Chapter - 3

Isolation and stereo chemical analysis of (2S, 3S) and (2S, 3R)-tetrahydro-3-hydroxy-5-oxo-2, 3 furan dicarboxylic acids
3.1 Introduction

The efficient construction of stereo chemically defined molecules is the fundamental challenge in organic synthesis. Synthesis of optically pure compounds has become focal point, in recent times, as the need for chiral drugs, natural products and fine chemicals is on the increase. In the interim several chiral molecules from chiral pool have been identified and employed in the synthesis of target molecule with excellent optical integrity. The \(\alpha\)-hydroxy citric acids are one of the select classes of molecules extensively used for synthesis of target molecule with mostly one or two chiral centers.

Considering the involvement of chiral \(\gamma\)-butyrolactone based molecules in various aspects of chirality, it is the objective of the present interest to utilize \((2S, 3S)\) and \((2S, 3R)\)-tetrahydro-3-hydroxy-5-oxo-2, 3 furan dicarboxylic acids (12 and 13) for the synthesis of chiral \(\gamma\)-butyrolactone based molecules. The direct access to the chiral \(\gamma\)-butyrolactone moiety with favourable orientation of the two carboxyl groups make them suitable precursor for several biologically active natural products. Convenient method for the large scale isolation of these molecules has been developed.

\((2S, 3S)\)-Tetrahydro-3-hydroxy-5-oxo-2,3- furan dicarboxylic acid (Garcinia acid, 12) is known to present in plant species *Garcinia cambogia* (Figure 3.1) which is extensively distributed across southern parts of India and the dried rind of the fruit, popularly known as “Malabar tamarind” is traditionally used as a condiment and is readily available in several markets in many Asian countries\(^{134,135}\).

\((2S, 3R)\)-Tetrahydro-3-hydroxy-5-oxo-2,3-furandicarboxylic acid (Hibiscus acid, 13) is present in the calyxes/leaves of *Hibiscus sabdariffa* (Mathippuli) and the leaves of *Hibiscus furcatus* (Uppanacham) and *Hibiscus cannabinus*\(^{15,151}\). All these plants are distributed across the country and the plant materials are available in large quantities throughout the seasons.
Garcinia Cambogia (Kudampuli)

Figure 3.1
Hibiscus furcatus (Upanacham)

Hibiscus sabdariffa (Mathippuli)

Figure 3.2
3.2 Results and Discussion

The plant material (dried fruit of *Garcinia cambogia*) for the isolation of garcinia acid (12) has been collected from different regions of South India covering different geographical indications. Though the percentage of acid content (6-7 percentage weight of the raw material) was varying from place to place, the [α]D observed for the pure sample irrespective of the region was identical [the plant material was collected from the high range (Mundakayam, Kottayam District) areas to that grown in the backwater (Kuttanad) areas of Kerala]. The malic acid content (usually not to the extent of isolable quantity according to the procedure developed in this laboratory) present in the plant grown in the Kuttanad area was slightly higher. *Garcinia* is a large genus of polygamous trees or shrubs which belong to the family *Clusiaceae* (*Guttiferae*) distributed in Asia, Africa and Polynesia. It consists of 180 species, of which ca. 30 species are found in India.

Hibiscus acid (13) was isolated from the leaves of *Hibiscus Furcatus* and from the calyces *Hibiscussabdariffa* and *Hibiscus cannabinus*, all belong to the family *Malvaceae*. *Hibiscus Sabdariffa* is known as 'gongura' in Telugu and its leaves are extensively used in south for the preparation of a variety of food products including 'gongura pickle'. Leaves are much used as diuretic, sedative and refrigerant. The fruits of *Hibiscus Sabdariffa* possess anti scorbutic properties. *Hibiscus cannabinus* also has a lot of medicinal importance. In the present study 12 and 13 were isolated from plant sources collected from the southern part of India following the procedure developed by Dr. Ibnusaud and co-workers16-18.

3.2.1 Isolation of (2S, 3S)-tetrahydro-3-hydroxy-5-oxo-2,3furan dicarboxylic acid (12)

1. The dried fresh rinds of the fruits of *Garcinia cambogia* was cut into small pieces and soaked in hot water. The water extract was collected after 10-20 hours. The extraction was repeated. The combined extract was evaporated to syrup (A).
2. To the syrup (A) sufficient quantity of methanol was added to remove pectin completely. The pectin free filtrate was concentrated to syrup (B).

3. After making syrup (B) alkaline by adding sufficient quantity of aqueous alkali at elevated temperatures, it is cooled and methanol was added to the solution. Separated thick mass was washed several times with various proportions of aqueous methanol to get a paste of alkali salt (C).

4. The alkali salt (C) on neutralization with mineral acid followed by evaporation gave the concentrate (D). The concentrate (D) was triturated with sufficient quantity of acetone to precipitate the insoluble parts. The filtrate on concentration yielded crude 12.

5. The crude acid 12 was purified by recrystallization from ether and the $[\alpha]_D^{25}$ observed for the pure sample is +103.5° (c 1.0, H$_2$O).

   Chemical and optical purity of the acid was assured by comparing the I.R., $^1$H NMR, $^{13}$C NMR and mass spectra (Figure 3.3a-d) and $[\alpha]_D$ values with that of the reported values. IR spectrum of the compound shows the characteristic absorption bands at $v_{max}$ 3450 (OH, broad), 1793 (γ-lactone carbonyl) and 1743 cm$^{-1}$ (carboxyl carbonyl groups). The $^1$H NMR spectrum of 12 shows an AB pattern at $\delta$ 2.6 (J =17.4 Hz) and 3.06 (J =17.4 Hz) corresponding to the methylene protons and a singlet at $\delta$ 4.80 due to methine protons. Six different signals in $^{13}$C NMR spectrum at $\delta$ 174.9, 171.9, 169.2, 84.0, 79.0, and 39.7 also confirm the structure.
KBr Pellet

\[ \text{Figure 3.3a} \]

\(^1\text{H NMR, 300 MHz, Solvent: Acetone-d}_6 \]

\[ \text{Figure 3.3b} \]
$^{13}$C NMR, 100 MHz, Solvent: DMSO-d$_6$

Figure 3.3c

Figure 3.3d
3.2.2 Determination of the enantiomeric purity of (2S, 3S)-Tetrahydro-3-hydroxy-5-oxo-2, 3-furandicarboxylic acid (12) by Vibrational Circular Dichroism (VCD)

The determination of absolute configuration of chiral compounds is always a difficult task in the structural elucidation of target molecules. The technique most often used was X-ray crystallography which required that the sample be a single crystal. Many organic and bio-organic compounds are difficult to crystallize, so an alternative technique for making the determination of absolute configuration was needed. Although polarimetry is still being used to characterize optically active compounds and as an enantiomeric purity criterion, the application of CD and chiral chromatography for these purposes is a far more effective means. Today, CD spectroscopy has completely replaced ORD (optical rotatory dispersion) mainly due to the easier interpretation of CD spectra.

Recent advances in chiroptical spectroscopic techniques have had a significant impact on the elucidation of intricate stereochemical problems in natural products chemistry and biology. Optical rotation and electronic circular dichroism, which are inherently sensitive to molecular chirality are referred to as chiroptical techniques that can be used independently to determine three-dimensional molecular structures. It is important to keep in mind that optical rotation (measured in polarimetry and ORD) is related to the difference (Dn) in refractive index of left and right circularly polarized light, whereas CD is based on the differential absorption (De) of the two light vectors.

Historically, electronic CD, measured in the near-infrared-visible-ultraviolet spectral region, has been the predominant choice for chemists. Vibrational CD (VCD), measured in the infrared spectral region, has been little utilized since the first measurements in the early 1970s. This can be attributed primarily to the absence of a practical methodology for reliably predicting VCD intensities. Recent developments in ab initio Density Functional Theory (DFT) now make practicable the prediction of VCD
intensities in large organic molecules with an accuracy sufficient to permit absolute configurations to be reliably deduced using VCD spectroscopy. The key to this advance has been the development and implementation of analytical derivative techniques for calculating Atomic Axial Tensors (AATs) using Gauge-Invariant Atomic Orbitals (GIAOs).

The advantage of vibrational CD over electronic CD$^{154}$ is that the widths of vibrational transitions are much narrower than of electronic transitions, leading to more highly resolved spectra (VCD can be measured for many transitions, in contrast to the small number of transitions generally accessible to electronic CD measurement). VCD intensities depend only on ground electronic state properties and are more easily and reliably predicted than electronic CD intensities which depend on excited electronic states in addition. The DFT/GIAO methodology for predicting VCD intensities is general and of high accuracy. Recent developments incorporated in GAUSSIAN 9816 have greatly enhanced the efficiency of DFT/ GIAO calculations. FTIR-VCD does the determination of the absolute stereochemistry of the chiral molecule by comparing experimental and quantum chemically (ab initio calculation) calculated spectra and predict the VCD spectrum of each configuration.$^{155}$

VCD spectra could be theoretically predicted with good accuracy which has opened up quite new possibilities to determine absolute configurations and conformations even in rather complex molecular structures. A pair of enantiomers has the same infrared spectrum but opposite VCD spectra (i.e. at any frequency the VCD intensities of the two enantiomers are equal in magnitude and opposite in sign), while the racemic sample possesses a null VCD spectrum. Today it has emerged as an important tool for the determination of absolute configuration and enantiomeric purity of optically active molecules or entities in the solution state where single X ray diffraction" the gold standard" fails$^{146-148}$. The magnitude of the VCD spectrum is directly proportional to the percent enantiomeric excess(%%ee).

Thus VCD offers a viable alternative to contemporary x-ray analysis. Since VCD spectra are usually measured in the solution phase, it is not
necessary to grow a crystal or dope the crystal for analysis. The new methodology for predicting the vibrational circular dichroism (VCD) spectra of chiral molecules using ab initio DFT/GIAO methodology permits the direct determination of the absolute configuration of organic molecules in solution.

Hence the study of chirooptical properties of the title lactones have been undertaken in order to ascertain the absolute configuration. Garcinia and hibiscus acids(12and13) have been subjected to a systematic conformational analysis using VCD technique and confirms these acids to be 2S, 3S and 2S, 3R respectively. The analysis results are discussed below.

A three dimensional view of the molecule (12), along with the bond torsions (rotational) and ring torsions (puckering) is shown in Figure 3.4 and 3.5. These torsions, in turn give rise to a large number of conformers in solution. The ring puckering shown in Figure 3.5 gives rise to twist angle of 14.55°.
Analysis of the lowest energy conformer of 12 reveals the familiar “envelope mode” arrangement of the lactone ring, adopting a conformation where the Cβ carbon is below the plane (Figure 3.6a and 3.6b) formed by Cα-CO-C (referred to as “conformer A” in lactone literature).

**Figure 3.6.a.** “Envelope” mode of arrangement in 12. Hydrogens are omitted for clarity and the ring carbonyl group is hidden in the view.

**b.** Configuration showing β carbon below the plane (indicated with green color) containing -O-CO- Cα.
Figure 3.7 Alligned view of the conformers of 12 (2S,3S configuration). The atoms are color coded: Carbon skeleton (grey); Oxygen (red); Hydrogen (white).

Most of the torsional motions occur in the side chains bearing the carboxylic acid groups. The conformer population for the molecule 12 is generated with MMFF calculation using the default parameters as outlined in the SPARTAN manual and the alignment of the conformers shown in the experimental VCD spectrum of 12 in DMSO matrix is shown in Figure 3.8.

This would mean that unlike the ring carbonyl group (lactone carbonyl), the acid carbonyl groups would distort the carbonyl peak in the IR and VCD. So one would expect to see a sharp band for the former and a broad band for the latter. Exactly this is what is observed in the IR and VCD spectrum. The IR spectrum of 12 is shown in Figure 3.8.
The residual (after background solvent subtraction) solvents peaks are indicated with a · while those regions marked with # denote excess background subtraction. The calculated IR spectrum of each conformer fits poorly (when compared individually) with the experimental spectrum, with distinct peaks for the three carbonyl groups as against the two seen in the experimental spectrum (Figs. 3.9 and 3.10). Differences are also observed in the 1100-1500 cm⁻¹ region. Thus it is clear that no single conformer uniquely defines the experimental behavior.
Absorption spectrum of (Garcinia acid) compared with the calculated spectra for the conformers

**Figure 3.9**

For clarity, the IR spectrum is the carbonyl region is shown in Fig. 3.10

**Figure 3.10**

An attempt was made to average all the IR spectra from the 7 conformers to mimic the solution phenomenon.
Barring the positional shifts (which arise because the gas phase calculations do not include solvent or intermolecular effects), the averaged spectrum (of all the conformers without any weighting) agrees well with the experimental behavior with two peaks in the carbonyl region, one being broader (carboxyl carbonyl) than the other (Figure 3.11). The experimental VCD spectrum of 12 in DMSO matrix is shown in Figure 3.12.
Figure 3.12

In DMSO, the carbonyl peaks were not well resolved, and only one positive phased signal could be discerned clearly. Hence spectra were measured in acetonitrile (100 % deuteriated).

Figure 3.13

As seen, in Figure 3.13 all the three carbonyl peaks could be resolved in this solvent and their spectral phases match well with the calculated spectra. Thus the assignment was made as SS. In addition, it appears that
the C-O stretching (band labeled 4) was blue shifted to 1320 cm⁻¹ and agreed well with the experimental band.

A peak-by-peak comparison of the IR and VCD spectra of 12 is shown below (Figure 3.14)
The spectra for the SR configuration is also generated (Figure 3.15).

Figure 3.15

Figure 3.16
The differences can be seen in the carbonyl region (Figure 3.16) which had negative phase for two of the carbonyl peaks unlike that of the experimental spectrum. The band patterns (position, phase and relative intensity distribution) seen in the SR do not match with the experiment. Thus SR configuration was ruled out for 12.

3.2.3 Isolation of (2S, 3R)-Tetrahydro-3-hydroxy-5-oxo-2, 3 furan dicarboxylic acid (13)

1. The fresh calyces or leaves of *Hibiscus sabdariffa* or leaves of *Hibiscus furcatus* were extracted with water and was concentrated to syrup.

2. To this concentrate, methanol was added to precipitate inorganic materials.

3. The organic layer was concentrated and aqueous alkali was added to yield the sodium salt of 13.

4. The salt was then washed several times with various proportions of aqueous methanol to get a thick paste of alkali salt.

5. The alkali salt on neutralization with mineral acid regenerated the acid, followed by concentration under vacuum gave the crude acid.

6. The crude acid residue was triturated with acetone or methanol to obtain 13. The final purification of 13 was done by repeated extraction and finally by crystallization from ether.

The chemical as well as optical purity of 13 was confirmed by comparing the IR, NMR and mass spectra with that of the reported values (Figure 3.17 a-d). IR spectrum of the compound shows the characteristic absorption bands at $\nu_{\text{max}}$ 3400, 1790 and 1735cm$^{-1}$. The diastereotopic methylene protons of 13 appeared as quartet at $\delta$ 2.8 ($J = 17.09$Hz) - 3.30 ($J = 17.09$Hz) and the methine protons at $\delta$ 5.36. The $^{13}$C NMR spectrum showed six signals at $\delta$ 173.2, 171.3, 167.1, 82.9, 78.4, 42.2 as expected.
Figure 3.17a

$\text{H NMR, 400 MHz, Solvent: Acetone-d}_6$
$^{13}$C NMR, 100 MHz, Solvent: Acetone-$d_6$

Figure 3.17c

Figure 3.17d
3.2.4 Determination of absolute configuration of (2S,3R)-Tetrahydro-3-hydroxy-5-oxo-2,3 furandicarboxylic acid by Vibrational Circular Dichroism

A three dimensional view of the molecule (13), along with the bond torsions and ring torsions is shown in Figure 3.18 and 3.19. Maximum movements of torsions or "torsional noise" occur in the acid and hydroxy side chains (along the yellow cylinders in Figure 3.18).

![Figure 3.18](image)

The lactone ring itself is distorted (and not planar) and for the lowest energy conformer the twist angle (defined as the angle between any two
planes in the ring) is 7 deg. The highest energy conformer has a distortion of about 16 deg. This is the well known ring strain energy that all the cyclic structure exhibit. These torsions give rise to a large number of conformers in solution.

![Image](image_url)

**Figure 3.20**

Conformer population analysis calculations were performed with SPARTAN using semi-empirical methods identified a total of 57 conformations (Figure 3.20). However not all of these conformer contribute to observed IR spectrum. As a rule of thump, conformers that differ in energy by 1 Kcal/mole are expected to dominate (about 19 in numbers) at room temperature. Ideally, one should calculate the IR and VCD of each of these 19 conformers and average them based on their Boltzmann distribution to reproduce the broad spectrum. The IR absorption spectrum of 13 is shown in Figure 3.21 along with the calculated IR absorbance spectra obtained from DFT calculation (B3LYP/6-31G**) of different configurations of 13.
From a comparison of IR spectra of 13 (the presence of broad vibration bands in solution) with that of the calculation, one can conclude that while the width of the bands do not match, the positions match thereby increasing the confidence on the method used for calculation. The functional region of the spectrum in Figure 3.22 further reinforces the presence of multiple conformations of 13 at room temperature in solution.
Experimental VCD spectrum of 13, like its IR spectrum, is very broad due to the presence of large numbers of conformers. For this reason a comparison of the VCD is made in the carbonyl region for the corresponding configurations to assign the absolute configuration without any ambiguity (Figure 3.23).
The above graphic gives a comparison of the experimental VCD with the calculated one and it is found that only in the case of (2S, 3R) the spectrum matches the position and phase correctly, thereby confirming the absolute configuration of 13 as (2S, 3R).

3.3 General Experimental Details

All commercial solvents were distilled prior to use. Dry solvents were prepared by following the procedures described in “Purification of Laboratory Chemicals” by D. D. Perrin and W. L. F. Armarego (3rd edition, Pergamon Press, 1988. Dried fruit rind of *Garcinia cambogia*, leaves and calyces of *Hibiscus sabdariffa* and the leaves of *Hibiscus furcatus* were procured from Kottayam and Pathanamthitta districts of Kerala, India. Chemical and optical purity of garcinia and hibiscus acids were verified using spectroscopic and analytical methods. Anhydrous sodium sulphate was used to dry organic extracts.

Melting points were determined on “Sunbim” make electrically heated melting point apparatus and are uncorrected. IR spectra were recorded using a Shimadzu IR 470 spectrophotometer as KBr pellets (solids) or thin films (liquids). $^1$H NMR and $^{13}$C NMR spectra were recorded on a Brucker W M 300 or Brucker Avance 300 or Jeol GSX 400 or Brucker AMX 400 NMR spectrometers. NMR spectra were recorded in appropriate solvents using tetramethylsilane as internal standard and the chemical shifts are shown in $\delta$ scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Electron impact mass spectra were recorded on a Finnigan MAT MS 8230 or jeol D-300. Specific rotations were recorded using Rudolph digital polarimeter. Elemental analyses were carried out on a Vario EL elemental analyzer.

3.4 Experimental procedures for the VCD study

The optically pure compound was dissolved in deuterated methanol(12mg/mL). The VCD and IR absorption spectra were obtained with a resolution of 4cm$^{-1}$ using a Chiral IR FT VCD spectrometer (Bomem/
Biotools, Illinois, USA) with an IR cell equipped with BaF$_2$ windows and a path length of 100$\mu$m. The VCD and IR were collected in 8 blocks of 60 minutes each with 150$\mu$L of the sample. The blocks were averaged to obtain the final VCD spectrum and appropriately subtracted with the noise obtained under the same conditions without the sample. The VCD intensities were then calibrated with the software supplied with Chiral IR.

The calculated VCD and IR spectra were obtained by following a series of computation steps as follows. With the aid of the Spartan program, lowest-energy conformations of the compounds were found using a systematic search procedure using molecular mechanics force fields (MMFF). The conformation search was performed with the molecule in a fully relaxed state without any constraints. The lowest energy conformer from the above calculation was then optimized with SCF/PM3 basis set using SPARTAN and also by Gaussian03 at the B3LYP/ 6-31+G** level on Linux cluster (4 Xeon nodes each with 2.4 GHz processors and parallelized with LINDA) and corresponding spectra were simulated using a Lorentzian band width of 6 cm$^{-1}$. The B3LYP DFT functional was selected since it provided excellent results for VCD simulations.

3.5 Experimental

3.5.1 (2S,3S)-Tetrahydro-3-hydroxy-5-oxo-2, 3-furandicarboxylic acid (12)

Dried rinds of the fruits of Garcinia cambogia (1 Kg) were cut into small pieces and soaked in hot water (1L). The extract was collected after 20 hours and the process was repeated 4-5 times. The combined extract was concentrated and methanol (2.5L) was added to precipitate pectin. Upon filtration the filtrate was concentrated to syrup. It was made alkaline with sufficient quantity of 10% aqueous sodium hydroxide, followed by the addition of methanol (1L) till two layers separated. Sodium salt separated as a paste (lower level) and was washed with 70%, 80%, 90% aqueous methanol (3x150mL) respectively, and finally with pure methanol. The pure
trisodium salt was dissolved in sufficient quantity of 2N hydrochloric acid to regenerate the free acid. It is concentrated and added acetone to precipitate the impurities. The filtrate on concentration yielded crude crystals of *Garcinia* acid. Pure crystals of 12 were obtained upon recrystallisation from acetone-chloroform mixture.

**Yield** : 72g

**Melting point** : 178-179 °C.  

**$[\alpha]_D^{25}$** : +103.5° (c 1.0, H2O)  

**IR (KBr)** : $\nu_{\text{max}}$ 3450 (OH, broad), 1793 (y-lactone) and 1743cm$^{-1}$ (carbonyl)

**$^1$H NMR (DMSO-$d_6$)** : 8 4.80 (s, IH), 3.06 (d, $J$ = 17.4Hz, IH), 2.60 (d, $J$ =17.4 Hz, IH)

**$^{13}$C NMR(DMSO-$d_6$)** : 8 174.9, 171.9, 169.2, 84.0, 79.0, 39.7

**Mass spectrum (E.I)** : m/z 191 (M+1) (2%), 162 (6%), 145 (35%), 127 (10%), 116 (48%), 99 (70%), 88(100%), 60 (40%) 55 (20%)

**Molecular formula** : C$_6$H$_6$O$_7$

**Molecular mass** : 190.107

**Elemental analysis** : Calculated : C 37.907, H 3.181

**Found** : C 36.770, H 3.788

### 3.5.2 (2S,3R)-Tetrahydro-3-hydroxy-5-oxo-2,3-furandicarboxylic acid(13)

**a) From leaves**

Fresh green leaves of *Hibiscus sabdariffa* or *Hibiscus furcatus* (1 Kg) were soaked in water (1L), ground well and extracted two times. The extract is concentrated under reduced pressure. After washing with hexane to remove chlorophyll, the concentrated extract was made alkaline with 8N sodium hydroxide solution (80mL). Methanol was added to precipitate the sodium salt followed by the addition of 2N hydrochloric acid to regenerate the
acid. Upon concentration followed by the addition of acetone precipitated the impurities. The residue obtained after concentration was further extracted with ether which on concentration yielded 10g of the acid 13.

b) From calyx

Fresh calyces of Hibiscus sabdariffa (1Kg) were dried, powdered and extracted with methanol several (6-8) times. The combined extract is concentrated under reduced pressure and the concentrate is washed with hexane to remove chlorophyll. The crude mass is then extracted with ether many times. The ether extract on concentration yielded 16 g of the acid 13.

Melting point : 180 -182 °C  
Reported: 182-183°C

$[\alpha]_D^{25}$ : +111° (c 1.0, H$_2$O)  
Reported: +110°

IR (KBr) : $\nu_{\text{max}}$ 3400 (OH, broad), 1790 (γ-lactone) and 1735 cm$^{-1}$

$^1$H NMR (acetone-d$_6$) : δ 5.36 (s, 1H), δ 3.3 (d, J =17.09, 1H), δ 2.8(d, J =17.09Hz,1H)

$^{13}$C NMR (DMSO-d$_6$) : δ 173.2, 171.3, 167.1, 82.9, 78.4, 42.2

Mass spectrum (E.I) : m/z191 (M+1) (2%), 162 (5%), 145 (60%), 127 (12%), 116 (38%), 99 (84%), 88(100%), 60 (48%), 55 (28%).

Molecular formula : C$_6$H$_6$O$_7$

Molecular mass : 190.107

Elemental analysis : Calculated : C 37.907, H 3.181  
Found : C 37.892, H 3.450