i-OH, b-OH, 3-OH, 5-OH and 7-OH, and eight hydrogen atoms from the C-H in the C and C' ring: 4' α -H, 4' β -H, 3'-H, 2'-H, 4 β -H, 4 α -H, 3-H and 2-H (Figure 5.1).

Figure 5.1 All possible hydrogen atom abstraction sites of antioxidant tea theaflavin with their corresponding labels (colouring and numbering system).

Figure 5.2 demonstrated the relative energy change produced as a result of H-abstraction to the lowest energy conformer (**1a**), at all possible sites enlisted in Figure 5.1. Abstraction of the H-atom at 2-H formed the most stable antioxidant radical. Subsequently, H-abstraction at 7'-OH and h-OH were the second and third

most energetically favourable sites, respectively. The least energetically favoured abstraction site corresponded to 3'-OH, trailed by 3-OH and then 3'-H. The difference in energy between the most stable and least stable antioxidant radical forms was 90.16 kJ mol⁻¹. For conformer **1a**, the results obtained showed the most likely H-abstraction site was at 2-H site, as the most stable antioxidant radical was formed. The least probable was at the 3'-OH site, corresponding to the highest energy antioxidant radical conformer (Figure 5.2).

Figure 5.2 Relative energies of the most stable neutral conformer **1a** at all possible 17 H-abstraction sites at the UB3LYP/6-31G(d,p) level of theory, in the gas phase. AO = antioxidant, RO•S = reactive oxygen species, AO• = antioxidant radical and OS = oxygen species. Key: left - neutral conformer and right - the corresponding HAB ring site. Relative energy in kJ mol⁻¹ and atoms are numbered according to the labelling in Figure 5.1.

Figure 5.3 Relative energies of the test set of 12 neutral theaflavin conformers **1a** – **1l** at all possible 17 H-abstraction sites at the UB3LYP/6-31G(d,p) level of theory, in the gas phase. AO = antioxidant, RO•S = reactive oxygen species, AO• = antioxidant radical and OS = oxygen species. Key: left - neutral conformer and right - the corresponding antioxidant radical conformer. Relative energy in kJ mol^{-1} and atoms are numbered according to the labelling in Figure 5.1.

An antioxidant's stability is vital to preventing further damage to biological systems. Thus, its antioxidant properties are affected by the hydroxyl side groups and the extent of charge delocalisation in the structure.^{8, 9} The most stable antioxidant radical formed in all 12 conformers and at all H-abstraction sites investigated was the conformer **1a** (as-ass-as) at the site 2-H. In general, the most preferred H-abstraction was at site 2-H site for the test set of 12 conformers (**1a** - **1k**). On the contrary, H-abstraction at 3'-OH site was the least favourable H-abstraction site, in the test set, as the antioxidant radical formed were the most energetically unstable.

Figure 5.4 The resonance structures of hydrogen atom abstraction at the 2-H and 3'-OH sites resulting in the lowest and highest energy antioxidant radical, respectively.

From Figure 5.4, H-abstraction at the 2-H site produced a large charge delocalisation through the molecule. Charge delocalisation is a stabilising force because it spreads energy over a larger area rather than keeping it confined to a small area. This is highlighted by the numerous resonance structures, and thus, resulting in an energetically stable antioxidant product, post-H-abstraction (Figure 5.3 and Figure 5.4).⁷ However, H-abstraction at the 3'-OH site resulted in no charge delocalisation, and therefore, formed the least stable antioxidant radical conformers. The structural instability of antioxidant radicals produced by HAB, at 3'-OH, was emphasised by the structures resultant energy (Figure 5.3 and Figure 5.4).

5.1.2 Hydrogen Atom Addition

The mechanism, hydrogen atom addition, was explored using the optimised structures from the test set of 12 neutral conformers, in the gas phase. The test set was computed for all potential H-addition sites with the UB3LYP^{1, 2} method accompanied by the 6-31G(d,p) basis set.

Figure 5.5 All seven possible hydrogen atom addition sites of antioxidant tea theaflavin with their corresponding labels.

Illustrated in Figure 5.5, are the seven possible competing H-addition sites in the antioxidant tea theaflavin. The hydrogen radical readily adds to unsaturated double bonds,⁷ and thus, the seven available H-addition were 6'-C, 8'-C, g-C, c-C, e-C, 6-C and 8-C sites (Figure 5.5).⁷ All seven possible sites of H-addition were analysed.

The various possible hydrogen atom addition sites were investigated by the lowest energy neutral conformer, compound **1a**, as shown in Figure 5.6. The H-addition were colour coded according to the ring where the radical scavenging took place: blue representing the A and A' rings, fuchsia for the B ring and green signifying the B' ring. In conformer **1a**, the most energetically favourable H-added antioxidant radical was at e-C site followed by c-C site, in the B ring. Hydrogen atom addition to the 8-C site in the A ring resulted in the least energetically favourable antioxidant radical. This was subsequently followed by 6'-C, 8'-C then 6-C, with marginal differences in the energies. However, the difference in energy between the most stable and least stable antioxidant radical was significant; the highest energy antioxidant radical was 90.16 kJ mol⁻¹ higher in energy than the most stable. From the results observed, the most probable H-addition site was at the B ring, attributable to the lowest energy antioxidant radical formed. Rings A and A' are the least probable addition site comprising of the highest energy antioxidant radical formed (Figure 5.6).

Figure 5.6 Relative energies of the most stable neutral conformer **1a** at all possible H-addition sites at the UB3LYP/6-31G(d,p) level of theory, in the gas phase. AO = antioxidant, $H^\bullet$ = hydrogen radical and $AO^\bullet-H$ = antioxidant H-added radical. Key: left - neutral conformer, right - the corresponding H-addition ring site. Relative energy in kJ mol^{-1} and atoms are numbered according to the labelling in Figure 5.5.

In the test set of 12 conformers, the following trend for the preferred H-addition site in terms of energetics emerged, e-C, c-C, g-C, 6'-C $\sim$ 8'-C $\sim$ 6-C $\sim$ 8-C, where e-C was the most energetically stable H-addition site (Figure 5.7). On the contrary, the least stable antioxidant radicals formed were from H-addition to the 6'-C, 8'-C, 6-C and 8-C sites, in the A and A' rings. In all the test set conformers (**1a** to **1l** in

Table 4.1), H-addition at the e-C site, in the B ring, resulted in the most stable antioxidant radical. The second most stable addition was c-C site, in the B, ring followed by g-C site, in the B' ring, while H-addition to 6'-C, 8'-C, 6-C and 8-C site led to negligible differences in the energetics of all 12 antioxidant radical conformers (Figure 5.7). The most energetically stable, and thus, most probable antioxidant radicals produced were from H-addition to e-C, c-C and g-C sites, in

the B and B' ring. Therefore, the important group for H-addition was the benzotropolone moiety, particularly, the seven-membered B ring.

Figure 5.7 Relative energies of the test set of 12 neutral theaflavin conformers **1a** – **1l** at all possible H-addition sites at the UB3LYP/6-31G(d,p) level of theory, in the gas phase. AO = antioxidant, H• = hydrogen radical and AO•–H = antioxidant H-added radical. Key: left - neutral conformer, right - the corresponding antioxidant radical. Relative energy in kJ mol^{-1} and atoms are numbered according to the labelling in Figure 5.5.

5.2 Scavenging Activity Solvent Phase

5.2.1 Hydrogen Atom Abstraction

The mechanism hydrogen atom abstraction was explored in an aqueous solvent using the test set of 12, highlighted in Table 4.2. The 12 conformers in the test set were re-optimised at all 17 possible hydrogen atom abstraction sites, illustrated in Figure 5.1, using the UB3LYP method and 6-31G(d,p) basis set. The results discussed below will use the colour coding from Figure 5.1.

The lowest energy conformation in the aqueous solvent, compound **1a-H₂O**, was investigated at all possible 17 hydrogen atom abstraction sites (Figure 5.8). Hydrogen atom abstraction to the hydroxyl group h-OH, in the B' ring, produced the most energetically stable antioxidant radical. Contrastingly, H-abstraction at 3-OH site, in the C ring, led to the least stable antioxidant radical. The difference in energy between the most stable and least stable antioxidant radical formed was 95.78 kJ mol⁻¹ (Figure 5.8). Abstraction at the b-OH (ζ6) site produced the second most stable antioxidant radical followed by H-abstraction at the 2-H site, in the C ring. The H-abstraction in solvent differed from the preference in the gas phase where the most energetically stable H-abstraction was at 2-H site, in the C ring, trailed by 7'-OH, in the A' ring, then the h-OH hydroxyl group site, in the B' ring (Figure 5.2). However, analysis of all 12 conformers in the test set, from the gas phase, indicated H-abstraction at site h-OH, in the B' ring, resulted in the second most stable antioxidant radical followed by the b-OH site (Figure 5.3). Thus, showing similarity to compound **1a-H₂O** (Figure 5.8). Nevertheless, the least stable antioxidant radicals formed were deduced from H-abstraction to the 3'-OH, 3'-H, 3-OH and 3-H sites, in the C' and C ring, for both the gas and aqueous environment (Figure 5.2 and Figure 5.8). In summary, the lowest energy conformer (**1a-H₂O**) preferred H-abstraction from the B' ring at the h-OH site (ζ4), then shadowed by the hydroxyl group b-OH (ζ6), in the B ring, as the most stable antioxidant radicals were produced.

The test set of 12 neutral antioxidant conformers, in Table 4.2, were analysed to see the preferred H-abstraction site and deduce a trend in the most stable antioxidant radicals (Figure 5.9). All the neutral antioxidant conformers, compound **1a-H₂O** – **1j-H₂O** and **1l-H₂O**, except the intermediate neutral conformation, compound **1k-H₂O**, resulted in the most stable antioxidant radical, from abstraction at the h-OH site, in the B' ring. However, compound **1k-H₂O** preferred H-abstraction at the b-OH site, in the B ring (Figure 5.9). In compound **1k-H₂O**, the conformation adopted was saaasa-ss, where the hydroxyl group (ζ4 - ζ6), in the B' and B ring, had the

configuration a,s,a (Figure 4.10). The preference for H-abstraction at the b-OH site in contrast to the h-OH site was probably due to the h-OH hydroxyl group participating in hydrogen bonding while the b-OH hydroxyl group was free (Figure 4.10).

Figure 5.8 Relative energies of the most stable neutral conformer **1a-H₂O** at all possible 17 H-abstraction sites at the UB3LYP/6-31G(d,p) level of theory, in an aqueous solvent. AO = antioxidant, RO•S = reactive oxygen species, AO• = antioxidant radical and OS = oxygen species. Key: left - neutral conformer and right - the corresponding HAB ring site. Relative energy in kJ mol⁻¹ and atoms are numbered according to the labelling in Figure 5.1.

The least preferred site of H-abstraction for all conformers in the test set were at the 3'-OH, 3'-H, 3-OH and 3-H site, in the C and C' ring, resulting in the highest energy antioxidant radicals (Figure 5.9). Overall, the most preferred H-abstraction site was the hydroxyl side chain group, h-OH in the B' ring, as this correlated with the most energetically stable antioxidant radical.

Figure 5.9 Relative energies of the test set of 12 neutral theaflavin conformers **1a** – **1l** at all possible 17 H-abstraction sites at the UB3LYP/6-31G(d,p) level of theory, in an aqueous solvent. AO = antioxidant, RO•S = reactive oxygen species, AO• = antioxidant radical and OS = oxygen species. Key: left - neutral conformer and right - the corresponding antioxidant radical conformer. Relative energy in kJ mol⁻¹ and atoms are numbered according to the labelling in Figure 5.1.

Transition States of H-radical abstractions

The relative free-energy barriers for the competing abstractions at the h-OH, b-OH and 2-H (C-H abstraction) sites were determined from the transition-state (TS) structures (Figure 5.10). These were found by exploring differing configurations of an approaching hydroxyl radical (HO•) to the H-atom to be abstracted from the respective h-OH, b-OH and 2-H sites. The barriers energetic ordering conserved the stability ordering of the AO•, with the barrier to **1a-H₂O** h-OH• abstraction as the most accessible ($\Delta G_{rel} = 4.39$ kJ mol⁻¹); an extremely low barrier, and resulting

in $-159.16 \text{ kJ} \cdot \text{mol}^{-1}$ of free-energy stabilisation. The **1a-H₂O** b-OH• abstraction barrier was likewise relatively low ($\Delta G_{\text{rel}} = 12.06 \text{ kJ} \cdot \text{mol}^{-1}$), with $-152.32 \text{ kJ} \cdot \text{mol}^{-1}$ of stabilisation resulting from this transformation. Finally, albeit non-traditionally explored in radical-scavenging studies, the **1a-H₂O** 2-H• abstraction showed a barrier of $55.89 \text{ kJ} \cdot \text{mol}^{-1}$, which albeit much higher than the alternative h-OH and b-OH sites, is still a reasonably accessible path, even at room temperature, with the **1a-H₂O** 2-C• + H₂O products $-149.62 \text{ kJ} \cdot \text{mol}^{-1}$ lower in free-energy (Figure 5.10).

Figure 5.10 Free-energy reaction profile ($\text{kJ} \cdot \text{mol}^{-1}$) of the H-radical abstraction events at the h-OH, b-OH and 2-H (C-H abstraction) sites, on the **1a-H₂O** conformer, as determined at the B3LYP/6-31G(d,p) (SCRF, PCM= H₂O) level of theory. AO = antioxidant, AO• = antioxidant radical.

Overall, the H• abstraction is relatively spontaneous at two –OH sites, with high stabilisation of products. The non-traditional alternative H• abstraction from a C-H site, albeit higher in free-energy, may perhaps occur even if rarely; further

characterisation of this process is required to characterise if it is at all competitive with –OH site abstractions.

5.2.2 Hydrogen Atom Addition

The test set of 12 neutral conformers (**1a-H₂O** – **1l-H₂O**) in Table 4.2 were re-optimised to investigate the mechanism hydrogen atom addition, in an aqueous solvent, using the UB3LYP method and basis set 6-31G(d,p). The seven competing H-addition sites and its complementary labelling, illustrated in Figure 5.5, was used in the analysis below.

Figure 5.11 Relative energies of the most stable neutral conformer **1a-H₂O** at all possible H-addition sites at the UB3LYP/6-31G(d,p) level of theory, in an aqueous solvent. AO = antioxidant, H[•] = hydrogen radical and AO[•]-H = antioxidant H-added radical. Key: left - neutral conformer, right - the corresponding H-addition ring site. Relative energy in kJ mol⁻¹ and atoms are numbered according to the labelling in Figure 5.5.

The lowest energy conformer, as-ass-as, (compound **1a-H₂O**) within the test set of 12 was investigated, at all possible hydrogen atom addition sites (Figure 5.11). Out of the seven competing addition sites, the most energetically stable H-added antioxidant radical formed was at the e-C site, in the B ring, trailed by H-addition to the c-C site, in the B ring. The highest energy antioxidant radical was produced via H-addition to the A ring at the position 8-C. The difference in energy between the most stable and least stable antioxidant radical formed was 90.16 kJ mol⁻¹ (Figure 5.11). The most probable site of H-addition to compound **1a-H₂O**, in the aqueous solvent, was at the e-C site in the B ring, resulting in the most stable antioxidant radical. With minute energy differences, the least probable H-addition were at 6'-C, 8'-C, 6-C and 8-C sites, in the A' and A rings, producing the highest energy antioxidant radicals formed (Figure 5.11). The order of preferred H-addition sites was the same, in both aqueous solvent and gas (Figure 5.6).

Figure 5.12 showed the relative energy change produced by hydrogen atom addition to the various sites on the test set of 12 neutral conformers (Table 4.2), in the aqueous solvent. The stability of the resulting antioxidant radical formed after hydrogen atom addition to the various competing sites emerged the following trend, in descending order: e-C, c-C, g-C, 6'-C ~ 8'-C ~ 6-C ~ 8-C (Figure 5.12). For all 12 neutral conformers in the test set (**1a-H₂O** – **1l-H₂O**), H-addition to the e-C site, in the B ring, resulted in the most stable antioxidant radical. This was subsequently followed by the c-C site, in the B ring, then the g-C site in the B' ring, and thus, they are the most probable sites of hydrogen atom addition. The least energetically favoured H-addition were the 6'-C, 8'-C, 6-C, 8-C sites, in the A' and A ring, where the energy difference between these H-added antioxidant radicals was minimal. The order of preference in the various H-addition sites was the same for the test set conformers, in both gas and aqueous solvent (Figure 5.7). From the results obtained, the benzotropolone moiety consisting of the B and B' rings are an essential aspect to the stability of the H-added antioxidant radical formed.

From a molecular perspective, theaflavin's unique structure, in particular, the B ring, are thought to underlie its superior ability to neutralise ROSs, through free-radical scavenging, – an overall proficient facilitation to electron-spin stabilisation.

Figure 5.12 Relative energies of the test set of 12 neutral theaflavin conformers **1a-H₂O** – **1l-H₂O** at all possible H-addition sites at the UB3LYP/6-31G(d,p) level of theory, in an aqueous solvent. AO = antioxidant, H[•] = hydrogen radical and AO[•]-H = antioxidant H-added radical. Key: left - neutral conformer, right - the corresponding antioxidant radical. Relative energy in kJ mol⁻¹ and atoms are numbered according to the labelling in Figure 5.5.

5.3 Conclusion

Theaflavin is a large and flexible molecule with numerous possible sites for radical scavenging. To investigate the radical scavenging mechanism hydrogen-atom abstraction (HAB), all possible H-abstraction sites were computed and analysed. Theaflavin had 17 possible sites for hydrogen abstraction; nine H-atom from

hydroxyl groups O-H and eight H-atoms from alkanes C-H. In the gas phase, the most stable antioxidant radical tea theaflavin produced were from H-abstraction at the 2-H in the C ring. Subsequently, the h-OH hydroxyl group, in the B' ring, was the second most stable antioxidant radical produced. The stability of the antioxidant radical from H-abstraction, at the 2-H position, originates from the extensive π -electron delocalisation from the 2-C, in the C ring, through to the B and B' ring. The least energetically stable antioxidant radical formed was from H-abstraction at the 3'-OH and 3'-H sites, in the C' ring, and the 3-OH and 3-H sites, in the C ring, in both gas and aqueous solvent. Radical scavenging at these sites resulted in no charge delocalisation, and thus, the antioxidant radical produced were less stable. In solvent, the most stable antioxidant radical was from H-abstraction of the hydroxyl group h-OH, in the B' ring, followed by the 2-H site, in the C ring, and then the b-OH hydroxyl, in the B ring. The most preferred site for the radical scavenging mechanism, hydrogen atom abstraction, was either in close proximity or in the vicinity of the benzotropolone moiety. The extensive π -electron delocalisation, from the B and B' ring, stabilised the antioxidant radical tea theaflavin by the abundant resonance structures formed.

To investigate the radical scavenging mechanism hydrogen atom addition (HAD), all possible H-addition sites were computed and analysed for the test set of 12 conformers, in both gas and the aqueous solvent. Theaflavin had a total of seven possible sites for hydrogen atom addition. Namely, the unsaturated carbon 6-C and 8-C, in the A ring at, 6'-C and 8'-C, in the A' ring, g-C, in the B' ring, and at e-C and c-C, in the B ring. The most stable hydrogen atom addition site, in all 12 conformers, in both the gas and the aqueous solvent phase, was in the B ring. Specifically, at the e-C site, being the most stable antioxidant radical, followed by the H-addition to the c-C site. The least stable hydrogen atom addition were the 6'-C, 8'-C, 6-C and 8-C sites, in the A' and A rings, for all test set conformers, in both the gas and aqueous solvent phase. H-addition to the benzotropolone moiety stabilised the antioxidant radical tea theaflavin, through the extensive charge

delocalisation of the B and B' ring, and thus, resulted in the antioxidant radicals with lowest energies.

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Chapter 6

Inelastic Neutron Scattering

This chapter builds on the structure-activity relationship database by arranging the theoretical vibrational spectra, in preparation for the inelastic neutron scattering spectra. A theoretical and experimental data comparison links the molecular geometry with the experimental results to affirm the conformational preference of the antioxidant tea theaflavin. The analysis of the low energy vibrational spectra, in the region $0 - 1000\text{ cm}^{-1}$, provides insight to the hydrogen bonding properties of theaflavin's lowest (as-ass-as), lowermost tenth (sa-ass-sa), mid (saaasa-ss) and highest energy (sa-saa-ss) conformational isomers.

6.1 Background

Vibrational spectroscopy is an excellent characterisation technique to obtain the molecular geometry of a molecule. The vibrational spectra analysis can be divided into two sections: functional groups ($1500\text{ cm}^{-1} - 4000\text{ cm}^{-1}$) and the fingerprint region ($0\text{ cm}^{-1} - 1500\text{ cm}^{-1}$).¹ Commonly in both, the wavenumber peaks allow for the identification of the chemical structure of the molecule. The fingerprint region of the spectra ($0\text{ cm}^{-1} - 1500\text{ cm}^{-1}$) is generally noisy, and thus, difficult to analyse.² Inelastic neutron scattering spectra has access to the fingerprint region.³⁻⁵ In addition, INS is sensitive to the motions of hydrogens, and thus, making it a complementary experiment for the conformational analysis study of hydrogen bonding interactions in antioxidant systems.⁶

The intramolecular interactions studied in chapter 4 was cross-referenced in the analysis of the theoretical vibrational spectra. Furthermore, the hydroxyl side chains and ketone functional group bond lengths were investigated to aid in the understanding of the strength of the intramolecular hydrogen bond interactions, in the chemical structures of the conformers investigated.

Figure 6.1 Schematic representation of tea theaflavin's skeleton with the labelling system applied for reference.

6.2 Hydrogen Bonding

Antioxidant activities of polyphenols are commonly attributable to the hydroxyl functional groups.⁶ The hydroxyl functional group is the source for hydrogen bonding motifs in polyphenols, and thus, stabilises the molecule.

Hydrogen bonding is typically described as an intermolecular interaction involving two molecules, due to the difference in the electronegativity of the atoms; the hydrogen act as a hydrogen bond donor, and the neighbouring atom (O, N, S) as a hydrogen bond acceptor.⁷ However, in large and flexible polyphenols, like theaflavin, the molecule can form intramolecular hydrogen bonding interactions due to the numerous hydroxyl groups within the molecule, therefore, stabilising the energy of the system. The strength of a hydrogen bond can be gauged by the length of the covalently bonded intra-atomic distance (Å), the length of the intramolecular H-Bond distance (Å) and the peak position of the vibrational mode deformation in the fingerprint region of vibrational spectra.^{2, 6, 8-10}

6.2.1 Intra-atomic Distance

The intra-atomic distance of this study refers to the distance between the two covalently bonded atoms which are either the hydroxyl side chain groups (O-H) or the carbonyl group (C=O). The length concerning the covalent bond entails the strength of the intra-molecular hydrogen bonding displayed; the longer the bond length, the stronger the hydrogen bond interaction.^{2, 8, 10} The intra-atomic distances of the test set of 12 conformers, in both gas and aqueous solvent, were analysed to understand the hydrogen bonding strengths in theaflavin.

6.2.1.1 Intra-atomic Distance Gas Phase

In the gas phase, the lowermost ten conformers **1a** to **1j** had the same intra-atomic distances in both the ketone functional group and the various hydroxyl groups (Table 6.1). The bond lengths of the hydroxyl atoms 5'-O-H and 7'-O-H, in the A'

ring, 3'-O-H, in the C' ring, 5-O-H and 7-O-H, in the A ring and 3-O-H, in the C ring, was 0.97 Å, in conformers **1a** - **1j**. The mid and high energy conformers (**1k**, and **1l**, respectively) had the same bond length (0.97 Å) for the hydroxyl groups as well. The length of the intra-atomic distance of these -OH groups' shows that they signify either free hydroxyls which are not participating in hydrogen bonding or hydroxyls involved in weak intramolecular interaction. Similarly, the intra-atomic distance of the hydroxyl h-O-H, in the B' ring, was also 0.97 Å for all the conformers in the test set, thus, indicating the intramolecular h-OH/i-O interaction is weak.

The length of the hydroxyl i-O-H, in the B' ring, was considerably longer with a length of 1.01 Å distance, for all conformers, apart from the highest energy conformer **1l** (Table 6.1). The shortened bond length of 0.97 Å present, in conformer **1l**, was because of the orientation of the ζ_6 dihedral angle. As a result, the i-OH group formed a weaker intramolecular i-OH/h-O interaction, rather than the stronger i-OH/C=O interaction existing in, the more stable energetic conformers **1a** - **1k** (Figure 4.11).

In structures, **1a** to **1j**, the bond lengths of the C=O ketone functional group was 1.28 Å and the hydroxyl b-O-H, in the B ring, was 0.98 Å (Table 6.1). The bond lengths were consistent in the lowermost ten conformers, however, in structures **1k** and **1l**, the hydroxyl b-O-H bond length was reduced to 0.97 Å and the C=O distance shortened to 1.25 Å. The decreased bond distance displayed was due to the change in orientation of the ζ_6 dihedral angle from syn (s) to anti (a). As a result, the earlier existing intramolecular b-OH/C=O interaction was absent in structures **1k** and **1l** (Figure 4.11). The b-OH hydroxyl, was thus, a free hydroxyl with a bond length of 0.97 Å. An additional loss of the adjacent hydrogen bonding interactions, i-OH/C=O, further reduced the C=O ketone functional group intra-atomic distance to 1.22 Å.

The strongest hydrogen bonding interaction in theaflavin was the hydroxyl group, i-O-H, in the B' ring (1.01 Å). The i-O-H hydroxyl group represented the lengthiest O-H intra-atomic distance with the most extended hydroxyl bond length. The length of the intra-atomic distance i-O-H group suggests that the most stabilising intramolecular interaction was the i-OH/C=O interaction. This was followed by the b-O-H hydroxyl, in the B ring, with a bond length of 0.98 Å. The results of the bond lengths substantiated the trends found in sections 4.1.2 and 4.3.1 page 54 and 63, respectively.

Table 6.1 Intra-atomic distance (Å) of the optimised test set of 12 conformers **1a** - **1l** at the B3LYP/6-31G(d,p) level of theory, in the gas phase. A', C', A, C, B' and B represents the rings in theaflavin, the intra-atomic distance (Å) of the atoms follows the labelling system in Figure 6.1.

	Intra-atomic Distance (Å)									
	A'		C'	B'		B	C	A		
	5'	7'	3'	h	i	a	b	3	5	7
	O-H	O-H	O-H	O-H	O-H	C=O	O-H	O-H	O-H	O-H
1a	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1b	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1c	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1d	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1e	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1f	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1g	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1h	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1i	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1j	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1k	0.97	0.97	0.97	0.97	1.01	1.25	0.97	0.97	0.97	0.97
1l	0.97	0.97	0.97	0.97	0.97	1.22	0.97	0.97	0.97	0.97

6.2.1.2 Intra-atomic Distance in Solvent

The intra-atomic distance of the lowermost ten conformers (**1a-H₂O** - **1j-H₂O**) in the aqueous solvent was consistent for each covalent bond in the study, with the exception of the 7-O-H bond (Table 6.2). A bond length of 0.97 Å was present between the O-H atoms at 5' and 7', in the A' ring, 3', in the C' ring, 5, in the A ring and h-O-H, in the B' ring. The 0.97 Å bond length suggests that these

hydroxyls are either free hydroxyls or are participating in weaker strength hydrogen bond interactions.

Depending on the conformer, the 7-O-H group in the A ring was either a free hydroxyl or involved in an intramolecular hydrogen bonding interaction. Specifically, the 7-OH/7'-O interaction, as seen in Figure 4.12. The lengthier 7-O-H bond length of 0.98 Å was found in structures **1a-H₂O**, **1b-H₂O**, **1f-H₂O** and **1g-H₂O** and the shorter bond length of 0.97 Å in structures **1c-H₂O**, **1d-H₂O**, **1e-H₂O**, **1h-H₂O**, **1j-H₂O**, **1k-H₂O** and **1l-H₂O** (Table 6.2). With the exception of the conformer **1l-H₂O**, the general trend demonstrates the conformers with the longer 7-O-H intra-atomic distance was involved in an intramolecular interaction (7-OH/7'-O). The shorter 7-O-H bond length, in conformer **1l-H₂O**, expressed the 7-OH/7'-O intramolecular interaction was weak. The weakened interaction was possibly the result of the angle of the hydrogen bond formation.¹¹ The general trend of the shortened 7-O-H bond length represented free hydroxyl groups, with the exception of conformers' **1i-H₂O** and **1j-H₂O** where an intramolecular 7-OH/6-C interaction was present. Thus, indicating the 7-OH/6-C intramolecular interaction was weak.

The longest intra-atomic distance in the test set was 1.01 Å and belonged to the hydroxyl group i-O-H, in the B' ring. The length concerning the i-O-H hydroxyl atoms was constant, in the conformers' **1a-H₂O** to **1k-H₂O**, but decreased in conformer **1l-H₂O** (Table 6.2). The alteration in the bond length was due to the i-OH/h-O hydrogen bond interaction. The orientation of the conformer **1l-H₂O** i-OH hydroxyl group was anti (a) at ζ5 resulting in the weaker i-OH/h-O intramolecular interaction, rather than the stronger i-OH/C=O interaction, as previously seen in conformers **1a-H₂O** - **1k-H₂O** (Figure 4.12).

An intra-atomic distance of 0.98 Å was established between the b-O-H atoms, in the B ring, of the lowermost ten conformers in the test set (**1a-H₂O** – **1j-H₂O**). The bond length then decreased to 0.97 Å, as seen in conformers **1k-H₂O** and **1l-H₂O**.

The longer bond length in the lowermost ten test set conformers was due to the b-OH hydroxyl group participating in the b-OH/C=O intramolecular H-bonding interaction. The b-OH/C=O intramolecular interaction was absent in both conformers **1k-H₂O** and **1l-H₂O**. Thus, the shorter bond lengths in conformers **1k-H₂O** and **1l-H₂O** represented free hydroxyl groups.

The ketone functional group's bond length fluctuates in the different structures of the test set. The C=O bond length was 1.28 Å, in the conformers **1a-H₂O** - **1j-H₂O**, 1.26 Å, in **1k-H₂O** and 1.23 Å, in **1l-H₂O**. The elongated C=O intra-atomic distance was attributable to its involvement in the intramolecular hydrogen bonding to both the i-OH and b-OH hydroxyl groups. The C=O intra-atomic distance was shortened when not participating in H-bonding, as shown in conformer **1l-H₂O** (Figure 4.12 and Table 6.2).

Table 6.2 Intra-atomic distance (Å) of the optimised test set of 12 conformers **1a** - **1l** at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. A', C', A, C, B' and B represents the rings in theaflavin, the intra-atomic distance (Å) of the atoms follows the labelling system in Figure 6.1.

	Intra-atomic Distance (Å)									
	A'		C'	B'		B	C	A		
	5'	7'	3'	h	I	a	b	3	5	7
	O-H	O-H	O-H	O-H	O-H	C=O	O-H	O-H	O-H	O-H
1a-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.98
1c-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1b-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.98
1f-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.98
1g-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.98
1d-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1h-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1i-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1e-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1j-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.28	0.98	0.97	0.97	0.97
1k-H ₂ O	0.97	0.97	0.97	0.97	1.01	1.26	0.97	0.97	0.97	0.97
1l-H ₂ O	0.97	0.97	0.97	0.97	0.97	1.23	0.97	0.97	0.97	0.97

In aqueous solvent, theaflavin's hydrogen bonding interactions are strengthened, and thus, the conformers more energetically stabilised. In particular, the intra-atomic distance of the ketone group in **1k-H₂O**, and the hydroxyl 7-O-H of the conformer **1a-H₂O**, **1b-H₂O**, **1f-H₂O** and **1g-H₂O** were 0.01 Å longer in the aqueous solvent than in the gas phase. Theaflavin's ketone functional group was the central hub for strong intramolecular hydrogen bonding interactions (OH/C=O). Adjacent to the carbonyl group was the hydroxyls i-OH and b-OH, both influencing the stability of theaflavin significantly through highly stabilising hydrogen bonds. This was evidenced by their longer intra-atomic distance which had shortened in the absence of the intramolecular OH/C=O interaction.

6.3 Vibrational Spectra

The vibrational spectra of the lowest energy theaflavin conformer **1a** (as-ass-as), lowermost tenth **1j** (sa-ass-sa), mid **1k** (saaasa-ss), and highest energy conformer **1l** (sa-saa-ss) were analysed. The vibrational spectra were analysed to determine the molecular geometry of the various conformational isomers of theaflavin, in preparation for the comparison with the experimental inelastic neutron scattering spectra. In addition, the hydrogen bonding properties and their effects on the vibrational frequency, namely, the intramolecular H-bond interactions and the intra-atomic distance (Å), were investigated. Thus far, the only dietary phenolic acids (Figure 6.2) study using INS in the literature was by Marques et al.⁶ (Table 6.3).

All four conformer's vibrational spectra were obtained at the same level of theory; B3LYP/6-31G(d,p), in the gas phase. A constant scaling factor 0.9627 was applied to the theoretical vibrational data above 500 cm⁻¹ and a factor of 1.0028 for the low energy vibrations, to correct for the anharmonicity of the normal modes of vibration.^{3, 12} The labelling system used was in accordance with Figure 6.1.

Note

- The vibrational spectra in the range $0\text{--}1500\text{ cm}^{-1}$, and its corresponding tabulated data is in the appendix (Figure A 1 and Table A 6).

Figure 6.2 Schematic representation of the dietary phenolic acids cinnamic acid, p-coumaric acid, m-coumaric acid, caffeic acid and ferulic acid with the labelling system applied for reference.

Table 6.3 Main experimental INS wavenumbers (cm^{-1}) for the dietary phenolic acids cinnamic acid (Cin), p-coumaric acid (p-C), m-coumaric acid (m-C) and caffeic acid (CA). The atoms description is according to the labelling system in Figure 6.2. δ = in-plane deformation and γ = out-of-plane deformation. ⁶

Phenolic Acids				
CA	Cin	p-C	m-C	
INS experimental				Approximate Description
	414/419			$\delta \text{O}^{(7)}\text{H}$
466	-	378	370	$\gamma \text{O}^{(7)}\text{H}$
~330	363	341/356	335/342	$\delta \text{O}^{13}\text{H}$
~330				$\delta \text{O}^7\text{H}$
240				$\gamma \text{O}^{(8)}\text{H}$

6.3.1 Frequency and Hydrogen Bonding

In regards to the low wavenumber region ($< 1000 \text{ cm}^{-1}$) of the spectra, theaflavin possessed two types of vibrational modes that were involved in hydrogen-bonding deformation. The normal modes included δ (in-plane deformation scissoring vibrations) and γ (out-of-plane deformation torsion vibrations) where the latter can be divided into γ - out-of-plane free hydroxyl torsion and γ - out-of-plane hydrogen bonded torsion. The δ (in-plane deformation scissoring vibrations) was in the lowermost region of the vibrational spectra investigated at $\sim 150 - 500 \text{ cm}^{-1}$ (Figure 6.3) and involved an in-line scissoring motion between the hydrogen bond donor and acceptor.^{2, 6, 8} Observed in the range of above $300 - 900 \text{ cm}^{-1}$ were the γ - out-of-plane free hydroxyl and hydrogen bonded torsion (Figure 6.3).^{2, 6, 8} The free γ - out-of-plane torsion represented the distortion of the hydroxyl where the oxygen atom was the hydrogen bond acceptor. On the other hand, the γ - hydrogen-bonded torsion, represented the distortion of the hydroxyl where the torsion involved the hydrogen bond donor.

The lowermost region of the vibrational spectra ($\sim 150\text{--}500\text{ cm}^{-1}$) was populated by δ - in-plane deformation scissoring vibrations (Figure 6.3 and Table 6.4). The highest of the δ peaks reflected the hydrogen bonding in the benzotropolone moiety. Specifically, $\delta\text{ O}^{(i,b)}\text{H}$ at 489 cm^{-1} , for both conformers **1a** and **1j**, but absent in conformers **1k** and **1l**. In conformers **1k** and **1l**, the $\text{O}^b\text{H}/\text{C}=\text{O}$ interaction was missing due to the anti (a) orientation of the b-OH hydroxyl group. Followed by the $\delta\text{ O}^{(i,b)}\text{H}$ at 489 cm^{-1} was the $\delta\text{ O}^i\text{H}$ peak at 465 cm^{-1} , 467 cm^{-1} , 490 cm^{-1} and 342 cm^{-1} for conformers **1a**, **1j**, **1k** and **1l**, respectively. The lower peak of 342 cm^{-1} displayed by conformer **1l** was from the weaker $\text{O}^i\text{H}/\text{O}^h$ interaction rather than, the stronger $\text{O}^i\text{H}/\text{C}=\text{O}$ interaction present in **1a**, **1j** and **1k**, and thus resulting in the redshift observed. At the stronger $\text{O}^i\text{H}/\text{C}=\text{O}$ hydrogen bonding interaction, present in conformers **1a**, **1j** and **1k**, it is resulting in the redshift observed. The $\text{O}^i\text{H}/\text{C}=\text{O}$ intramolecular interaction hydrogen bond distance was $1.5\text{ \AA}$ which formed a meta-stable six-membered ring while the $\text{O}^i\text{H}/\text{O}^h$ interaction had an intramolecular hydrogen bond distance of $2.1\text{ \AA}$ and formed a lesser stable meta-five membered ring (Figure 4.11 and Appendix Table A 4). An $\delta\text{ O}^h\text{H}$ peak was observed for conformers **1a**, **1j** and **1k** at 413 cm^{-1} , 409 cm^{-1} , 417 cm^{-1} , respectively. The $\delta\text{ O}^h\text{H}$ peak was absent for the **1l** conformer as the O^hH was a free hydroxyl group (Figure 6.3 and Table 6.4).

Detected at 323 cm^{-1} was the in-plane deformation peak $\delta\text{ O}^7\text{H}$, for conformer **1a**, where a $7\text{-OH}/7'\text{-O}$ interaction was present. Although a $7\text{-OH}/7'\text{-O}$ interaction was also seen in conformer **1l**, a strong peak could not be located (Figure 6.3 and Table 6.4). Experimentally, the following polyphenolic acids, caffeic a, cinnamic acid, p-coumaric acid and m-coumaric acid showed $\delta\text{ O}^{13}\text{H}$ peak at $\sim 330\text{ cm}^{-1}$, 363 cm^{-1} , $341/356\text{ cm}^{-1}$ and $335/342\text{ cm}^{-1}$, respectively (Table 6.3).⁶ The polyphenolic acids $\delta\text{ O}^{13}\text{H}$ peaks displayed hydrogen bonding interactions similarly to the $\delta\text{ O}^7\text{H}$ peak in theaflavin conformer **1a**.

At 279 cm^{-1} , 284 cm^{-1} and 189 cm^{-1} the δ (in-plane scissoring deformation peaks) of O^3H for conformers **1a**, **1j** and **1l**, respectively, were observed. In close proximity at 188 cm^{-1} and 171 cm^{-1} were the $\delta\text{ O}^3\text{H}$ peak of conformer **1j** and **1k**. The $\delta - \text{O}^3\text{H}$ peaks reflected the stronger $\text{O}^3\text{H}/\text{O}^{1'}$ interaction as compared to the $\delta\text{ O}^3\text{H}$ peaks from the $\text{O}^3\text{H}/\text{O}^1$ interaction; the strength of the hydrogen bonding was deduced by their intramolecular H-bond distance⁹ as the intra-atomic distance ($3'\text{-O-H}$ and 3-O-H) were the same $0.97\text{ \AA}$ (Figure 4.11, Appendix Table A 4 and Table 6.1). No $\delta\text{ O}^3\text{H}$ peak was observed for conformer **1k**, as the orientation of its $3'\text{-OH}$ was in an anti (a) orientation, and thus, devoid of the $\text{O}^3\text{H}/\text{O}^{1'}$ hydrogen bonding interaction. For structures **1a** and **1l**, the $\delta\text{ O}^3\text{H}$ peak was not detected possibly due to weak and negligible hydrogen bonding interactions.

In the vibrational spectra region of $300\text{ cm}^{-1} - 900\text{ cm}^{-1}$ the γ - out-of-plane deformation peaks were detected (Figure 6.3 and Table 6.4).^{6, 8, 9} The highest γ (out-of-plane torsion) peaks reflected was the $\gamma\text{ O}^i\text{H}$ at 862 cm^{-1} , 861 cm^{-1} , 895 cm^{-1} and 456 cm^{-1} for conformers **1a**, **1j**, **1k** and **1l**, in that order. For all four conformers, the peaks corresponded to the hydrogen bonded out-of-plane torsions. However, a significant redshift was seen for conformer **1l**. This is in agreement with the $\gamma\text{ O}^i\text{H}$ peak having the strongest $\text{O}^i\text{H}/\text{C}=\text{O}$ interaction; present in conformers **1a**, **1j** and **1k** and lacking in conformer **1l** with its $\gamma\text{ O}^i\text{H}$ peak representing the weaker $\text{O}^i\text{H}/\text{O}^h$ interaction. In conformers **1a**, **1j** and **1k**, the $i\text{-O-H}$ bond length was $1.01\text{ \AA}$ and the intramolecular $\text{O}^i\text{H}/\text{C}=\text{O}$ H-bond distance was $1.5\text{ \AA}$ whilst in conformer **1l**, the $i\text{-O-H}$ bond length was $0.97\text{ \AA}$, and the intramolecular $\text{O}^i\text{H}/\text{O}^h$ hydrogen bond interaction distance was $2.1\text{ \AA}$ (Table 6.1, Figure 4.11 and Appendix Table A 4).

Followed by the $\gamma\text{ O}^i\text{H}$ peak was the $\gamma\text{ O}^b\text{H}$ peak at 679 cm^{-1} , 674 cm^{-1} , 440 cm^{-1} and 435 cm^{-1} , for structures **1a**, **1j**, **1k** and **1l** (Figure 6.3 and Table 6.4). The redshift in the peaks of the latter two conformers were because they represented the γ - free hydrogen bonded out-of-plane torsion) whilst conformers **1a** and **1j** peaks

corresponded to the γ - hydrogen bonded out-of-plane torsion. The disruption of the hydrogen bond represented by a hydrogen-bonded out-of-plane torsion distorts the stability of the antioxidant more than the γ free OH torsion, thereby, requiring more energy and thus located further upfield.

The γ O^hH peaks were detected on the vibrational spectra at 496 cm⁻¹, 492 cm⁻¹, 492 cm⁻¹ and 226 cm⁻¹ for conformers **1a**, **1j**, **1k** and **1l**, respectively (Figure 6.3 and Table 6.4).⁶ The red-shifted γ O^hH peak for conformer **1l** was owed to the hydroxyl being a free hydrogen bonded out-of-plane torsion. Therefore disrupting the hydrogen bonding interaction to a lesser degree than the hydrogen bonded out-of-plane torsion in structures **1a**, **1j** and **1k**.

Conformer **1l**'s γ O^hH (226 cm⁻¹) and γ OⁱH (456 cm⁻¹) (Table 6.4) peaks were comparable to caffeic acid's (CA) γ O⁸H (240 cm⁻¹) and γ O⁷H (466 cm⁻¹) experimental data peaks (Table 6.3), respectively.⁶ The structure of these polyphenols are similar as the hydroxyls groups are adjacent and oriented in a similar fashion (Figure 6.2 and Figure 6.3). Thus, the γ O⁸H from CA and γ O^hH from TF (**1l**) represented free out of plane distortion peaks whilst the γ O⁷H from CA and γ OⁱH from TF (**1l**) represented bonded out of plane distortion peaks and hence they have comparable peaks.

Collectively, red-shifts were observed in the γ OH peaks of the hydroxyls in the A, C, A' and C' rings (Figure 6.3 and Table 6.4). Out-of-plane deformation (γ) of the γ O³H peaks were observed at 420 cm⁻¹, 433 cm⁻¹, 273 cm⁻¹ and 423 cm⁻¹ for conformers **1a**, **1j**, **1k** and **1l**, respectively. The lower position of the γ O³H peak seen in **1k** was a result of the anti (a) orientation of the 3'-OH hydroxyl group, and thus, the γ O³H peak represents a free hydrogen bonded out-of-plane torsion (Figure 6.3 and Figure 4.11). The γ O³H peak position of conformers **1a**, **1j** and **1l** were the hydrogen bonded out-of-plane torsion corresponding to the intramolecular O³H/O¹' interaction (Figure 6.3 and Figure 4.11).

The out-of-plane deformation (γ) representing the γ O³H peaks were observed at 300 cm⁻¹, 310 cm⁻¹, 449 cm⁻¹ and 420 cm⁻¹ for conformers **1a**, **1j**, **1k** and **1l**, respectively (Figure 6.3 and Table 6.4). The blue-shift in conformers **1k** and **1l** may be attributable to the stabilising effect of the c-CH/3-O interaction forming a meta-stable six-membered ring with the additional O³H/O¹ hydrogen bond interaction for conformer **1a** and the possible O³H/ π interaction for **1l** (Figure 4.11). The γ O³H peak position in conformer **1a** and **1j** may be further red-shifted compared to conformer **1k**, and **1l** as the intramolecular hydrogen bond distance is greater (2.6 Å and 2.4 Å, respectively), thus, reflecting a weaker hydrogen bond interaction on top of the lack of a pseudo-six-membered ring.

The vibrational spectra signifying the γ O⁷H peaks were most varied. The peaks appeared at 649 cm⁻¹, 358 cm⁻¹, 482 cm⁻¹ and 367 cm⁻¹ for conformers **1a**, **1j**, **1k** and **1l**, respectively (Figure 6.3 and Table 6.4). The vast range in the peak positions detected for γ O⁷H was a result of the various type of intramolecular interactions, namely an O⁷H/O^{7'} interaction in conformers **1a** and **1l**, an O⁷H/C⁶ interaction in conformer **1k** and a free out-of-plane deformation for conformer **1j**. The lowermost peak was a reflection of the free normal mode out-of-plane deformation, in conformer **1k**, and the highest due to the O⁷H/O^{7'} interaction, in conformer **1a**. However, the generally strong OH/O interaction was also present in conformer **1l** although the γ O⁷H peak was at the second lowest range. This was due to the angle of the hydrogen bond donor and acceptor resulting in a weaker hydrogen bond interaction.¹¹

In the vibrational spectra the γ O⁷H peaks were established at 406 cm⁻¹, 371 cm⁻¹, 347 cm⁻¹ and 334 cm⁻¹ for conformers **1a**, **1j**, **1k** and **1l**, respectively. The 406 cm⁻¹ and 334 cm⁻¹ peaks represented the γ (free hydrogen bonded out-of-plane torsion) in conformers **1a** and **1l**, respectively. However, the red-shifted peak seen in **1l** reaffirms the weak OH/O interaction displayed due to the angle of hydrogen bond formation. On the contrary, the blue-shift in conformer **1a** reinstates the stronger O⁷H/O^{7'} hydrogen bonding present. The γ O⁷H peaks of conformers **1j** and **1k** represent a free normal mode out-of-plane deformation. Similarly, at the lowermost frequency for out-of-plane deformation (γ) torsions were the γ O⁵H and γ O⁵H peaks representing free normal mode out-of-plane deformations (Figure 6.3, Table 6.4 and Figure 4.11). The 5 and 5' hydroxyl groups are free hydroxyls not partaking as hydrogen bond donors or receptors.

Table 6.4 Main theoretically calculated wavenumbers in the region 0-1000 cm^{-1} for tea theaflavin conformers **1a** (as-ass-as), **1j** (sa-ass-sa), **1k** (saaasa-ss), and **1l** (sa-saa-ss) performed at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The atoms description is according to the labelling system in Figure 6.1. s = syn, a = anti, - = gauche-; δ = in-plane deformation, γ = out-of-plane deformation and Δ = in-plane deformation of skeleton atoms.

Theaflavin Conformers				Approximate Description
1a	1j	1k	1l	
as-ass-as	sa-ass-sa	saaasa-ss	sa-saa-ss	
Theoretically calculated				
924	910	915	892	$\gamma \text{C}^{\text{g}}\text{H}$
898/ 884	891 /875	862/ 849	898 ^e /864 ^c	$\gamma \text{C}^{(\text{c,e})}\text{H}$
873	875	880	877	$\delta \text{C}^{\text{d}}\text{H}_2 \text{C}' \text{ ring}$
862	861	895	456	$\gamma \text{O}^{\text{i}}\text{H}$
838	836	833	855	$\delta \text{C}^{\text{2'}}\text{C}^{\text{3'}}\text{C}^{\text{4'}} \text{C}' \text{ ring}$
814	818	819	842	$\delta \text{C}^{\text{c}}\text{C}^{\text{d}}\text{C}^{\text{e}} \text{B ring}$
785	792	806	800	$\gamma \text{C}^{\text{8}}\text{H}$
779	796	805	796	$\gamma \text{C}^{\text{8}}\text{H}$
763	771	770	768	$\gamma \text{C}^{\text{6}}\text{H}$
761	767	785	770	$\gamma \text{C}^{\text{6}}\text{H}$
721	717	748	735	$\gamma \text{C}^{\text{i}}\text{C}^{\text{a}}\text{C}^{\text{b}} \text{B ring}$
695	693	695	683	$\Delta \text{A ring \& C ring}$
679	674	440	435	$\gamma \text{O}^{\text{b}}\text{H}$
649	358	482	367	$\gamma \text{O}^{\text{7}}\text{H}$
647	648	652	637	$\gamma \text{C}^{\text{h}}\text{C}^{\text{i}}\text{C}^{\text{j}} \text{B' ring}$
583	582	580	580	$\Delta \text{CCC B',A', C' ring}$
528	525	529	524	$\Delta \text{CCC C' ring}$
508	509	509	502	$\Delta \text{CCC A' ring}$
496	492	492	226	$\gamma \text{O}^{\text{b}}\text{H}$
489	489	-	-	$\delta \text{O}^{(\text{i,b})}\text{H}$
			486	$\gamma \text{O}^{(7,7')}\text{H}$
465	467	490	342	$\delta \text{O}^{\text{i}}\text{H}$
462	463	468		$\Delta \text{CCC A,C ring}$
420	433	273	423	$\gamma \text{O}^{\text{3}}\text{H}$
413	409	417	-	$\delta \text{O}^{\text{b}}\text{H}$

406	371	347	334	γ O ⁷ H
385	296	286		γ O ⁵ H
344	328	337	291	γ O ⁵ H
323	-	-		δ O ⁷ H
300	310	449	420	γ O ³ H
		307		γ O ^(7,5) H
279	284	-	189	δ O ³ H
-	188	171	-	δ O ³ H

6.4 Conclusions

To build on the structure-activity relationship database for theaflavin, the intra-atomic distance of the ketone and hydroxyl functional groups and theoretical vibrational spectra in the range 0 – 1000 cm⁻¹ were analysed. A hydrogen bond strength is associated with the length of the O-H atom bond distance, where a longer distance reflects a stronger hydrogen bond.⁸

In both gas and aqueous solvent the strongest hydrogen bond formation involved the hydroxyl group i-OH as the hydrogen bond donor and the C=O oxygen atom as the hydrogen bond acceptor; the intramolecular hydrogen bond interaction involving i-OH/C=O. The longest hydroxyl intra-atomic distance belonged to the i-O-H hydroxyl group with a bond length of 1.01 Å. The 1.01 Å bond length was consistent in all conformers with the exception of the highest energy conformer (**11** and **11-H₂O**) where the bond length was shortened to 0.97 Å. Similarly, the bond distance of the C=O was 1.28 Å for the lowermost ten conformers, in gas and aqueous solvent, but decreased with the loss of the adjacent b-OH/C=O intramolecular interaction and reduced further when the i-OH/C=O interaction was absent (1.25 Å and 1.22 Å, in gas and 1.26 Å and 1.23 Å, in the aqueous solvent, respectively).

The subsequent strongest hydrogen bond was the b-OH hydroxyl group where the b-OH hydrogen atom was the hydrogen bond donor and the ketone oxygen atom as

the hydrogen bond acceptor (b-OH/C=O). The intra-atomic distance of the b-O-H group was 0.98 Å and reduced to 0.97 Å when the intramolecular b-OH/C=O hydrogen bonding interaction was lost. The reduced length of 0.97 Å was seen in the least energetically stable conformers (**1k** and **1l**, in the gas phase and **1k-H₂O** and **1l-H₂O**, in the aqueous solvent).

The vibrational spectra can be used to gauge the hydrogen bond strength. The lowermost region of the vibrational spectra ~150 - 500 cm⁻¹ was populated by δ - in-plane deformation scissoring vibrations. The highest of the δ peaks reflected the hydrogen bonding in the benzotropolone moiety. Specifically, the δ O^(i,b)H peaks at 489 cm⁻¹ for both conformers **1a** and **1j**, followed by the δ OⁱH peak, then the δ O^bH peak. The anomaly red-shifted peak seen in conformer **1l** at the δ OⁱH peak was due to the alteration of the i-OH hydroxyl group orientation, resulting in a weaker hydrogen bond.

In the vibrational spectra region of 300 - 900 cm⁻¹ the γ - out-of-plane deformation peaks were detected.⁸ Similarly to the δ - in-plane deformation peaks, the highest out-of-plane torsion peaks were from the hydroxyl groups, in the B and B' ring; in corroboration with the intra-atomic distance.⁸ The highest out-of-plane torsion was the γ OⁱH peak, followed by the γ O^bH peak, then the γ O^hH peak. However, in conformer **1a**, the γ O⁷H peak was higher than the γ O^bH peak. The blue-shift identified may be attributable to a stabilising effect from the pseudo-eight-membered ring formation by the 7-OH/7'-O and 8-CH/8'C intramolecular interaction (Figure 4.11).

Similarities in the peaks were γ - out-of-plane deformation peaks were detected in our theoretically calculated data and scarce INS experimental data, specifically concerning conformer **1l** and polyphenolic caffeic acid (Table 6.3 and Table 6.4).

The h-O-H bond distance of 0.97 Å was identical to the hydroxyl groups in the A, C, A' and C' rings, yet, the out-of-plane hydrogen bonded torsion was blue-shifted in the vibrational spectra of the conformers investigated, except the γ O⁷H peak in conformer **1a**. The general blue-shift observed reflects a stronger H-bond possibly due to the π -electron delocalisation of the B and B' ring. Therefore, resulting in a stabilising effect on the hydrogen bonding formations in the benzotropolone moiety. In addition, the formation of the hydrogen bonding interactions, in the B and B' rings, formed meta-stable five and six-membered rings.

The lowermost out-of-plane torsion peaks are from the γ O⁵ & ^{5'}H peaks. The peak represented a free normal mode out-of-plane torsion, where the 5 and 5' hydroxyl group were not participating in intramolecular hydrogen bonding interactions, and thus, were the furthest red-shifted hydroxyl out-of-plane torsion.⁸

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Chapter 7

Implications, Impact and Future Work

To conclude this thesis, this chapter confers the problem, provides a brief description of the investigation and lists the findings; highlighting the fundamental structural properties of theaflavin's structure-activity relationship (SAR) database in place. The thesis will end with suggestions to further aid in the SAR of the antioxidant tea theaflavin.

7.1 Conclusion

An excess of reactive species damages vital core components in the body causing a variety of ageing diseases. Naturally occurring antioxidants found in fruits, vegetables, wine and tea alleviates oxidative stress by scavenging reactive species. The beneficial makeup of biomolecules are established at a physiological scale, yet atomistic understanding is lacking. Therefore, the development of a rational structure-activity relationship database will be beneficial for promising new drug development. Investigating the structural properties and establishing the conformational preference of theaflavin is the key to understanding its antioxidant activity performance. Thus, the SAR of theaflavin provides a platform for a better understanding of the nutraceutical health benefits; to optimise the efficacy and minimise the toxicity for potential future therapeutic applications.^{1, 2}

This thesis aided in the rational design of a structure-activity relationship of the antioxidant tea theaflavin. Tea theaflavin is a large and flexible molecule that provides a vast variety of biological functions due to its ability to scavenge reactive species. The design of a rational structure-activity relationship database through conformational analysis of the neutral antioxidant theaflavin, the antioxidant radical theaflavin and the vibrational spectra.

7.1.1 The Start

Tea antioxidant theaflavin had 512 possible conformational isomers as a result of the free rotation around the hydroxyl group sigma bond. All 512 conformers were modelled, computed and analysed at a low level of theory, to save time and cost. This generated a refined shortlist to be investigated at the higher level of theory, and thus, the initial scope was reduced from 512 conformers to 153. The 153 conformers were successfully optimised at a higher level of theory, in both gas and the aqueous solvent. Within a small energy range, theaflavin could adopt numerous

stable conformational isomers. This suggests that theaflavin may exist as a complex conformational mixture where the health properties of theaflavin could be attributed to its conformational flexibility. Provided each conformational isomer possessed different structural properties, therefore, can impart differing chemical and biological functions.

A test set of 12 conformers, out of the 153 conformers, of theaflavin, were selected to study the contributing factors associated with conformation and energy, in detail. The test set of 12 contained the lowermost ten, intermediate, and highest energy conformers of the gas phase. For consistency, the same conformers were used as the test set for the study in the aqueous solvent. The change in environment from gas to solvent did not influence the structural dynamics of the conformers but did influence the energetics. With the exception of conformer **1b-H₂O**, all conformers were more energetically stable in the aqueous solvent than in gas. The change in energy can be accounted for by the inclusion of aqueous solvent, therefore, stabilising the many hydroxyl side chain groups, and thus, being a more energetically stable conformer.

7.1.2 The Findings

7.1.2.1 Benzotropolone Moiety

The benzotropolone moiety constituent is a characteristic found in black tea and is a vital component to the stability of theaflavin. There was a trend in the energetic stability of the conformer and the conformation adopted, in the hydroxyl groups of the B and B' ring. The hydroxyl side chains h-OH, i-OH, b-OH in the benzotropolone moiety, comprised of theaflavin's strongest hydrogen bonding interactions; having the longest intra-atomic distance and shortest intramolecular hydrogen bonding interaction. In addition, the intramolecular interaction formed meta-stable five and six-membered rings which further stabilised the system. Furthermore, the B and B' rings, possessed an extensive π -electron delocalisation network. Therefore, the most energetically stable conformers of theaflavin, in both

gas and the aqueous solvent, adopted the conformation a,s,s, in their B and B' rings. The a,s,s arrangement produced the maximum amount of hydrogen bond formations and minimum steric hindrance. On the contrary, the least stable conformers adopted the conformation s,a,a in the ζ_4 - ζ_6 of the benzotropolone moiety; the least amount and the weakest hydrogen bond formation with maximum steric hindrance.

The importance of the benzotropolone moiety was emphasised in the vibrational spectra where the higher out-of-plane hydrogen bonded torsion peaks detected, belonged to the B and B' ring hydroxyl side chain groups. Also, the h-O-H bond distance of 0.97 Å was identical to the hydroxyl groups in the A, C, A' and C' rings, yet, the γ O^hH hydrogen bonded torsion peak were blue shifted in the conformers investigated. Thus, reflecting the existence of a stronger H-bond interaction. The stronger hydrogen bond was possibly due to the stabilising effect elicited from the π -electron delocalisation of the B and B' ring and the formation of a pseudo-five-membered ring. The benzotropolone moiety was an essential component in tea theaflavin's structural stability, and consequently, affects the antioxidant's properties. This statement was further verified by the preferred radical scavenging site which was in and about the vicinity of the B and B' rings.

7.1.2.2 Intramolecular Interaction

The functional groups in theaflavin formed an extensive electronic network within the structure, in turn, stabilising the biomolecule. The electronic network of theaflavin was created by the formation of the OH/O, CH/O, CH/C, H/H and OH/C type interactions. The different type of interactions ranged from weak electrostatic forces to stronger hydrogen bonds; the cumulative effect highly stabilised the energetics of theaflavin, as shown in all conformers investigated. These intramolecular interactions formed, created meta-stable five, six and eight-membered ring. As a result, theaflavin assembled an encapsulated molecular structure type ring system which was only possible with large, floppy and flexible structures.

The strength of a hydrogen bond may be gauged from the length of the intramolecular OH/O interaction, where a shorter distance between the hydrogen bond donor and acceptor reflected a stronger hydrogen bond.³ Theaflavin's ketone functional group; located in the benzotropolone moiety, was the central hub for strong intramolecular hydrogen bonding interactions (OH/C=O), in theaflavin. Adjacent to the ketone group was the hydroxyls i-OH and b-OH, both influencing the stability of theaflavin significantly, through highly stabilising hydrogen bonds. This was evident by their longer intra-atomic distance which shortened in the absence of the intramolecular OH/C=O interaction, as displayed in the higher energy conformers.

7.1.2.3 Intra-Atomic Distance

The hydrogen bond strength was associated with the length between the hydroxyl group atoms (O-H) where a more extended length reflected a stronger hydrogen bond.³ The elongation of the bond length entailed the participation of the hydroxyl group in hydrogen bonding. A longer bond length reduced the hydrogen bond distance between the H-donor and H-acceptor, thus, forming stronger H-bond interactions. The longest hydroxyl intra-atomic distance belonged to the i-O-H group with a bond length of 1.01 Å, followed by the b-O-H hydroxyl bond length of 0.98 Å. Thus, in both gas and the aqueous solvent, the strongest hydrogen bond formation involved the hydroxyl group i-OH as the hydrogen bond donor and the C=O oxygen atom as the hydrogen bond acceptor (i-OH/C=O). The subsequent strongest hydrogen bond was the b-OH hydroxyl group as the hydrogen bond donor and the ketone oxygen atom as the hydrogen bond acceptor (b-OH/C=O).

The predicted strength of the i-OH and b-OH hydrogen bond were supported by the vibrational spectra where the highest γ - out-of-plane torsion was the γ OⁱH peak, followed by the γ O^bH peak; in corroboration with the intra-atomic distance.⁴ However, a shortened intra-atomic distance of either the i-O-H or b-O-H hydroxyl group entailed a loss or weakened hydrogen bonding interaction. As a result, the

antioxidant's structure was destabilised, as seen in the highest energy conformers, specifically **1k**, **1l**, **1k-H₂O** and **1l-H₂O**.

In the solvent, theaflavin's hydrogen bonding interactions were strengthened, and thus, the conformers were more energetically stabilised. In particular, the intra-atomic distance of the ketone group in conformer **1k-H₂O** and the hydroxyl 7-O-H of the conformer **1a-H₂O**, **1b-H₂O**, **1f-H₂O** and **1g-H₂O** were 0.01 Å longer in the aqueous solvent than in the gas phase.

7.1.2.4 Ring Puckering

Puckering in the benzotropolone moiety reduced the stability of the antioxidant tea theaflavin. Ring puckering disrupted the planar structure of the B ring, consequently, reducing the extensive π -electron delocalisation of the structure. The deformation occurred to reduce the steric hindrance between the C=O and b-O atom, and the C=O and i-O atom caused by the ζ_5 and ζ_6 dihedral angle rotation. The destabilising effect caused by the steric hindrance was highlighted on the energetics of the mid, and highest energy conformers. Consequently, the distortion of the planarity in the benzotropolone ring significantly impacted the stability of the structure.

7.1.2.5 Formation of Pseudo Rings

Theaflavin was stabilised by the formation of pseudo rings. The pseudo rings were formed by the vast intramolecular interactions adopted in theaflavin, namely the CH/O, OH/O, OH/C, H/H and CH/C intramolecular interactions. These interactions formed meta-stable five-membered, six-membered and also eight-membered rings, thus, creating a vast electronic network within the antioxidant structure and stabilising the molecule. The stabilising effects of the pseudo rings were evidenced in the strong hydrogen bonds of the benzotropolone moiety where the strongest i-OH/C=O and second strongest b-OH/C=O intramolecular interaction formed meta-stable six-membered rings and the h-OH/i-O interaction formed a pseudo-five-membered ring. Furthermore, the γ O³H peak and γ O³H peak illustrated that the

formation of meta-stable six-membered rings stabilised the hydroxyl group, resulting in a blue shift in the vibrational spectra and a redshift when the pseudo ring formation was absent.

7.1.2.6 The Angle of Hydrogen Bond Formation

The angle of the hydrogen bond formation was essential in establishing a strong H-bond interaction.⁵ This was apparent in the vibrational spectra of the γ O⁷H peak corresponding to the 7-OH/7'-O intramolecular hydrogen bond interaction, for conformers **1a** and **1l**. The peak at 649 cm⁻¹ belonged to the γ O⁷H hydroxyl group of conformer **1a** whilst the γ O⁷H peak for conformer **1l** was much lower (367 cm⁻¹). The vast difference in the peak position can be partly owed to the bad angle for hydrogen bond formation and the slight increase in the intramolecular H-bond distance for conformer **1l**. Also, the red-shift of the γ O⁷H peak shown by conformer **1l** reaffirmed the weak OH/O interaction present. The γ O⁷H peak for both conformers represented the free OH out-of-plane torsion. Both conformers had identical intra-atomic distance length, yet, the peaks for conformer **1a** was at 406 cm⁻¹ and for conformer **1l** at 334 cm⁻¹. The variation in the peaks displayed in the 7-OH/7'-O interaction was due to the angle of hydrogen bond formation and the increase in intramolecular H-bond distance.

7.1.2.7 Vibrational Spectra

Vibrational spectra analysis in the region of 150 - 900 cm⁻¹ allowed for the characterisation of the hydrogen bonding interactions. A δ - in-plane scissoring vibrational motion indicated the presence of a hydrogen bond, a low vibrational γ - out-of-plane torsion peak indicated a free H-bond and a high γ - out-of-plane torsion peak was an indication of a strong hydrogen bond presence.^{4, 6} The peaks were influenced by the hydroxyl group O-H intra-atomic distance, the intramolecular interaction distance, meta-stable ring formation and the extent of π -electron delocalisation. These factors influenced the stability and the strength of the hydrogen bonds where a stronger hydrogen bond resulted in a blue-shift on the

vibrational spectra and a weakened hydrogen bond resulted in a red-shift. The furthest red-shifted γ - out-of-plane torsion peaks were from free hydroxyls not involved in hydrogen bonding interactions, as shown in the γ O⁵H and γ O⁵H peaks.

7.1.2.8 Antioxidant Radical Tea Theaflavin

Theaflavin is a large and flexible molecule with numerous possible radical scavenging sites. Theaflavin had 17 possible hydrogen atom abstraction and seven possible hydrogen atom addition sites.

The most preferred site of the radical scavenging mechanism hydrogen atom abstraction was either in the vicinity of the benzotropolone moiety or in close proximity. Specifically, the preferred hydrogen atom abstraction in the conformers of theaflavin was either the 2-H site, in the C ring, or the h-O-H site, in the B' ring, for both the gas and aqueous solvent phase. H-abstraction at these sites yielded the most energetically stable antioxidant radical. The extensive π -electron delocalisation from the B, and B' ring stabilised the antioxidant radical tea theaflavin by the abundant resonance structures formed.

The B ring was the most stable of hydrogen atom addition site, in all 12 conformers investigated, in both the gas and aqueous solvent. Specifically, H-addition to the e-C site produced the most stable antioxidant radical, and subsequently, followed by the H-addition to the c-C site, also in the B ring. H-addition to the benzotropolone moiety stabilised the antioxidant radical tea theaflavin through the extensive charge delocalisation of the B and B' ring.

From the results obtained, the benzotropolone moiety consisting of the B and B' rings are an essential aspect for the energetic stability of the antioxidant radical tea theaflavin formed, post-radical capture. The benzotropolone moiety characteristic to black tea is thought to underlie its superior ability to neutralise ROSs through the extensive electronic network formed - making theaflavin an overall proficient facilitator to electron-spin stabilisation.

7.1.3 The Continuation – Future Work

To further aid in the structure-activity relationship database of antioxidant tea theaflavin, the following should be investigated:

7.1.3.1 Large & Flexible Antioxidants

Theaflavin's antioxidant activity arose from its large and flexible structure. The intramolecular interactions formed produced an electronic cage like system which stabilised the neutral conformer and stabilised the antioxidant radical. Therefore, a comparison of other antioxidants with numerous free rotations in its structure will help affirm a trend between an antioxidant's activity and large and flexible structures.

7.1.3.2 Environment

The stability of the antioxidant was essential to retard chain reactions from damaging the biological system. To understand the dynamics of theaflavin to the changing environment, theaflavin conformers were studied in the gas and aqueous solvent. Further studies, including the use of explicit solvent particles and important biological ions, are valid aspects to be explored. Yet are much more complex determinations due to a large number of configurations or 'poses' available to the theaflavin and H₂O molecules.

7.1.3.3 Theaflavin Dimers

In this study, a database was created for the monomer conformers of theaflavin, to understand the stability of the individual conformational isomers. The study established that the conformers are stabilised via numerous intra-molecular interaction. To build the database further, the study of antioxidant dimer formations should be modelled and analysed. Theaflavin conformers may be further stabilised through inter-molecular interactions by forming OH/O interactions possibly with the free hydroxyl groups (5'-OH and 5-OH in the A' and A ring respectively) and π/π interactions through the ring system.^{1, 2, 7}

7.1.3.4 The Synergy between Theory and Experiment

To aid the current findings of the antioxidant radical structures generated by hydrogen atom addition (HAD), avoided level crossing muon spin resonance spectroscopy ALC- μ SR, can be employed to verify the radical scavenging site. Thus, building on to the structure-activity relationship of the antioxidant radical in tea theaflavin. The theory of the experimental technique was discussed below.

Muon Spin Spectroscopy

Oxidative stress has been implemented as the cause of various degenerate diseases and ageing. The protective effects of antioxidants from oxidative stress, through their interaction with reactive species, is thus vital to the maintenance of one's health. Therefore, the stability of an antioxidant pre and post-radical capture is essential to deter further damage to biological systems. And thus, the conformation of the antioxidant plays an important role. It is necessary to determine the antioxidant's post-radical structure, dynamics and energetics to comprehend the importance of the antioxidant mechanism involved. The study of free radicals is somewhat tricky due to their highly reactive nature. Electron spin resonance (ESR) is the principle spectroscopy technique employed to investigate the formation of radical species.⁸ An alternative spectroscopy technique is μ SR which is based on the positron muon μ^+ . μ SR spectroscopy can be used to study free radicals as the muon behaves like a light proton and can be substituted into molecules.⁹ The short lifetime of the muon makes it suitable for the study of fast chemical reactions and species that are short-lived. An advantage of μ SR over the commonly used ESR technique is the primary radical product of the Mu addition reaction is static thus, do not undergo rearrangement.⁹ The rearrangement of radicals results in simultaneous detection of generated secondary products resulting in the harder identification of radical-capture site. Therefore, μ SR is an excellent tool for eliciting the critical atomic position on the antioxidant via the preferred and specific site of radical addition.

Muon (μ)

A muon (μ) is an elementary particle discovered in 1937 by physicists Anderson and Neddermeyer.¹⁰ The muon has a mass of which is ~ 200 times heavier than an electron and ~ 9 times lighter than a proton and carry a charge (positive μ^+ or negative μ^-).^{11, 12} Muons are unstable, having a lifetime of $2.2 \mu\text{s}$ before decaying into a positron (e^+) and two neutrinos (ν_e and ν_μ).¹¹ It has a spin of $\frac{1}{2}$ and a magnetic moment more significant than a proton.^{11, 12}

The characteristic spin and charge make muons very sensitive to their local environment and an extremely sensitive probe of magnetism. Its features also give them the ability to mimic H-atoms making muon spectroscopy an excellent candidate for the study of material science, chemistry and physics.

Muon Spin Rotation, Relaxation or Resonance (μ SR) Spectroscopy

μ S spectroscopy is commonly referred to as μ SR: rotation, relaxation or resonance and describes the muon spin in the study. This is dependent on the sample investigated and also determines the muon beam criteria (high energy, low energy or surface).

Muon Production

Figure 7.1 Schematic of muon production involving a high energy proton impacting an intermediate target (graphite) resulting in the production and decay of a pion.

Muon production (Figure 7.1) for experiments requires protons that have been accelerated to high energies to collide with the nuclei of an intermediate target such as graphite, a light element.¹¹ The proton interacts with the subatomic particles (proton/ neutron) in the nuclei producing positive pions (π^+):

$$p + p_1 \rightarrow \pi^+ + p + n$$

$$p + n_1 \rightarrow \pi^+ + p + n$$

Where p = proton from beam, p_1 = proton from target, π^+ = pion, n = neutron and n_1 = neutron of target.

Pions (π^+) are unstable and have a mean lifetime of 26 ns before they decay into a muon and a muon neutrino:¹¹

$$\pi^+ \rightarrow \mu^+ + \nu_\mu$$

Where π^+ = pion, μ^+ = positive muon and ν_μ = muon neutrino.

Parity violation in the weak interaction of pion decay creates only left-handed neutrinos (ν_μ). Pions are spinless, therefore, at rest, both the emitted muon neutrino and positive muon helicity are anti-parallel (Figure 7.2).¹³ This characteristic provides spin-polarised muon beams, where the muon spin (S_μ) is antiparallel to the muon linear momentum (P_μ).

Depending on the momentum of the pion, a variety of muon beams are available: high energy, surface and low energy beams. High energy muon beams are a result of high energy pions escaping the intermediate target towards a long superconducting solenoid to decay. The muons produced are 80% polarised and are high in energy, thereby, requiring fairly large samples but suited to study samples that need to be contained.¹¹ The surface muon beams are produced in the surface

layer of the intermediate target.^{11, 12} It is a 100% polarised muon beam, as the pions decay at rest, generating low momentum muons.¹⁴ The resulting muons can thus be applied to fairly small samples. Low energy muon beams are attained by adding moderators to a surface beam. The reduction in energy of the muon beam facilitates analysis of surface and thin-film samples.¹⁵

Figure 7.2 An illustration of the decay of a pion at rest and the products produced. π^+ = pion, μ^+ = muon, ν_{μ} = muon neutrino, S_{π} = pion spin, S_{μ} = muon spin and S_{ν} = neutrino spin, P_{μ} = muon linear momentum and P_{ν} = neutrino linear momentum. Reproduced from S. F. J. Cox¹¹

As the production of muon beams require a high energy particle accelerator, there are only a few facilities in the world for this instrumentation: TRIUMF in Vancouver Canada,¹⁶ Paul Scherrer Institut (PSI) in Switzerland,¹⁷ Japan-Proton Accelerator Research Complex (J-PARC) in Japan¹⁸ and ISIS at the RAL in the UK.¹⁹

Avoided Level Crossing Muon Spin Resonance Spectroscopy (ALC- μ SR)

Muon Spin (μ S) spectroscopy is an experimental technique based on the implantation of muons into a sample target where the influence of the sample's environment on the polarisation of the implanted muon is determined.

The muon decay:

$$\mu^+ \rightarrow e^+ + \nu_e + \bar{\nu}_{\mu}$$

Where μ^+ = positive muon, e^+ = positron, ν_e = electron neutrino and $\bar{\nu}_{\mu}$ = muon neutrino.

The positive muon has a mean lifetime of 2.2 μs before decaying into a positron (e^+), and two neutrinos (ν_e and $\bar{\nu}_\mu$).^{11, 12} The positron (e^+) during the muon decay is detected. The direction of the positron emission is anisotropic concerning the muon spin (S_μ) and tends to be emitted along the direction of μ^+ spin.¹³ The interaction between the muon and the sample's environment is provided through analysis of the positron (e^+).

In this type of μSR experiment, an avoided level crossing muon resonance takes place when the muon Zeeman splitting matches the Zeeman splitting of a neighbouring spin system, by tuning the magnetic field. Here, the muons become depolarised in a flip-flop transition with its neighbour via their hyperfine interactions.¹¹ This resonance can be detected in the spectrum. Analysis of the hyperfine coupling provides information on the formation of the antioxidant radical by H-addition (location and environment).

Muonium (μ^+e^-)

Figure 7.3 The muonium $\equiv$ positive muon and an electron (μ^+e^-). The electron is orbiting the muon.

ALC- μSR will be used to determine the stability of the antioxidant radical through means of quantifying the influence of the antioxidants electronic and magnetic properties, reactivity and functionality at the atomic scale. To examine the properties of hydrogen atom addition to the antioxidant, the muonium (μ^+e^-) as a light hydrogen isotope will be exploited (Figure 7.3).^{20, 21} Muoniums are formed when a positive muon (μ^+) picks up an electron resulting in a one-electron atom orbiting the positive muon as a nucleus (Figure 7.3). They behave chemically like a proton (H^+) and so are used in the study of chemical reaction mechanisms.

Muoniums are very reactive as they contain an unpaired electron and can bind to a molecule resulting in a spin-labelled radical.²⁰ They readily add to double bonds resulting in the formation of free radicals, similarly to H-addition. Thus, the μ SR technique can inspect the reactivity of hydrogen atoms, in such materials. Muonium addition has been studied for various organic compounds including polyaromatic hydrocarbons (PAH): fluoranthene²² and triphenylene,²³ in the liquid state. From the experimental results conducted, muonium addition to the unsaturated secondary carbons of PAH was preferable; addition to the unsaturated tertiary carbon takes place only in the absence of unsaturated secondary carbons. μ SR spectroscopy has been successful in elucidating the structure and dynamics of compounds in various environments, but to the best of my knowledge, this experimental technique has not been used to exploit the role of antioxidants. This will be the first study on the antioxidant tea using the muon as a probe.

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APPENDIX

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Table A 1 512 possible conformers of theaflavin. A', C' B', B, C, A represent the rings in theaflavin, $\zeta 1$ - $\zeta 9$ = dihedral angle of the hydroxyl side chains where s = syn, a = anti and $\phi 1$ and $\psi 2$ = C-C rotamers.

CONFORMATIONS											
Dihedral angle - syn (s), $\pm$ gauche (+/-) and anti (a).											
No.	A'		C'	B'		B	C	A		$\phi 1$	$\psi 2$
	$\zeta 1$	$\zeta 2$	$\zeta 3$	$\zeta 4$	$\zeta 5$	$\zeta 6$	$\zeta 7$	$\zeta 8$	$\zeta 9$		
1	s	s	s	s	s	s	s	s	s	180	180
2	a	s	s	s	s	s	s	s	s	180	180
3	s	a	s	s	s	s	s	s	s	180	180
4	s	s	a	s	s	s	s	s	s	180	180
5	s	s	s	a	s	s	s	s	s	180	180
6	s	s	s	s	a	s	s	s	s	180	180
7	s	s	s	s	s	a	s	s	s	180	180
8	s	s	s	s	s	s	a	s	s	180	180
9	s	s	s	s	s	s	s	a	s	180	180
10	s	s	s	s	s	s	s	s	a	180	180
11	a	a	s	s	s	s	s	s	s	180	180
12	a	s	a	s	s	s	s	s	s	180	180
13	a	s	s	a	s	s	s	s	s	180	180
14	a	s	s	s	a	s	s	s	s	180	180
15	a	s	s	s	s	a	s	s	s	180	180
16	a	s	s	s	s	s	a	s	s	180	180
17	a	s	s	s	s	s	s	a	s	180	180
18	a	s	s	s	s	s	s	s	a	180	180
19	s	a	a	s	s	s	s	s	s	180	180
20	s	a	s	a	s	s	s	s	s	180	180
21	s	a	s	s	a	s	s	s	s	180	180
22	s	a	s	s	s	a	s	s	s	180	180
23	s	a	s	s	s	s	a	s	s	180	180
24	s	a	s	s	s	s	s	a	s	180	180
25	s	a	s	s	s	s	s	s	a	180	180
26	s	s	a	a	s	s	s	s	s	180	180
27	s	s	a	s	a	s	s	s	s	180	180
28	s	s	a	s	s	a	s	s	s	180	180
29	s	s	a	s	s	s	a	s	s	180	180
30	s	s	a	s	s	s	s	a	s	180	180
31	s	s	a	s	s	s	s	s	a	180	180
32	s	s	s	a	a	s	s	s	s	180	180
33	s	s	s	a	s	a	s	s	s	180	180
34	s	s	s	a	s	s	a	s	s	180	180
35	s	s	s	a	s	s	s	a	s	180	180
36	s	s	s	a	s	s	s	s	a	180	180
37	s	s	s	s	a	a	s	s	s	180	180
38	s	s	s	s	a	s	a	s	s	180	180

39	s	s	s	s	a	s	s	a	s	180	180
40	s	s	s	s	a	s	s	s	a	180	180
41	s	s	s	s	s	a	a	s	s	180	180
42	s	s	s	s	s	a	s	a	s	180	180
43	s	s	s	s	s	a	s	s	a	180	180
44	s	s	s	s	s	s	a	a	s	180	180
45	s	s	s	s	s	s	a	s	a	180	180
46	s	s	s	s	s	s	s	a	a	180	180
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353	s	a	s	a	s	a	a	a	s	180	180
354	s	a	s	a	s	a	a	s	a	180	180
355	s	a	s	a	s	a	s	a	a	180	180
356	s	a	s	a	s	s	a	a	a	180	180
357	s	a	s	s	a	a	a	a	s	180	180
358	s	a	s	s	a	a	a	s	a	180	180
359	s	a	s	s	a	a	s	a	a	180	180
360	s	a	s	s	a	s	a	a	a	180	180
361	s	a	s	s	s	a	a	a	a	180	180
362	s	s	a	a	a	a	a	s	s	180	180
363	s	s	a	a	a	a	s	a	s	180	180
364	s	s	a	a	a	a	s	s	a	180	180
365	s	s	a	a	a	s	a	a	s	180	180
366	s	s	a	a	a	s	a	s	a	180	180
367	s	s	a	a	a	s	s	a	a	180	180
368	s	s	a	a	s	a	a	a	s	180	180
369	s	s	a	a	s	a	a	s	a	180	180
370	s	s	a	a	s	a	s	a	a	180	180
371	s	s	a	a	s	s	a	a	a	180	180
372	s	s	a	s	a	a	a	a	s	180	180
373	s	s	a	s	a	a	a	s	a	180	180
374	s	s	a	s	a	a	s	a	a	180	180

375	s	s	a	s	a	s	a	a	a	180	180
376	s	s	a	s	s	a	a	a	a	180	180
377	s	s	s	a	a	a	a	a	s	180	180
378	s	s	s	a	a	a	a	s	a	180	180
379	s	s	s	a	a	a	s	a	a	180	180
380	s	s	s	a	a	s	a	a	a	180	180
381	s	s	s	a	s	a	a	a	a	180	180
382	s	s	s	s	a	a	a	a	a	180	180
383	a	a	a	a	a	a	s	s	s	180	180
384	a	a	a	a	a	s	a	s	s	180	180
385	a	a	a	a	a	s	s	a	s	180	180
386	a	a	a	a	a	s	s	s	a	180	180
387	a	a	a	a	s	a	a	s	s	180	180
388	a	a	a	a	s	a	s	a	s	180	180
389	a	a	a	a	s	a	s	s	a	180	180
390	a	a	a	a	s	s	a	a	s	180	180
391	a	a	a	a	s	s	a	s	a	180	180
392	a	a	a	a	s	s	s	a	a	180	180
393	a	a	a	s	a	a	a	s	s	180	180
394	a	a	a	s	a	a	s	a	s	180	180
395	a	a	a	s	a	a	s	s	a	180	180
396	a	a	a	s	a	s	a	a	s	180	180
397	a	a	a	s	a	s	a	s	a	180	180
398	a	a	a	s	a	s	s	a	a	180	180
399	a	a	a	s	s	a	a	a	s	180	180
400	a	a	a	s	s	a	a	s	a	180	180
401	a	a	a	s	s	a	s	a	a	180	180
402	a	a	a	s	s	s	a	a	a	180	180
403	a	a	s	a	a	a	a	s	s	180	180
404	a	a	s	a	a	a	s	a	s	180	180
405	a	a	s	a	a	a	s	s	a	180	180
406	a	a	s	a	a	s	a	a	s	180	180
407	a	a	s	a	a	s	a	s	a	180	180
408	a	a	s	a	a	s	s	a	a	180	180
409	a	a	s	a	s	a	a	a	s	180	180
410	a	a	s	a	s	a	a	s	a	180	180
411	a	a	s	a	s	a	s	a	a	180	180
412	a	a	s	a	s	s	a	a	a	180	180
413	a	a	s	s	a	a	a	a	s	180	180
414	a	a	s	s	a	a	a	s	a	180	180
415	a	a	s	s	a	a	s	a	a	180	180
416	a	a	s	s	a	s	a	a	a	180	180
417	a	a	s	s	s	a	a	a	a	180	180
418	a	s	a	a	a	a	a	s	s	180	180
419	a	s	a	a	a	a	s	a	s	180	180
420	a	s	a	a	a	a	s	s	a	180	180
421	a	s	a	a	a	s	a	a	s	180	180
422	a	s	a	a	a	s	a	s	a	180	180
423	a	s	a	a	a	s	s	a	a	180	180
424	a	s	a	a	s	a	a	a	s	180	180
425	a	s	a	a	s	a	a	s	a	180	180
426	a	s	a	a	s	a	s	a	a	180	180
427	a	s	a	a	s	s	a	a	a	180	180
428	a	s	a	s	a	a	a	a	s	180	180
429	a	s	a	s	a	a	a	s	a	180	180
430	a	s	a	s	a	a	s	a	a	180	180

431	a	s	a	s	a	s	a	a	a	180	180
432	a	s	a	s	s	a	a	a	a	180	180
433	a	s	s	a	a	a	a	a	s	180	180
434	a	s	s	a	a	a	a	s	a	180	180
435	a	s	s	a	a	a	s	a	a	180	180
436	a	s	s	a	a	s	a	a	a	180	180
437	a	s	s	a	s	a	a	a	a	180	180
438	a	s	s	s	a	a	a	a	a	180	180
439	s	a	a	a	a	a	a	s	s	180	180
440	s	a	a	a	a	a	s	a	s	180	180
441	s	a	a	a	a	a	s	s	a	180	180
442	s	a	a	a	a	s	a	a	s	180	180
443	s	a	a	a	a	s	a	s	a	180	180
444	s	a	a	a	a	s	s	a	a	180	180
445	s	a	a	a	s	a	a	a	s	180	180
446	s	a	a	a	s	a	a	s	a	180	180
447	s	a	a	a	s	a	s	a	a	180	180
448	s	a	a	a	s	s	a	a	a	180	180
449	s	a	a	s	a	a	a	a	s	180	180
450	s	a	a	s	a	a	a	s	a	180	180
451	s	a	a	s	a	a	s	a	a	180	180
452	s	a	a	s	a	s	a	a	a	180	180
453	s	a	a	s	s	a	a	a	a	180	180
454	s	a	s	a	a	a	a	a	s	180	180
455	s	a	s	a	a	a	a	s	a	180	180
456	s	a	s	a	a	a	s	a	a	180	180
457	s	a	s	a	a	s	a	a	a	180	180
458	s	a	s	a	s	a	a	a	a	180	180
459	s	a	s	s	a	a	a	a	a	180	180
460	s	s	a	a	a	a	a	a	s	180	180
461	s	s	a	a	a	a	a	s	a	180	180
462	s	s	a	a	a	a	s	a	a	180	180
463	s	s	a	a	a	s	a	a	a	180	180
464	s	s	a	a	s	a	a	a	a	180	180
465	s	s	a	s	a	a	a	a	a	180	180
466	s	s	s	a	a	a	a	a	a	180	180
467	a	a	a	a	a	a	a	s	s	180	180
468	a	a	a	a	a	a	s	a	s	180	180
469	a	a	a	a	a	a	s	s	a	180	180
470	a	a	a	a	a	s	a	a	s	180	180
471	a	a	a	a	a	s	a	s	a	180	180
472	a	a	a	a	a	s	s	a	a	180	180
473	a	a	a	a	s	a	a	a	s	180	180
474	a	a	a	a	s	a	a	s	a	180	180
475	a	a	a	a	s	a	s	a	a	180	180
476	a	a	a	a	s	s	a	a	a	180	180
477	a	a	a	s	a	a	a	a	s	180	180
478	a	a	a	s	a	a	a	s	a	180	180
479	a	a	a	s	a	a	s	a	a	180	180
480	a	a	a	s	a	s	a	a	a	180	180
481	a	a	a	s	s	a	a	a	a	180	180
482	a	a	s	a	a	a	a	a	s	180	180
483	a	a	s	a	a	a	a	s	a	180	180
484	a	a	s	a	a	a	s	a	a	180	180
485	a	a	s	a	a	s	a	a	a	180	180
486	a	a	s	a	s	a	a	a	a	180	180

487	a	a	s	s	a	a	a	a	a	180	180
488	a	s	a	a	a	a	a	a	s	180	180
489	a	s	a	a	a	a	a	s	a	180	180
490	a	s	a	a	a	a	s	a	a	180	180
491	a	s	a	a	a	s	a	a	a	180	180
492	a	s	a	a	s	a	a	a	a	180	180
493	a	s	a	s	a	a	a	a	a	180	180
494	a	s	s	a	a	a	a	a	a	180	180
495	s	a	a	a	a	a	a	a	s	180	180
496	s	a	a	a	a	a	a	s	a	180	180
497	s	a	a	a	a	a	s	a	a	180	180
498	s	a	a	a	a	s	a	a	a	180	180
499	s	a	a	a	s	a	a	a	a	180	180
500	s	a	a	s	a	a	a	a	a	180	180
501	s	a	s	a	a	a	a	a	a	180	180
502	s	s	a	a	a	a	a	a	a	180	180
503	a	a	a	a	a	a	a	a	s	180	180
504	a	a	a	a	a	a	a	s	a	180	180
505	a	a	a	a	a	a	s	a	a	180	180
506	a	a	a	a	a	s	a	a	a	180	180
507	a	a	a	a	s	a	a	a	a	180	180
508	a	a	a	s	a	a	a	a	a	180	180
509	a	a	s	a	a	a	a	a	a	180	180
510	a	s	a	a	a	a	a	a	a	180	180
511	s	a	a	a	a	a	a	a	a	180	180
512	a	a	a	a	a	a	a	a	a	180	180

Appendix

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Table A 2 Relative energies of the 153 optimised conformers of theaflavin with its corresponding numerical dihedral angle at the B3LYP/6-31G(d,p) level of theory, in the gas phase. A', C', B', B, C, A represent the rings in theaflavin, ζ_1 - ζ_9 = dihedral angle of the hydroxyl side chains, ϕ_1 and ψ_2 = C-C rotamers and R. E = Relative Energy in kJ mol⁻¹.

OPTIMISED CONFORMATIONS - B3LYP/6-31G(d,p)													
Dihedral angle - syn (s), $\pm$ gauche (+/-) and anti (a)													
	A'		C'	B'		B	C	A		ϕ_1	ψ_2	Hartrees	R. E kJ mol ⁻¹
	ζ_1	ζ_2	ζ_3	ζ_4	ζ_5	ζ_6	ζ_7	ζ_8	ζ_9				
<i>as-ass-as</i>	-179.1	16.1	-37.3	-179.4	0.1	0.6	-74.0	-177.9	11.0	-159.8	91.1	-2021.81	0.00
<i>sa-ass-as</i>	0.3	170.5	-35.6	-178.9	0.5	1.1	-75.7	-178.0	14.7	-157.8	87.0	-2021.80	1.80
<i>as-ass-aa</i>	-177.9	1.8	-38.7	-179.5	0.8	0.7	-64.3	-177.8	-179.7	-164.3	-92.9	-2021.80	6.68
<i>as-ass-sa</i>	-177.7	5.4	-39.2	-179.7	0.4	0.5	-55.1	1.6	-179.7	-164.3	-89.9	-2021.80	9.98
<i>aa-ass-ss</i>	-178.3	179.0	-37.4	-179.5	0.9	0.7	-57.2	0.6	1.1	-166.6	-92.8	-2021.80	10.07
<i>asaass-as</i>	-179.6	17.0	-174.2	-179.6	0.2	0.6	-73.1	-177.9	11.0	-158.0	91.8	-2021.80	10.08
<i>aaaass-as</i>	-178.6	169.1	-173.7	-179.1	0.6	1.0	-75.1	-178.2	13.1	-156.1	87.6	-2021.80	10.91
<i>aa-ass-sa</i>	-178.3	178.7	-37.2	-179.4	0.9	0.7	-55.3	0.4	-179.8	-166.3	-92.2	-2021.80	11.14
<i>aa-ass-as</i>	-177.9	178.9	-37.8	179.9	-0.4	0.0	-79.6	-177.8	6.2	-159.0	102.4	-2021.80	11.68
<i>sa-ass-sa</i>	-2.0	179.1	-35.5	-179.5	0.9	0.7	-56.0	1.3	-179.7	-166.9	-92.1	-2021.80	12.99
<i>saaass-as</i>	6.1	170.6	-178.7	-179.2	0.6	1.1	-75.4	-178.0	14.7	-155.6	87.2	-2021.80	15.14
<i>aaaass-ss</i>	-179.1	169.9	-173.3	-179.1	0.7	1.0	-67.5	4.7	11.8	-156.0	89.6	-2021.80	15.36
<i>as-assass</i>	-178.0	4.8	-39.8	-179.2	0.9	0.6	179.6	7.5	1.3	-161.9	-98.6	-2021.80	18.17
<i>aa-assass</i>	-178.5	179.1	-37.3	-179.5	1.1	0.3	177.8	5.4	1.7	-166.8	-94.6	-2021.80	18.78
<i>as-assasa</i>	-178.0	4.8	-39.7	-179.1	0.9	0.6	178.8	6.8	-179.9	-161.7	-98.7	-2021.80	19.13
<i>s--assaaa</i>	-3.2	-137.6	-36.3	179.9	-0.7	0.8	-172.1	-177.6	-179.2	-160.1	87.7	-2021.80	21.34
<i>ss-assass</i>	0.1	5.3	-37.3	-179.4	0.7	0.6	-179.6	8.7	1.3	-162.8	-97.6	-2021.80	22.53
<i>ss-ass-aa</i>	-13.4	2.5	-52.5	-177.0	1.9	2.5	-71.1	-170.7	164.5	-123.0	89.7	-2021.79	28.48
<i>aa-sss-as</i>	-178.0	-179.4	-39.5	1.2	1.1	1.4	-68.9	-177.5	0.5	-161.5	-98.9	-2021.79	32.44
<i>ss-ass-as</i>	-0.5	-25.0	-47.5	-176.6	3.1	2.6	-68.6	-174.1	-9.7	-143.7	91.4	-2021.79	32.49
<i>ss-ass-sa</i>	-0.7	-18.7	-42.4	-177.6	2.3	2.1	-62.4	-3.8	176.0	-153.1	95.4	-2021.79	33.79
<i>as-ass-ss</i>	-177.9	-25.4	-53.7	-176.7	3.3	2.5	-62.9	-4.6	-11.0	-146.6	93.3	-2021.79	34.88
<i>aa-sss-sa</i>	-178.5	179.0	-38.4	1.2	1.0	0.9	-54.4	0.2	-179.9	-164.3	-92.0	-2021.79	35.10

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aa-sss-aa	-178.0	-179.6	-39.4	1.2	1.1	1.4	-66.3	-177.1	-180.0	-161.4	-98.3	-2021.79	35.52
sa-assass	2.4	167.8	-36.6	-179.2	0.6	0.8	168.5	-7.6	18.7	-157.5	103.7	-2021.79	36.20
ss-assaas	-1.1	-24.9	-46.2	-176.5	3.1	2.6	175.1	-173.0	-8.8	-142.4	92.3	-2021.79	36.39
ss-assasa	2.0	-11.7	-47.8	-177.2	2.0	2.3	168.3	-5.0	166.1	-144.7	96.2	-2021.79	36.90
aa-sssaaas	-178.2	-179.4	-39.5	1.0	1.1	1.0	-174.3	-177.5	1.3	-161.8	-98.9	-2021.79	36.97
sa-sss-aa	0.4	-179.5	-37.4	1.9	0.7	1.9	-65.3	-178.5	-179.8	-159.7	68.4	-2021.79	38.09
as-sssaaa	-178.3	1.7	-41.0	0.7	1.0	1.1	-173.4	-177.7	-180.0	-160.2	-99.6	-2021.79	39.84
ss-ass-ss	-0.1	2.9	57.0	179.4	-1.1	-1.9	-50.1	2.9	7.4	71.6	114.7	-2021.79	40.43
aa-sssasa	-178.8	179.4	-38.4	0.8	1.3	0.4	176.7	4.8	179.7	-164.5	-95.1	-2021.79	42.58
aa-sssass	-178.8	179.5	-38.5	0.9	1.2	0.5	177.4	5.1	1.8	-164.9	-95.1	-2021.79	42.67
ss-sssaaa	0.8	1.9	-38.8	1.0	1.0	1.1	-172.9	-178.1	-179.8	-160.2	-99.8	-2021.79	44.14
sa-asa-as	0.0	170.9	-35.0	-179.5	-1.9	179.2	-74.7	-177.7	15.4	-157.3	89.9	-2021.79	45.18
sa-sssasa	-1.0	179.4	-36.6	1.1	1.2	0.4	177.6	5.7	179.8	-165.0	-94.7	-2021.79	45.26
as-asa-aa	-177.1	-8.5	-38.8	-178.9	-1.2	179.2	-74.9	-176.2	178.1	-158.1	75.3	-2021.79	48.91
as-sss-aa	-172.3	-4.8	-64.2	1.0	1.1	2.9	-70.4	-174.7	167.1	-137.3	90.5	-2021.79	49.03
sa-sss-as	1.3	169.1	-39.5	1.4	0.7	1.2	-71.5	176.3	19.4	-155.2	103.5	-2021.79	50.18
as-sss-sa	-177.9	-19.9	-49.5	1.6	1.9	2.5	-62.0	-4.4	179.6	-151.0	95.7	-2021.79	50.49
as-sss-as	-175.8	-24.3	-56.4	1.5	2.6	3.0	-67.8	-176.8	-10.1	-144.6	93.1	-2021.79	50.56
as-asa-sa	-177.7	4.6	-39.5	-179.5	-1.4	178.6	-65.5	2.2	-179.2	-160.4	-101.0	-2021.79	50.68
as-sss-ss	177.8	12.2	-39.8	0.8	0.2	0.3	-64.8	-3.5	16.8	-160.2	103.2	-2021.79	51.74
ss-asa-aa	-1.1	-7.9	-36.7	-179.0	-1.4	179.1	-75.8	-175.3	177.2	-157.9	74.8	-2021.79	51.86
ss-sss-aa	-10.0	0.2	-54.2	1.1	0.9	3.1	-70.3	-173.4	164.6	-129.8	90.3	-2021.79	51.94
as-asa-as	-179.2	13.7	-38.8	-178.3	-2.2	179.7	-43.0	-178.5	-8.5	-151.7	158.2	-2021.79	52.40
asaasa-as	-179.4	17.4	-174.9	-179.9	-1.4	179.9	-71.8	-177.7	11.3	-157.3	94.5	-2021.78	53.58
sa-sssaaas	0.8	169.1	-38.6	1.3	0.5	1.2	176.0	177.1	20.1	-155.0	103.9	-2021.78	53.93
as-sssaaas	-175.4	-24.4	-54.7	1.3	2.5	3.1	175.9	-175.4	-9.1	-143.4	94.0	-2021.78	54.43
aaaasa-as	-178.5	169.3	-174.4	-179.6	-1.6	179.3	-73.9	-178.0	13.6	-155.4	90.6	-2021.78	54.54
ss-asa-sa	0.1	5.0	-37.3	-179.6	-1.5	178.5	-66.5	3.1	-179.1	-160.6	-101.3	-2021.78	54.73
as-sssasa	-178.4	-15.2	-53.0	1.2	1.5	2.9	168.2	-9.3	172.5	-146.0	96.3	-2021.78	55.06
sa-asa-aa	-0.5	-179.8	-35.4	-179.1	-1.4	178.6	-65.4	-178.1	179.6	-161.2	68.3	-2021.78	55.67
ss-sss-as	0.2	-25.7	-49.5	1.8	2.3	3.2	-68.2	-174.4	-9.7	-142.9	92.4	-2021.78	55.76
aa-asa-aa	-178.4	-179.7	-37.2	-179.0	-1.3	178.7	-63.8	-178.8	-180.0	-161.3	67.5	-2021.78	55.98
asaass-ss	178.3	-15.4	174.5	-177.3	1.7	2.6	-61.1	-3.3	-15.3	-164.3	75.9	-2021.78	56.06
as-sssass	178.1	12.5	-38.9	0.7	0.0	0.3	169.0	-9.7	17.4	-159.9	103.9	-2021.78	57.11
sa-sss-s+	-13.2	-176.8	-43.0	0.3	-1.9	0.5	-68.2	-0.5	146.8	-149.3	102.3	-2021.78	57.20
asaasa-ss	-179.8	17.1	-174.6	-179.9	-1.2	179.7	-55.1	1.7	9.2	-157.7	100.3	-2021.78	57.42
as-asaass	-177.9	4.4	-39.2	-179.4	-1.4	178.2	178.2	7.7	0.2	-160.0	-103.0	-2021.78	58.03
ssaass-sa	-10.9	-10.0	167.9	-177.4	1.8	2.6	-62.7	-1.2	-177.7	-164.0	84.7	-2021.78	58.18

Appendix

<i>ssaass-as</i>	-10.2	-14.2	166.5	-177.2	1.7	2.6	-67.0	-179.9	-14.7	-163.2	76.5	-2021.78	58.33
<i>as-asa-ss</i>	-179.2	13.3	-39.2	-178.3	-2.2	179.7	-40.6	-2.4	-8.9	-151.9	160.0	-2021.78	58.43
<i>saaasa-as</i>	5.5	170.9	-179.6	-179.7	-1.7	179.2	-74.4	-177.7	15.4	-155.0	90.1	-2021.78	58.65
<i>aa-asa-sa</i>	-178.3	-178.0	-37.4	-179.1	-1.3	178.5	-60.6	1.4	-179.7	-162.1	68.0	-2021.78	58.84
<i>aaaasa-ss</i>	-179.0	170.2	-174.0	-179.6	-1.5	179.2	-66.2	4.4	12.6	-155.6	92.1	-2021.78	59.61
<i>ss-sssaas</i>	-0.4	-25.2	-48.3	1.6	2.2	3.3	175.3	-173.3	-8.8	-142.0	93.5	-2021.78	59.83
<i>ssaasa-as</i>	4.0	17.6	179.9	-180.0	-1.5	179.7	-72.9	-177.5	12.8	-157.0	94.5	-2021.78	60.23
<i>sa-sssass</i>	3.3	169.4	-39.4	1.4	0.6	1.1	168.5	-7.0	18.8	-155.4	104.5	-2021.78	60.82
<i>s--sss-sa</i>	-3.0	-140.8	-37.8	1.4	-0.2	0.5	-62.0	-2.5	-179.2	-160.8	101.4	-2021.78	61.41
<i>as-sas-as</i>	-179.8	14.9	-38.8	-2.4	176.3	4.9	-74.0	-177.9	12.1	-159.8	94.1	-2021.78	66.15
<i>aa-asa--s</i>	179.4	165.8	-38.6	-179.8	-1.1	178.6	-69.2	-3.9	18.6	-158.1	103.5	-2021.78	70.30
<i>ss-asa-as</i>	-1.2	-24.0	-45.1	-178.1	-1.5	177.2	-74.0	-173.4	-8.7	-144.5	94.2	-2021.78	71.82
<i>sa-sas-ss</i>	1.1	173.6	-37.7	-0.8	176.8	4.9	-67.4	5.0	14.3	-157.2	92.4	-2021.78	71.95
<i>sa-asa-ss</i>	2.4	167.8	-36.3	-179.8	-1.1	178.6	-69.7	-2.2	19.2	-158.5	103.6	-2021.78	72.60
<i>aa-sas-as</i>	-178.4	-178.9	-39.2	2.4	-176.1	-3.1	-65.9	-177.1	0.9	-161.9	-96.9	-2021.78	72.78
<i>saaasa-ss</i>	2.3	-160.0	179.4	-179.3	-2.3	179.6	-40.0	-2.6	-2.0	-153.4	174.1	-2021.78	73.91
<i>as-ssa-aa</i>	-178.0	2.0	-40.8	0.5	-2.7	177.7	-72.9	-177.9	-179.2	-158.3	-105.5	-2021.78	74.54
<i>aa-ssa-as</i>	-177.7	-179.8	-39.7	0.9	-2.5	176.9	-76.0	-177.9	0.0	-159.2	-107.5	-2021.78	74.54
<i>ss-asaaas</i>	-1.7	-23.8	-44.4	-177.8	-1.4	178.7	178.5	-172.6	-7.9	-142.9	96.0	-2021.78	74.76
<i>as-ssaaas</i>	-178.1	1.9	-40.3	0.5	-2.7	177.4	-175.3	-178.1	0.5	-158.5	-105.0	-2021.78	75.08
<i>ssaasa-ss</i>	6.1	-2.3	-179.1	-179.4	-2.2	-179.2	-40.7	-2.6	-9.8	-152.1	168.0	-2021.78	75.14
<i>aa-sas-aa</i>	-178.9	-178.9	-39.4	-0.6	177.6	4.3	-61.7	-179.3	-178.8	-159.1	72.2	-2021.78	76.79
<i>asasss-as</i>	178.2	-14.1	168.2	1.6	1.1	3.3	-66.2	-179.9	-15.2	-162.8	77.9	-2021.78	77.34
<i>asasss-sa</i>	178.1	-10.0	169.4	1.7	1.3	3.2	-62.0	-1.7	-175.9	-163.7	86.1	-2021.78	77.52
<i>sa-sas-aa</i>	1.2	-179.2	-37.7	0.1	177.6	4.5	-63.4	-178.5	-179.3	-158.7	72.6	-2021.78	78.59
<i>ss-ssa-aa</i>	0.6	2.2	-38.6	0.8	-2.8	177.5	-73.5	-178.2	-179.0	-158.2	-106.1	-2021.77	79.66
<i>sa+sss-sa</i>	-1.1	174.1	56.1	2.8	2.2	3.0	-67.0	5.6	159.1	-100.0	94.6	-2021.77	79.69
<i>ss-ssaaas</i>	0.5	2.1	-38.2	0.8	-2.7	177.3	-174.8	-178.5	0.4	-158.5	-105.0	-2021.77	79.88
<i>aa-ssa-aa</i>	-178.6	-179.5	-38.6	1.3	-2.6	178.8	-63.0	-178.9	-179.7	-159.5	70.5	-2021.77	81.29
<i>aa-sas-ss</i>	-179.7	179.5	-40.0	-1.7	176.7	3.9	-72.0	4.9	6.4	-158.9	106.5	-2021.77	81.41
<i>sa-ssa-aa</i>	0.4	-179.8	-37.0	1.6	-2.7	178.6	-64.6	-178.2	179.8	-159.1	70.9	-2021.77	81.50
<i>asasss-ss</i>	177.8	-15.0	169.4	1.8	1.0	3.3	-60.6	-2.8	-15.5	-164.4	77.3	-2021.77	82.04
<i>saasas-as</i>	7.6	172.5	-172.6	7.7	178.8	4.2	-74.2	-178.1	15.6	-153.2	91.2	-2021.77	82.25
<i>as-ssaasa</i>	-178.2	3.6	-40.6	0.2	-3.0	178.3	175.7	6.1	-179.6	-157.8	-103.7	-2021.77	82.56
<i>as-ssa-ss</i>	-179.5	12.8	-40.6	0.7	-3.3	179.4	-40.3	-2.2	-8.4	-151.9	161.1	-2021.77	82.71
<i>as-ssaaas</i>	-178.1	3.5	-40.8	0.3	-2.9	177.8	176.5	6.6	0.7	-157.9	-104.6	-2021.77	82.74
<i>ss+sss-ss</i>	-4.8	-22.7	57.4	2.2	2.8	2.9	-66.0	-0.9	-6.8	-111.2	94.8	-2021.77	82.77
<i>aa-ssa-sa</i>	-178.5	-178.0	-38.8	1.4	-2.6	178.6	-59.8	1.5	-179.5	-160.3	71.4	-2021.77	82.92

Appendix

<i>ssasas-as</i>	5.8	15.9	-172.7	5.8	178.0	4.5	-74.0	-177.7	14.1	-156.3	94.7	-2021.77	84.09
<i>aa-ssaass</i>	-178.1	179.7	-39.7	0.6	-2.4	177.8	175.0	6.0	0.9	-159.9	-103.0	-2021.77	84.09
<i>aa-sasass</i>	-179.0	-180.0	-38.9	1.4	-176.1	-3.8	177.9	5.3	1.8	-163.9	-95.9	-2021.77	85.24
<i>as-sas-aa</i>	176.9	-0.3	-40.6	-1.6	176.4	5.7	-38.5	-177.7	-179.0	-157.3	178.3	-2021.77	85.31
<i>ssasss-ss</i>	-9.8	-7.0	167.3	-1.1	-3.2	-3.0	-48.1	0.9	9.3	76.1	103.2	-2021.77	85.34
<i>as-ssa-sa</i>	179.1	-0.4	-40.3	0.7	-3.4	179.3	-38.5	-2.0	-178.3	-156.3	-176.6	-2021.77	86.37
<i>as-sasass</i>	-178.3	3.3	-41.1	-1.8	176.8	4.8	178.2	6.6	1.2	-160.0	-100.7	-2021.77	86.41
<i>ss-ssaasa</i>	0.8	3.9	-38.3	0.5	-3.0	178.2	176.4	7.0	-179.5	-158.0	-103.8	-2021.77	87.35
<i>ssassa-as</i>	4.5	17.3	-175.4	2.8	-3.0	179.5	-73.7	-177.5	13.9	-154.9	96.4	-2021.77	87.69
<i>asasssass</i>	177.7	-16.0	170.0	1.9	1.6	3.3	168.1	-8.0	-16.0	-161.4	83.4	-2021.77	87.92
<i>as-sas-sa</i>	-178.6	-20.9	-49.1	-1.0	176.8	5.8	-60.3	-4.1	179.2	-152.1	96.5	-2021.77	88.38
<i>ss+ssasasa</i>	-3.5	-9.6	55.1	2.0	1.2	3.3	166.1	-5.2	-178.1	-161.0	88.5	-2021.77	89.77
<i>as-ssa-as</i>	-175.5	-23.5	-51.5	0.9	-2.7	177.1	-73.6	-175.6	-8.7	-146.0	96.0	-2021.77	90.61
<i>s--sasasa</i>	-2.5	-137.2	-37.8	1.2	177.1	5.2	-179.9	8.1	-179.3	-159.2	94.0	-2021.77	90.61
<i>sa-sas-as</i>	0.7	170.6	-38.8	0.5	176.6	5.9	-68.6	175.4	20.5	-158.8	102.5	-2021.77	90.92
<i>ss-sas-aa</i>	-10.0	-1.4	-51.5	-2.1	175.5	7.0	-68.1	-172.8	164.4	-135.2	92.5	-2021.77	91.47
<i>ss-sasass</i>	1.2	3.6	-38.9	-0.9	176.9	4.9	179.0	7.5	1.2	-160.1	-100.6	-2021.77	92.01
<i>sa-ssa-as</i>	0.5	170.1	-38.7	0.9	-3.2	178.4	-75.1	176.6	21.3	-156.1	104.9	-2021.77	92.56
<i>sa-sas-sa</i>	26.4	179.9	-52.4	-2.4	176.8	4.8	-61.4	10.1	177.3	-82.2	97.4	-2021.77	94.50
<i>ss-sas-as</i>	0.9	-24.3	-52.9	-0.4	-178.4	-0.5	-65.6	-175.8	-9.5	-139.1	92.4	-2021.77	94.70
<i>as-sas-ss</i>	-178.7	-25.2	-59.6	-0.8	-178.2	-1.1	-60.5	-3.6	-10.8	-141.6	94.1	-2021.77	95.37
<i>ss-sasaaa</i>	-11.0	-0.1	-50.2	-3.0	175.2	7.3	177.2	-171.0	163.9	-133.9	93.8	-2021.77	95.57
<i>ss-sas-sa</i>	8.3	-4.1	-58.5	-1.5	175.8	6.4	-64.8	6.6	159.8	-138.3	98.4	-2021.77	96.08
<i>saassa-as</i>	4.8	-171.2	-176.2	4.0	-3.3	179.3	-42.4	-179.7	-4.9	-150.4	171.2	-2021.77	96.22
<i>aa-ssa-ss</i>	179.0	168.5	-41.5	0.7	-2.9	178.3	-69.4	-3.5	19.4	-156.6	104.5	-2021.77	96.35
<i>sa-ssaaaa</i>	-20.7	178.2	-48.8	0.9	-3.0	178.7	178.8	-164.3	176.1	-89.9	96.9	-2021.77	96.96
<i>ss-ssa-sa</i>	-0.6	-17.3	-43.3	1.2	-3.4	177.2	-65.9	-3.9	178.1	-152.8	97.7	-2021.77	97.79
<i>sa-ssa-ss</i>	3.4	170.3	-39.4	1.0	-2.9	178.2	-70.0	-1.5	20.0	-156.7	104.4	-2021.77	98.93
<i>ss-sasaas</i>	-0.1	-27.2	-47.8	-2.3	176.4	6.3	175.6	-173.0	-8.9	-143.5	96.1	-2021.77	99.59
<i>sa-ssaass</i>	3.1	172.2	-40.1	0.8	-3.7	179.7	170.9	-8.1	20.8	-154.5	108.4	-2021.77	102.21
<i>ss-sas-ss</i>	1.5	-24.0	-52.7	0.2	-177.9	-1.3	-60.8	-2.9	-10.9	-141.9	95.0	-2021.77	102.84
<i>sa-sasass</i>	4.1	171.7	-39.2	0.5	176.5	6.2	168.7	-5.3	20.1	-158.5	104.6	-2021.77	102.89
<i>as-saa-as</i>	-179.8	15.3	-38.0	1.0	177.9	177.5	-73.4	-177.6	12.7	-161.1	98.7	-2021.76	105.74
<i>sa-saa-as</i>	0.2	172.0	-36.0	2.2	178.0	177.0	-74.4	-177.8	15.3	-159.6	95.2	-2021.76	107.56
<i>ss-ssaass</i>	-1.6	-24.1	-46.4	1.3	-2.5	178.3	170.4	-10.0	-8.4	-145.1	100.7	-2021.76	108.92
<i>as-saa-aa</i>	-177.7	-11.7	-39.4	1.5	177.3	176.6	-73.7	-176.4	177.5	-159.9	81.9	-2021.76	109.41
<i>as-saa-ss</i>	180.0	12.6	-39.1	1.0	177.4	178.4	-40.2	-1.8	-5.2	-156.7	153.5	-2021.76	112.36
<i>ss-saa-aa</i>	-0.8	-11.1	-37.6	2.3	177.5	176.5	-74.9	-175.4	176.6	-159.6	81.1	-2021.76	112.59

Appendix

<i>aa-saa-aa</i>	-178.3	-178.6	-38.5	1.9	177.6	176.7	-57.0	-178.5	-178.5	-160.1	78.8	-2021.76	115.19
<i>sa-saa-aa</i>	0.9	-179.0	-36.8	2.8	177.8	176.6	-58.9	-177.9	-179.1	-159.7	77.9	-2021.76	116.37
<i>asasaa-as</i>	179.8	16.2	-169.3	6.8	178.9	177.4	-72.4	-177.7	12.7	-158.6	99.5	-2021.76	117.37
<i>ss-saa-ss</i>	-0.9	12.3	-37.2	1.8	177.6	178.1	-40.7	-1.8	-4.9	-156.8	155.1	-2021.76	117.74
<i>aa-saa-ss</i>	-177.4	-178.0	-39.5	2.2	178.2	176.4	-41.5	0.8	1.3	-160.8	-158.8	-2021.76	118.54
<i>as-saa-sa</i>	178.5	-0.7	-39.7	1.3	177.6	177.8	-37.6	-1.7	-177.9	-158.6	178.2	-2021.76	119.90
<i>as-saaass</i>	-178.4	2.5	-40.4	0.8	177.7	174.9	173.1	5.6	0.8	-160.1	-107.2	-2021.76	119.99
<i>ss-saa-sa</i>	2.7	1.0	-39.4	1.8	178.1	176.5	-41.9	0.7	-178.5	-160.2	-156.7	-2021.76	121.08
<i>asasas-ss</i>	177.7	-14.9	168.8	-2.5	174.4	7.6	-59.0	-2.1	-15.5	-173.1	75.2	-2021.76	121.96
<i>asassa-ss</i>	177.9	-14.2	170.5	1.5	-3.9	178.0	-64.1	-3.7	-15.3	-166.1	83.7	-2021.76	122.28
<i>saasaa-as</i>	6.4	172.1	-172.7	9.2	179.1	176.9	-74.2	-177.9	15.5	-156.7	95.4	-2021.76	123.70
<i>ssasaa-as</i>	4.3	16.3	-174.0	8.6	179.2	177.2	-73.6	-177.5	14.7	-158.2	98.8	-2021.76	124.64
<i>ssasaa-ss</i>	5.1	16.1	-173.3	9.0	179.5	177.3	-47.7	2.1	11.1	-158.8	109.8	-2021.76	128.69
<i>ss-saa-as</i>	-0.7	-26.7	-42.9	1.5	177.6	175.1	-72.3	-173.6	-8.9	-151.2	94.8	-2021.76	128.94
<i>ss-saaaas</i>	-1.1	-26.5	-42.5	0.6	177.1	176.2	178.7	-172.7	-8.1	-149.3	97.7	-2021.75	132.66
<i>sa-saa-ss</i>	3.9	173.7	-38.6	4.4	179.6	175.2	-69.7	0.1	22.2	-161.4	103.3	-2021.75	133.79

Appendix

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Table A 3 Relative energies of the 153 optimised conformers of theaflavin with its corresponding numerical dihedral angle at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. A', C', B', B, C, A represent the rings in theaflavin, ζ_1 - ζ_9 = dihedral angle of the hydroxyl side chains, ϕ_1 and ψ_2 = C-C rotamers and R. E = Relative Energy in kJ mol⁻¹.

OPTIMISED CONFORMATIONS - B3LYP/6-31G(d,p)													
Dihedral angle - syn (s), $\pm$ gauche (+/-) and anti (a)													
	A'		C'	B'		B	C	A		ϕ_1	ψ_2	Hartrees	R. E kJ mol ⁻¹
	ζ_1	ζ_2	ζ_3	ζ_4	ζ_5	ζ_6	ζ_7	ζ_8	ζ_9				
as-ass-as	-179.3	16.3	-38.9	-179.5	0.2	0.5	-66.8	-177.8	10.8	-159.1	92.8	-2021.83	0.00
as-ass-aa	-178.6	0.6	-39.2	-179.6	0.8	0.4	-68.4	-178.3	-179.6	-164.8	-95.3	-2021.83	0.12
sa-ass-as	2.7	166.0	-38.1	-178.9	0.9	0.6	-66.8	-178.2	13.0	-158.5	92.7	-2021.83	2.63
asaass-as	-179.2	17.2	178.0	-179.4	0.3	0.5	-66.5	-177.8	10.8	-158.7	92.9	-2021.83	3.59
aaaass-as	-178.6	166.1	178.2	-178.9	1.0	0.6	-67.8	-178.1	12.9	-157.8	91.8	-2021.83	3.65
as-ass-sa	-178.6	0.6	-39.2	-179.5	0.8	0.4	-64.7	2.3	-179.6	-164.7	-95.2	-2021.83	4.45
aa-ass-sa	-178.4	-178.9	-38.7	-179.5	0.9	0.4	-64.7	2.3	-179.6	-165.4	-95.0	-2021.83	5.26
aa-ass-as	-177.4	-179.6	-39.8	179.5	-0.6	-0.2	-73.5	-177.4	4.7	-161.7	102.5	-2021.83	5.95
as-assasa	-178.9	1.1	-39.4	-179.5	0.9	0.4	171.9	4.0	-179.6	-163.9	-97.3	-2021.83	6.01
aa-ass-ss	-178.4	-178.9	-38.8	-179.6	0.9	0.4	-65.1	2.5	1.0	-165.5	-94.6	-2021.83	6.02
aa-assass	-178.5	-178.9	-38.9	-179.6	0.9	0.3	171.9	4.1	1.1	-165.6	-96.0	-2021.83	6.29
as-assass	-178.9	1.1	-39.3	-179.6	0.9	0.3	172.0	4.4	1.2	-164.4	-96.9	-2021.83	6.85
saaass-as	4.1	166.6	175.4	-178.9	1.0	0.6	-67.9	-178.1	13.1	-157.9	91.7	-2021.83	7.23
aaaass-ss	-178.6	166.2	178.2	-178.9	1.0	0.6	-62.9	4.0	12.4	-157.7	93.1	-2021.83	7.88
sa-ass-sa	1.4	-179.0	-37.7	-179.5	0.9	0.4	-64.7	2.2	-179.6	-165.8	-95.0	-2021.83	8.61
ss-assass	3.1	1.1	-38.2	-179.6	0.9	0.3	172.0	4.4	1.2	-164.6	-96.8	-2021.83	10.08
s--assaaa	0.9	-133.7	-37.7	-179.9	-0.6	0.6	176.2	-177.1	178.4	-159.8	86.1	-2021.83	14.88
aa-sss-as	-178.5	-178.9	-39.4	-0.8	0.8	0.4	-68.6	-178.3	1.0	-164.7	-95.1	-2021.83	15.18
aa-sss-aa	-178.5	-178.9	-39.3	-0.8	0.8	0.4	-68.2	-178.3	-179.7	-164.6	-95.2	-2021.83	15.60
aa-sssas	-178.9	-179.6	-39.5	-0.4	0.8	0.4	176.5	-178.1	1.2	-164.0	-96.4	-2021.83	15.85
as-sssaaa	-179.0	1.0	-40.0	-0.4	0.8	0.5	176.5	-178.0	-179.6	-163.3	-96.8	-2021.83	16.38
aa-sssasa	-178.8	-178.9	-39.6	-0.7	0.9	0.5	172.0	3.7	-179.7	-164.1	-96.8	-2021.83	18.90
aa-sss-sa	-178.4	-178.9	-39.2	-0.5	0.9	0.5	-64.4	2.3	-179.7	-164.5	-95.0	-2021.83	19.35
ss-sssaaa	3.1	1.0	-38.9	-0.4	0.8	0.5	176.5	-178.1	-179.7	-163.5	-96.8	-2021.83	19.40
aa-sssass	-178.8	-179.0	-39.6	-0.7	0.9	0.4	172.0	4.1	1.3	-164.3	-96.4	-2021.83	19.48

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sa-sssasa	2.0	-178.9	-38.5	-0.7	0.9	0.5	172.0	3.7	-179.7	-164.5	-96.6	-2021.82	24.03
sa-sss-aa	1.5	-179.0	-38.6	0.9	1.0	1.6	-66.7	-178.0	-179.3	-159.5	70.1	-2021.82	25.14
as-asa-as	-179.2	16.8	-38.9	-179.7	-1.6	-180.0	-66.4	-177.8	11.7	-158.3	95.0	-2021.82	28.19
ss-ass-aa	-24.9	1.1	-54.0	-177.3	2.2	1.8	-73.6	-163.9	179.2	-90.0	87.8	-2021.82	30.75
sa-asa-as	2.6	166.3	-37.9	-179.2	-0.9	179.8	-66.4	-178.2	13.5	-158.0	94.8	-2021.82	31.15
as-asa-ss	-179.3	16.7	-38.7	-179.8	-1.5	180.0	-60.7	3.9	10.9	-158.6	96.1	-2021.82	31.63
asaasa-as	-179.1	17.7	177.7	-179.7	-1.3	180.0	-66.3	-177.7	11.5	-157.9	95.0	-2021.82	31.96
as-ass-ss	-178.5	-28.9	-51.0	-176.7	3.4	2.0	-70.1	-2.7	-8.5	-145.7	93.8	-2021.82	32.14
ss-ass-as	3.5	-28.5	-47.7	-176.7	3.4	2.1	-72.1	179.7	-8.3	-145.7	93.5	-2021.82	32.49
aaaasa-as	-178.6	166.3	178.3	-179.2	-0.7	179.8	-66.8	-178.1	13.2	-157.3	94.3	-2021.82	32.73
sa-assass	3.1	166.1	-37.8	-179.2	0.7	0.5	165.2	-5.7	21.2	-159.2	101.5	-2021.82	33.07
ss-assaas	3.5	-29.0	-47.8	-176.6	3.3	2.1	167.4	179.7	-8.0	-145.0	94.1	-2021.82	33.61
as-asa-sa	-178.6	1.2	-40.0	-179.5	-0.9	179.7	-66.7	3.8	-179.4	-161.1	-99.5	-2021.82	34.15
aa-asa-aa	-179.1	-178.9	-38.7	-178.9	-1.1	179.7	-66.3	-178.3	-179.3	-159.3	72.6	-2021.82	34.66
as-asa-aa	-178.5	-15.1	-39.5	-178.5	-0.5	180.0	-75.1	-177.4	-179.3	-155.9	80.3	-2021.82	35.09
ssaasa-as	2.2	17.6	174.5	-179.7	-1.3	180.0	-66.3	-177.8	11.6	-158.0	95.1	-2021.82	35.30
ss-ass-sa	3.4	-24.1	-42.2	-177.6	2.5	1.5	-68.5	-3.6	-177.3	-153.2	94.5	-2021.82	35.34
asaasa-ss	-179.3	17.5	177.9	-179.7	-1.2	180.0	-60.4	4.0	10.6	-158.2	96.2	-2021.82	35.48
as-asaass	-178.9	0.9	-39.4	-179.5	-0.8	179.7	172.0	4.9	0.6	-161.3	-100.8	-2021.82	35.80
sa-sss-ss	6.2	-179.9	-38.5	-0.5	-0.6	-1.3	-77.7	-4.5	5.5	-165.3	110.3	-2021.82	36.06
saaasa-as	4.1	166.8	175.4	-179.2	-0.7	179.8	-66.9	-178.1	13.3	-157.3	94.3	-2021.82	36.10
ss-assasa	3.2	-25.2	-43.2	-177.5	2.5	1.7	163.2	-4.2	-176.6	-152.3	95.4	-2021.82	36.66
aaaasa-ss	-178.6	166.5	178.2	-179.2	-0.7	179.8	-61.9	4.1	12.8	-157.3	95.2	-2021.82	36.79
ss+ass-ss	1.7	1.5	65.3	179.0	-1.4	-1.6	-63.3	4.3	7.3	71.8	112.5	-2021.82	37.31
ss-asa-sa	3.0	1.2	-39.0	-179.5	-0.9	179.6	-66.7	3.8	-179.4	-161.1	-99.5	-2021.82	37.67
ss-asa-aa	3.3	-15.1	-38.4	-178.5	-0.5	180.0	-75.2	-177.4	-179.5	-156.1	80.2	-2021.82	38.81
sa-asa-aa	1.4	-179.0	-37.9	-178.9	-1.1	179.7	-66.5	-178.1	-179.3	-159.6	72.0	-2021.82	38.92
aa-asa-sa	-179.1	-178.9	-38.7	-178.9	-1.1	179.7	-64.3	2.3	-179.3	-159.5	72.1	-2021.82	39.41
as-sss-ss	-179.1	12.7	-38.7	0.7	0.7	0.2	-71.3	-4.9	21.3	-160.2	101.9	-2021.82	39.93
as-sss-aa	-178.3	1.3	-64.0	1.7	1.8	2.2	-74.4	-179.8	163.7	-134.0	90.8	-2021.82	40.13
ss-sss-aa	-4.5	1.1	-62.8	2.0	1.8	2.2	-75.2	-177.4	170.0	-121.7	91.3	-2021.82	40.94
sa-sss-as	3.0	166.4	-39.5	0.7	1.1	0.6	-73.6	178.6	21.2	-157.8	101.9	-2021.82	41.12
as-sss-as	-178.5	-29.0	-52.8	2.4	3.1	2.4	-72.1	179.7	-8.0	-144.5	94.0	-2021.82	41.36
as-sssass	-179.2	12.6	-38.6	0.7	0.5	0.3	164.7	-5.7	21.7	-160.2	102.1	-2021.82	42.25
as-sssaas	-178.6	-29.6	-53.2	2.3	3.0	2.5	167.6	179.6	-7.7	-144.0	94.5	-2021.82	42.86
sa-sssaas	3.0	167.0	-39.5	0.6	0.8	0.7	168.7	178.6	21.7	-157.8	101.7	-2021.82	43.07
as-sss-sa	-178.1	-24.7	-46.5	1.8	2.5	1.8	-68.7	-3.7	-176.2	-151.7	95.3	-2021.82	43.68
aa-ssa-as	-178.6	-179.8	-40.3	-0.3	-2.0	179.6	-69.9	-177.9	0.6	-160.0	-100.0	-2021.82	44.38
ss-sss-as	3.4	-28.6	-49.4	2.4	3.1	2.4	-72.2	179.7	-8.0	-144.8	94.2	-2021.82	45.12
as-ssa-aa	-178.7	0.8	-40.5	-0.4	-1.9	179.6	-69.4	-177.8	-179.7	-160.0	-99.8	-2021.82	45.41

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as-sssasa	-178.3	-25.6	-47.6	1.7	2.4	2.0	163.7	-4.4	-175.5	-150.8	96.1	-2021.82	45.46
as-ssaaas	-178.8	0.7	-40.0	-0.1	-1.7	179.6	175.8	-177.7	0.6	-160.2	-101.0	-2021.82	45.85
ssaasa-ss	5.3	0.2	175.5	179.7	0.1	-179.5	-71.7	5.2	5.2	-160.0	101.8	-2021.82	45.90
ss-sssaas	3.5	-29.2	-49.5	2.4	3.0	2.5	167.7	179.7	-7.7	-144.2	94.7	-2021.82	46.42
sa-sssass	3.1	166.8	-39.3	0.7	0.8	0.7	165.2	-5.7	21.6	-158.1	102.0	-2021.82	47.01
as-ssa-ss	-179.3	16.7	-39.3	-0.7	-2.4	180.0	-60.8	4.1	11.4	-158.4	96.9	-2021.82	47.42
as-sas-as	-179.3	15.8	-38.9	-6.7	174.5	4.7	-66.8	-177.9	11.9	-160.3	95.9	-2021.82	48.16
aa-asa-ss	-178.3	-178.9	-40.0	-179.0	-2.2	179.5	-42.2	1.0	-6.4	-155.5	164.7	-2021.82	48.67
as-ssa-sa	-178.6	1.0	-40.7	-0.4	-2.0	179.4	-66.1	3.9	-179.6	-160.1	-99.1	-2021.82	48.84
ss-ssa-aa	3.2	0.9	-39.5	-0.4	-1.9	179.5	-69.4	-177.8	-179.7	-160.1	-99.8	-2021.82	48.97
ss-ssaaas	3.0	0.7	-38.9	-0.1	-1.7	179.6	175.8	-177.7	0.7	-160.4	-100.9	-2021.82	48.97
ssassa-as	2.1	17.4	174.4	-0.2	-2.0	179.9	-66.6	-177.8	11.9	-157.6	95.9	-2021.82	49.59
aa-ssa-aa	-178.3	-179.2	-39.7	0.5	-2.2	179.8	-68.1	-178.2	-179.3	-156.3	81.5	-2021.82	50.02
as-ssaass	-178.8	0.7	-40.1	-0.1	-1.8	179.6	171.4	5.2	0.6	-160.3	-101.0	-2021.82	50.19
saaasa-ss	4.5	-179.1	174.3	-179.2	-2.3	179.4	-42.4	0.8	-6.4	-154.0	161.1	-2021.82	50.19
aa-ssaass	-178.7	-179.8	-40.0	-0.2	-1.8	179.6	171.5	5.2	0.6	-160.3	-101.0	-2021.81	50.30
ssaass-as	-6.0	-14.3	164.3	-176.9	2.4	2.2	-71.6	179.0	-15.8	-159.3	83.1	-2021.81	50.53
s--sss-sa	-0.2	-136.6	-37.4	0.3	0.2	0.4	-69.4	-4.9	-179.6	-160.5	99.1	-2021.81	50.57
as-ssaasa	-178.8	0.8	-40.1	-0.2	-1.8	179.6	171.4	5.1	-179.5	-160.3	-101.0	-2021.81	50.77
asaass-ss	177.2	-14.8	167.1	-176.9	2.4	2.1	-69.4	-2.9	-15.9	-158.9	84.4	-2021.81	50.84
aa-ssa-sa	-179.2	-178.8	-39.2	0.8	-2.0	179.8	-63.9	2.2	-179.3	-158.7	74.7	-2021.81	50.93
aa-sas-as	-178.5	-179.0	-38.9	4.0	-174.8	-3.5	-67.8	-178.4	0.6	-163.4	-96.5	-2021.81	51.43
ssaass-sa	-5.6	-13.8	164.6	-177.2	2.2	2.0	-69.9	-4.0	-174.0	-161.8	86.5	-2021.81	53.40
as-sas-aa	-178.3	0.9	-40.0	-4.8	176.0	3.9	-66.5	-177.8	-179.3	-161.8	-99.0	-2021.81	53.41
sa-ssa-aa	3.1	-179.1	-38.7	0.6	-2.2	179.8	-68.7	-178.2	-179.3	-156.4	81.1	-2021.81	53.51
ss-ssaasa	3.2	0.9	-39.1	-0.2	-1.8	179.6	171.5	5.2	-179.5	-160.4	-101.1	-2021.81	54.00
ssasas-as	2.2	16.8	174.8	-4.7	175.6	4.0	-65.0	-178.1	11.9	-158.6	96.9	-2021.81	55.53
saasas-as	4.0	166.6	175.9	-6.3	175.2	4.3	-67.7	-177.6	14.6	-158.3	94.5	-2021.81	56.65
aa-sas-ss	-177.5	-179.6	-39.9	-7.8	175.1	3.1	-66.5	5.0	4.8	-163.2	106.1	-2021.81	56.77
sa-sas-ss	2.4	166.4	-38.3	-5.8	175.2	4.5	-61.0	4.5	13.5	-159.2	97.4	-2021.81	57.37
aa-sasass	-178.5	-179.1	-39.0	4.1	-174.8	-3.6	172.4	3.8	0.6	-163.5	-97.1	-2021.81	57.56
ss-asa-as	3.6	-28.6	-46.8	-177.9	-0.7	178.2	-74.8	179.9	-7.3	-146.0	95.4	-2021.81	58.10
as-sasass	-178.5	0.9	-40.3	-4.7	176.1	3.7	173.1	4.4	0.6	-162.0	-99.4	-2021.81	58.20
aa-sas-aa	-178.5	-177.9	-38.8	-6.0	174.7	4.9	-64.9	-178.5	-179.5	-159.4	83.3	-2021.81	58.66
ss-asaaas	3.6	-29.2	-46.8	-177.8	-0.6	178.3	167.9	179.8	-7.2	-145.4	96.0	-2021.81	59.23
sa-asa-ss	2.9	166.5	-37.7	-179.6	-0.7	179.3	-73.3	-5.2	21.7	-159.1	102.4	-2021.81	59.50
asasss-as	177.1	-14.4	167.1	2.0	1.8	2.6	-71.6	179.0	-15.5	-159.1	84.4	-2021.81	59.63
sa-sas-aa	3.2	-177.9	-37.8	-5.9	174.7	5.0	-65.4	-178.5	-179.5	-159.6	83.0	-2021.81	61.36
ss-sasass	2.7	0.9	-39.3	-4.4	176.1	3.8	173.1	4.6	0.6	-162.0	-99.5	-2021.81	62.63
asasss-sa	176.9	-13.8	167.5	2.0	1.8	2.5	-69.9	-4.1	-173.9	-161.6	87.3	-2021.81	64.04

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ss-sss-ss	-3.6	-23.1	63.0	1.5	3.0	2.2	-74.2	-3.6	-5.9	-117.6	95.5	-2021.81	64.83
sa-sss-sa	-0.9	176.7	65.1	2.0	2.7	2.2	-74.0	-2.7	155.8	-102.3	92.5	-2021.81	65.25
saassa-as	4.6	-179.1	174.0	0.9	-2.8	179.5	-43.2	-179.0	-6.2	-153.8	163.3	-2021.81	65.75
asasss-ss	178.6	-18.0	167.0	1.7	2.4	2.2	167.6	-4.6	-9.5	-134.9	95.5	-2021.81	66.32
ssasss-ss	-3.0	-10.5	164.2	-1.5	-3.1	-3.0	-49.8	3.9	9.7	73.1	111.1	-2021.81	66.52
as-ssa-as	-178.7	-29.0	-50.6	1.9	-1.3	178.3	-75.2	179.8	-7.0	-145.4	96.1	-2021.81	67.43
ss+sssasa	-4.6	-13.6	62.6	2.0	1.8	2.5	165.7	-4.4	-174.1	-161.3	87.2	-2021.81	67.71
s--sasasa	0.7	-134.4	-37.8	-5.6	174.5	4.9	172.2	4.4	178.5	-160.6	90.7	-2021.81	67.82
sa-ssa-as	2.7	168.2	-40.4	0.4	-1.9	179.4	-75.6	178.5	22.4	-157.3	103.5	-2021.81	68.61
aa-ssa-ss	-178.6	167.4	-41.8	0.4	-1.7	179.3	-73.8	-5.4	22.1	-157.7	103.3	-2021.81	69.05
sa-ssaaaa	-23.5	-178.6	-54.2	1.5	-2.2	179.0	170.5	-164.4	179.3	-92.1	94.3	-2021.81	71.39
a-ssss-ss	177.2	-14.8	167.0	2.0	1.8	2.6	-69.5	-3.0	-15.6	-158.6	85.7	-2021.81	72.30
as-saa-as	-179.4	16.4	-38.6	-2.7	174.6	178.3	-66.8	-177.9	13.0	-161.0	99.7	-2021.81	72.86
sa-ssa-ss	2.9	167.9	-39.9	0.4	-1.7	179.3	-73.8	-5.3	22.4	-157.8	103.4	-2021.81	72.93
ss-ssa-sa	2.9	-23.6	-44.3	1.1	-2.2	178.5	-71.1	-3.5	-175.2	-152.4	97.2	-2021.81	73.42
sa-ssaass	2.8	169.6	-41.5	0.4	-2.4	179.9	165.4	-6.3	23.1	-156.5	104.5	-2021.81	73.58
ss-saa-ss	0.3	16.5	-37.6	-1.9	174.8	178.2	-60.5	5.0	12.7	-161.4	101.1	-2021.80	76.50
sa-saa-as	1.9	166.3	-37.5	-3.2	174.6	178.1	-69.2	-177.1	16.0	-161.9	96.6	-2021.80	76.78
asasaa-as	-179.3	17.0	177.5	-2.8	174.5	178.3	-67.3	-177.8	12.8	-160.2	99.6	-2021.80	77.66
as-saa-ss	-179.5	16.6	-38.5	-1.9	174.8	178.2	-60.3	4.9	12.6	-161.1	101.3	-2021.80	77.96
sa-sas-as	2.7	166.4	-37.4	-8.0	174.0	4.9	-71.5	178.3	21.0	-162.6	101.5	-2021.80	78.68
as-saa-aa	-178.7	-17.2	-39.0	-6.9	173.4	177.6	-73.7	-177.4	179.8	-159.1	85.2	-2021.80	79.02
as-saa-sa	-178.3	0.9	-39.2	-4.6	174.2	177.6	-62.1	3.0	-179.4	-162.2	-103.0	-2021.80	79.46
+a-sas-sa	30.3	-179.1	-52.2	-4.3	175.2	5.2	-71.8	11.4	179.6	-83.8	96.0	-2021.80	79.59
aa-saa-ss	-178.2	-179.7	-39.1	-4.5	174.2	177.6	-62.3	2.9	0.6	-162.3	-102.9	-2021.80	79.90
ss-sas-as	3.3	-26.9	-52.0	0.2	-177.1	-1.5	-70.2	179.7	-8.2	-140.9	93.9	-2021.80	79.90
as-sas-sa	-178.3	-24.2	-44.8	-6.9	175.1	5.1	-67.4	-3.4	-177.0	-153.8	96.4	-2021.80	80.03
as-sas-ss	-178.6	-27.1	-55.2	0.2	-177.0	-1.6	-68.8	-2.5	-8.3	-140.9	94.4	-2021.80	80.05
ss-sas-sa	4.0	-0.2	-57.4	-4.8	173.9	5.9	-72.3	-3.1	167.3	-143.2	97.4	-2021.80	80.20
ss-sas-aa	1.1	2.8	-56.9	-3.5	174.9	5.9	-72.7	-178.2	161.3	-138.2	92.5	-2021.80	80.57
ss-saa-ss	0.3	16.5	-37.6	-1.9	174.8	178.2	-60.5	5.0	12.7	-161.4	101.1	-2021.80	80.71
ssasaa-as	1.4	16.9	174.5	-2.5	174.6	178.3	-67.6	-177.8	13.0	-160.3	99.3	-2021.80	80.72
ss-sasaaa	1.0	2.9	-57.7	-3.8	174.9	5.9	171.4	-178.7	161.7	-137.2	93.5	-2021.80	80.73
as-saaass	-178.4	1.0	-39.0	-5.7	174.2	177.3	172.5	4.3	0.5	-163.5	-103.8	-2021.80	80.98
ss-sasaas	3.1	-27.3	-52.2	0.3	-177.2	-1.3	168.4	179.7	-7.6	-140.5	94.2	-2021.80	81.26
saasaa-as	3.3	167.2	175.9	-3.1	174.6	178.1	-69.4	-177.2	15.8	-161.2	96.3	-2021.80	81.82
aa-saa-aa	-178.8	-177.9	-38.2	-4.6	174.0	177.8	-62.0	-178.4	-179.7	-160.7	89.5	-2021.80	82.40
sa-sasass	2.7	167.9	-37.7	-8.0	173.8	5.3	166.5	-5.8	21.4	-162.2	102.1	-2021.80	82.42
ss-saa-aa	2.9	-17.1	-38.0	-6.8	173.4	177.6	-74.0	-177.3	179.6	-159.2	85.1	-2021.80	82.85
ss-saa-sa	2.3	0.9	-38.3	-4.4	174.2	177.6	-62.2	2.9	-179.4	-162.2	-103.1	-2021.80	83.25

Appendix

sa-saa-aa	2.7	-177.5	-37.0	-4.5	174.3	177.9	-63.0	-178.2	-179.4	-162.3	89.2	-2021.80	83.93
ss-sas-ss	3.3	-26.8	-51.3	0.4	-177.0	-1.7	-68.8	-2.4	-8.5	-141.4	94.7	-2021.80	84.81
ssasaa-ss	1.4	17.3	174.6	-1.6	174.8	178.3	-60.3	5.0	12.5	-160.5	101.2	-2021.80	85.57
asassa-ss	177.1	-12.9	167.8	1.8	-2.7	178.7	-70.6	-2.7	-15.1	-161.9	86.4	-2021.80	91.90
asasas-ss	177.5	-13.5	168.9	-3.9	173.1	7.1	-67.8	-2.9	-14.3	-170.3	77.0	-2021.80	101.82
s--saa-as	3.2	-30.6	-42.3	-5.4	173.6	176.9	-74.5	179.9	-7.4	-153.6	96.8	-2021.80	102.01
s--saaaas	3.3	-31.0	-42.5	-5.7	173.4	176.8	169.7	180.0	-7.1	-152.7	97.8	-2021.80	102.42
sa-saa-ss	2.1	171.0	-38.3	-4.8	174.5	177.5	-74.6	-4.8	24.3	-163.2	103.4	-2021.79	103.54

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Table A 4 Tabulated intramolecular interactions of optimised theaflavin conformers **1a** – **1k** at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The intramolecular interaction distance is in Å. NA - no possible intramolecular interaction present.

Intramolecular H-Bond distance (Å)							
	7'-OH/7-O	3'-OH/1'-O	h-OH/i-O	i-OH/C=O	b-OH/C=O	3-OH/1-O	7-OH/7'-O
	i-OH/h-O ^a						
1a	NA	2.25	2.04	1.52	1.80	2.61	2.00
1b	NA	2.25	2.05	1.52	1.80	2.63	2.03
1c	NA	2.27	2.04	1.52	1.80	2.54	NA
1d	NA	2.28	2.04	1.52	1.80	2.42	NA
1e	NA	2.24	2.05	1.52	1.80	2.43	NA
1f	NA	NA	2.04	1.52	1.80	2.61	2.01
1g	NA	NA	2.05	1.52	1.80	2.64	2.03
1h	NA	2.25	2.05	1.52	1.80	2.41	NA
1i	NA	2.29	2.04	1.52	1.79	2.78	3.47
1j	NA	2.22	2.05	1.52	1.80	2.42	NA
1k	NA	NA	2.05	1.51	NA	2.25	NA
1l	NA	2.27	NA	2.07 ^a	NA	NA	2.23

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Table A 5 Tabulated intramolecular interactions of optimised theaflavin conformers **1a-H₂O** – **1l-H₂O** at the B3LYP/6-31G(d,p) level of theory, in an aqueous solvent. The intramolecular interaction distance is in Å. NA - no possible intramolecular interaction present.

Intramolecular H-Bond distance (Å)							
	7'-OH/7-O	3'-OH/1'-O	h-OH/i-O	i-OH/C=O	b-OH/C=O	3-OH/1-O	7-OH/7'-O
	i-OH/h-O ^a						
1a-H₂O	NA	2.26	2.06	1.51	1.80	2.57	1.97
1c-H₂O	NA	2.27	2.06	1.51	1.80	2.60	NA
1b-H₂O	NA	2.27	2.06	1.51	1.80	2.57	2.01
1f-H₂O	NA	NA	2.06	1.51	1.80	2.57	1.97
1g-H₂O	NA	NA	2.06	1.51	1.80	2.59	2.01
1d-H₂O	NA	2.28	2.06	1.52	1.80	2.55	NA
1h-H₂O	NA	2.27	2.06	1.52	1.80	2.55	NA
1i-H₂O	NA	2.31	2.06	1.52	1.80	2.72	3.51
1e-H₂O	NA	2.27	2.06	1.51	1.80	2.55	NA
1j-H₂O	NA	2.26	2.06	1.51	1.80	2.55	NA
1k-H₂O	NA	NA	2.07	1.50	NA	2.30	NA
1l-H₂O	NA	2.30	NA	2.05 ^a	NA	NA	2.20

Figure A 1 Vibrational spectra in the region 0 – 1500 cm⁻¹ for tea theaflavin conformers **1a** (as-ass-as), **1j** (sa-ass-sa), **1k** (saaasa-ss), and **1l** (sa-saa-ss). The theoretical results were performed at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The atoms description is according to the labelling system in Figure 6.1. s = syn, a = anti, - = gauche-; δ = in-plane deformation, γ = out-of-plane deformation and Δ = in-plane deformation of skeleton atoms.

Table A 6 Theoretically calculated wavenumbers in the region 0-1500 cm^{-1} for tea theaflavin conformers **1a** (as-ass-as), **1j** (sa-ass-sa), **1k** (saaasa-ss), and **1l** (sa-saa-ss) performed at the B3LYP/6-31G(d,p) level of theory, in the gas phase. The atoms description is according to the labelling system in Figure 6.1. s = syn, a = anti, - = gauche; ν = stretching, ν_s = symmetric stretching, ν_a = asymmetric stretching, δ = in-plane deformation, γ = out-of-plane deformation and Δ = in-plane deformation of skeleton atoms.

Theaflavin Conformers				Approximate Description
as-ass-as	sa-ass-sa	saaasa-ss	sa-saa-ss	
Theoretically Calculated Wavenumber (cm^{-1})				
1477	1491	1461	-	$\nu_a \text{ C}=\text{C}$ B ring
1456	1458	1456	1469	$\nu_a \text{ C}=\text{C}$ C ring
1448	1448	1454	1452	$\nu_a \text{ C}=\text{C}$ B' ring + $\delta \text{ O}^i\text{-H}$
1417	1426	1433	1416	$\nu_a \text{ C}=\text{C}$ B' ring
1405	1404	1409	1429	$\delta \text{ C}^{(c,a)}\text{H} + \delta \text{ O}^b\text{-H}$
1378	1379	1390	1381	$\delta \text{ C}^3\text{H} + \delta \text{ O}^3\text{-H}$
1330	1336	1342	1320	$\nu_s \text{ C}=\text{C}$ B' ring + $\delta \text{ C}^8\text{H}$
1319	1317	1324	1313	$\delta \text{ C}^{(2,3)}\text{H} + \gamma \text{ C}^4\text{-H}_2$
1312	1304	1309	1290	$\delta \text{ C}^5\text{H} + \delta \text{ O}^i\text{-H}$
1267	1268	1260	-	$\delta \text{ C}^5\text{H} + \delta \text{ O}^b\text{-H} + \nu \text{ C}^b\text{-O}$
1253	1241	1246	1258	$\delta \text{ C}^{(2,6)}\text{H} + \delta \text{ O}^7\text{-H}$
1220	1215	1214	1217	$\delta \text{ O}^{(5,7)}\text{-H}$
1194	1194	1199	1200	$\delta \text{ C}^{(g,2)}\text{H}$
1165	1175	1167	1155	$\gamma \text{ C}^4\text{H}_2 + \delta \text{ C}^3\text{H} + \delta \text{ O}^3\text{-H}$
1143	1120	1127	1126	$\delta \text{ C}^8\text{H} + \nu \text{ C}^7\text{-O}$
1122	1140	1138	1138	$\delta \text{ C}^{(6,8)}\text{H} + \delta \text{ O}^{5'}\text{-H}$
1088	1071	1069	1090	$\nu \text{ O}^1\text{-C}^2$
1072	1089	1054	1060	$\Delta \text{ CC}_{\text{B, B' ring}} + \delta \text{ O}^i\text{-H}$
1059	1060	1073	1037	$\nu \text{ C}^{3'}\text{-O} + \gamma \text{ C}^4\text{H}_2$
1039	1040	987	1028	$\nu \text{ C}^{5'}\text{-O} + \nu \text{ C}^{\text{Ba}'}\text{-O}^{1'} + \gamma \text{ C}^{\text{Ba}'}\text{H}$
1022	1020	992	1008	$\delta \text{ C}^{(6,8)}\text{H} + \nu \text{ C}^5\text{-O}$
987	988	1049	988	$\delta \text{ C}^6\text{C}^7\text{C}^8\text{ A' ring}$
924	910	915	892	$\gamma \text{ C}^8\text{H}$
898/ 884	891 / 875	862/ 849	898 ^a /864 ^c	$\gamma \text{ C}^{(c,a)}\text{H}$
873	875	880	877	$\delta \text{ C}^4\text{H}_2 \text{ C' ring}$

1039	1040	987	1028	$\nu \text{C}^{5'}\text{-O} + \nu \text{C}^{8\text{a}'}\text{-O}^{1'} + \gamma \text{C}^{4\text{a}'}\text{H}$
1022	1020	992	1008	$\delta \text{C}^{(6,8)}\text{H} + \nu \text{C}^{5'}\text{-O}$
987	988	1049	988	$\delta \text{C}^{6'}\text{C}^{7'}\text{C}^{8'}_{\text{A' ring}}$
924	910	915	892	$\gamma \text{C}^{\text{g}}\text{H}$
898/ 884	891 /875	862/ 849	898 ^e /864 ^c	$\gamma \text{C}^{(\text{c,e})}\text{H}$
873	875	880	877	$\delta \text{C}^{4'}\text{H}_2_{\text{C' ring}}$
862	861	895	456	$\gamma \text{O}^{\text{i}}\text{H}$
838	836	833	855	$\delta \text{C}^{2'}\text{C}^{3'}\text{C}^{4'}_{\text{C' ring}}$
814	818	819	842	$\delta \text{C}^{\text{c}}\text{C}^{\text{d}}\text{C}^{\text{e}}_{\text{B ring}}$
785	792	806	800	$\gamma \text{C}^{8'}\text{H}$
779	796	805	796	$\gamma \text{C}^{8'}\text{H}$
763	771	770	768	$\gamma \text{C}^{6'}\text{H}$
761	767	785	770	$\gamma \text{C}^{6'}\text{H}$
721	717	748	735	$\gamma \text{C}^{\text{j}}\text{C}^{\text{a}}\text{C}^{\text{b}}_{\text{B ring}}$
695	693	695	683	$\Delta \text{A ring} \& \text{C ring}$
679	674	-	435	$\gamma \text{O}^{\text{b}}\text{H}$
649	358	482	367	$\gamma \text{O}^{7'}\text{H}$
647	648	652	637	$\gamma \text{C}^{\text{h}}\text{C}^{\text{i}}\text{C}^{\text{j}}_{\text{B' ring}}$
583	582	580	580	$\Delta \text{CCC}_{\text{B',A', C' ring}}$
528	525	529	524	$\Delta \text{CCC}_{\text{C' ring}}$
508	509	509	502	$\Delta \text{CCC}_{\text{A' ring}}$
496	492	492	226	$\gamma \text{O}^{\text{h}}\text{H}$
489	489	-	-	$\delta \text{O}^{(\text{i,b})}\text{H}$
-	-	-	486	$\gamma \text{O}^{(7,7')}\text{H}$
465	467	490	342	$\delta \text{O}^{\text{j}}\text{H}$
462	463	468		$\Delta \text{CCC}_{\text{A,C ring}}$
420	433	273	423	$\gamma \text{O}^3\text{H}$
413	409	417	-	$\delta \text{O}^{\text{h}}\text{H}$
406	371	347	334	$\gamma \text{O}^{7'}\text{H}$
385	296	286	-	$\gamma \text{O}^5\text{H}$
344	328	337	291	$\gamma \text{O}^5\text{H}$
323	-	-	-	$\delta \text{O}^{7'}\text{H}$
300	310	449	420	$\gamma \text{O}^3\text{H}$
-	-	307	-	$\gamma \text{O}^{(7',5)}\text{H}$
279	284	-	189	$\delta \text{O}^3\text{H}$
-	188	171	-	$\delta \text{O}^3\text{H}$