CHAPTER II

ISOLATION AND STRUCTURAL INVESTIGATION OF (2S,3R)-TETRAHYDRO-3-HYDROXY-5-OXO-2,3-FURANDICARBOXYLIC ACID

II.1 Introduction

(2S,3R) Tetrahydro-3-hydroxy-5-oxo-2,3-furandicarboxylic acid (Hibiscus acid, 166) is present in the calyces/leaves of *Hibiscus sabdariffa* (Mathipuli) and the leaves of *Hibiscus furcatus* (Uppanacham) and *Hibiscus cannabinus* (Figs. II.1a and II.1b). All these plants are distributed throughout India and the leaves are available in large quantities in Kerala in all seasons.

The fruits of *Hibiscus sabdariffa* possess anti-scorbutic properties. The leaves are regarded as emollient. *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 the famous ‘gongura pickle’. The leaves were used in Kerala till a few decades ago for the preparation of ‘fish curry’. In bilious conditions, a diet drink is made in boiling water. Leaves are much used as diuretic, sedative and refrigerant.

*Hibiscus cannabinus* also has a lot of medicinal importance. Seed is acrid and sour; stomachic, appetiser; removes diseases due to kapha and vata, cures earache, used as an external application to pains and bruises, and are said to be aphrodisiac and fattening. Leaves used in dysentery, cure diseases of the blood, bile and throat. The juice of flowers with sugar and black pepper is a popular remedy for biliousness with acidity.
Hibiscus sabdariffa

Fig. II.1a
Hibiscus furcatus
The roots of *Hibiscus furcatus* as an infusion in water is a good cooling drink for hot weather and is given to cure internal poison and swellings, to cleanse the kidneys. Leaves eaten cooked improve digestion, cure eye diseases and are anthelmintic. Leaves made into a paste and swallowed to remove small fish bones stuck in the throat<sup>114</sup>.

It is believed that the organic acids present in the plant is responsible for their medicinal properties. The major acid component has been mistakenly identified as malic acid in the past<sup>113</sup>.

Garcinia acid (167) one of the optical isomers of hydroxycitric acid finds extensive application in the pharmacological as well as synthetic fronts<sup>115-119</sup>. However only very little information is available on Hibiscus acid. The potential of the molecule is not yet explored due to the non-availability of the compound in the market. This is due to the lack of any economically viable large-scale isolation procedure for this compound. The unique structure and stereochemistry of 166 with a large number of complex naturally occurring molecules makes this molecule highly important in the pharmacological as well as organic synthetic fronts.

In spite of the ready accessibility in the optically pure form, from the chiral pool no effort has been made towards the use of 166 in the wide area of asymmetric synthesis.

Though, Lewis *et al.* reported the presence of Hibiscus acid in the leaves of *Hibiscus furcatus* and *Hibiscus cannabinus*, no method is described for the isolation of the compound. The method reported by Per.M.Boll, Else Sorensen and Eric Balieu for the isolation of 166 is from the calyces of the fruits of *Hibiscus sabdariffa*. However the acid was isolated as its dimethyl ester.

The dried, ground calyces of *Hibiscus sabdariffa* were extracted at room temperature for 3 days with methanolic hydrogen chloride. After repeated extraction, diethyl ether was added to deposit the colouring matter as a dark red syrupy mass. The pooled ether extracts were evaporated and the resulting residue
was dissolved in methanol. Upon cooling crystals of dimethyl ester of the acid (168) were collected. The absolute configuration of the molecule is determined by conventional methods. Hydrolysis of 168 followed by concentration and storage over Drierite for 2 months yield 166.

Another method for the laboratory-scale production of DL-166 described by Martius et al. is purely a synthetic one. The process involves oxidation of a mixture of cis and trans aconitic anhydride using silver chlorate and osmium tetroxide to give after preparative thin layer chromatography a mixture of DL-166 and 167 (Scheme II.1). The oily material obtained is dried over P$_2$O$_5$ and recrystallised.

Scheme II.1

It has been found that the Per Boll et al.'s method fails to give pure acid when the leaves of the plants are used and is applicable in the case of Hibiscus sabdariffa calyces only. Hibiscus sabdariffa is a seasonally flowering plant and hence the raw-calyxes may not be available at any given time. Large quantities of expensive solvent ether is required for the process. Moreover crystallization was effected only on prolonged storage over drierite. The chemical synthesis described by Martius in the racemic form is not economically viable.

The present method is novel and general for the isolation of Hibiscus acid from the fresh or dried leaves of Hibiscus furcatus, Hibiscus sabdariffa and Hibiscus cannabinus which is available in plenty as a commonly grown shrub throughout South India. This is economic, simple and less time consuming. The
leaves are available in all seasons. The method can be scaled up for large-scale isolation of 166. Incidentally the same process works for Hibiscus sabdariffa calyxes also.

The purity of the title compound has been improved and hence the compound is isolated in the stable crystalline form. Complete characterisation of the compound, using IR, $^1$H NMR, $^{13}$C NMR, HMBC NMR, mass spectra and other analytical methods has been done for the first time. A large number of novel derivatives of the compound were also prepared to confirm the structure of the molecule. The use of undesirable solvent methanol is totally replaced by water and use of solvent ether is reduced considerably. This process is entirely new from the method described in any of the prior methods which fails when leaves of Hibiscus furcatus, Hibiscus sabdariffa and Hibiscus cannabinus were used.

II.2 Results and Discussion

Hydroxycitric acid contains two centers of asymmetry and hence four stereomeric forms are possible (Scheme II.2). Two optically active isomers, (2S, 3R) and (2S, 3S) hydroxycitric acids are present in the nature and can be isolated as their lactone form namely hibiscus acid (166) and garcinia acid (167).
The process of isolation of 166 following Per Boll et al.’s\textsuperscript{110} method is applicable only in the case of calyxes of \textit{Hibiscus sabdariffa}. However it is not possible for the direct isolation of the acid following Per Boll’s method which \textit{is} resulted in the isolation of 166 as its dimethyl ester(168).

The modified method is more convenient and economically viable for the isolation of (2S, 3R)-Tetrahydro-3-hydroxy-5-oxo-2, 3,furandicarboxylic acid.

Two independent methods have been developed for the isolation of hibiscus acid from the calyxes/leaves of \textit{Hibiscus sabdariffa} and the leaves of \textit{Hibiscus furcatus}

\textbf{Method - A}

1. Fresh calyxes or leaves of \textit{Hibiscus sabdariffa} or leaves of \textit{Hibiscus furcatus} and \textit{Hibiscus cannabinus} is extracted using water. The extraction is repeated thrice and the combined extract is concentrated to syrup.

2. To the aqueous concentrate organic solvents like methanol or acetone is added to remove insoluble inorganic materials.

3. After evaporating the organic layer, aqueous alkali is added to the concentrate to yield the alkali salt. It is washed several times with 100\% alcohol.

4. The pH of dry alkali salt is readjusted by the addition of mineral acids to regenerate the acid.

5. The solution is concentrated and extracted with organic solvents like acetone or methanol and concentrated to a syrup.

6. This is further extracted with solvents like ether, acetone or methanol followed by concentrations, resulted in the isolation of the lactone in the pure form.
Method - B

1. Fresh or dried leaves of *Hibiscus furcatu*, *Hibiscus sabdariffa* or *Hibiscus cannabinus* is cut into small pieces and soaked in acidic alcohols like methanol, ethanol etc for 12-36 hrs and the extract is collected. The process is repeated many times and the combined extract is concentrated to a thick syrup (A). Alternatively the leaves can be subjected to exhaustive soxhlet extraction with acidic alcohols to get the extract.

2. Sufficient water is used to precipitate organic impurities from the syrup (A) and filtrate is concentrated to get syrup (B).

3. Syrup (B) is triturated with appropriate solvents like methanol, ethanol, acetone etc. several times and combined extracts on concentration gives a residue(C).

4. Residue(C) is triturated several times with suitable solvents like ethyl acetate, ether or chloroform. The extract on concentration furnished crude acid.

5. The crude product is further purified by converting it into the ester followed by acid hydrolysis.

The purity is confirmed by IR, $^1$H NMR, specific rotation and melting point. IR, $^{13}$C NMR and mass spectra were recorded for the first time.

IR spectrum of the compound show the characteristic peaks 3400(OH, broad), 1790(ylactone) and 1735(carbonyl) cm$^{-1}$

The low resolution $^1$H NMR spectrum of Hibiscus acid reported by Per Boll et al. shows some anomaly with the high resolution $^1$H NMR spectrum of the acid(166) recorded again.

The values are given in Table II.1.
The $^{13}$C NMR spectrum of 166 shows six different peaks corresponding each carbon atoms. The lactone carbonyl appeared at $\delta$ 173.2 ppm to contrary of the deserved value of $\delta$ 177 ppm. To further confirm the assignment of $^{13}$C NMR values, derivatives like dimethyl ester (168) and methyl thiomethyl ether (169) were prepared and fully characterised. It is clear that the esterification of carbonyl groups and protection of hydroxyl group as MTM ether has no effect on the lactone carbonyl. On this basis the $\delta$ values of the lactone carbonyl is assigned.

The carbonyl carbon atom, attached to the secondary carbon is assigned $\delta$ 167.1 ppm and the other attached to the tertiary carbon atom at $\delta$ 172.3 ppm. A doublet at $\delta$ 82.9 ppm in the proton-coupled $^{13}$C NMR spectrum confirms the presence of secondary carbon atom. A change in the $\delta$ 78.43 ppm to $\delta$ 81.7 ppm upon protection of the hydroxyl group as MTM ether also confirms the tertiary carbon $^{13}$C NMR value.

Table II.1

<table>
<thead>
<tr>
<th>$^1$Hnmr(acetone-$d_6$)</th>
<th>Instrument</th>
<th>$^1$C</th>
<th>$^1$C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$H_A$</td>
<td>$H_B$</td>
</tr>
<tr>
<td>Reported $\delta$ values</td>
<td>Varian A 60 A</td>
<td>3.1(d)</td>
<td>3.6(d)</td>
</tr>
<tr>
<td>Recorded $\delta$ values</td>
<td>Jeol GSX 400</td>
<td>2.8(d)</td>
<td>3.3(d)</td>
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</table>
The $\delta$ values are further confirmed by the HMBC spectrum of 166. Fig. II.5b shows the $^{1}H$-$^{13}C$ long-range correlation observed in the HMBC spectrum of Hibiscus acid. The carbonyl peak at $\delta$ 167.8 ppm shows correlation with the oxymethine peak at $\delta$ 5.2 ppm while the peak at $\delta$ 172.9 ppm shows correlation with oxymethine proton and one of the ketomethylene proton showing peaks at $\delta$ 3.14 ppm. However, the lactone carbonyl shows correlation with only the methylene proton. This clearly confirms the $\delta$ values of all carbon.
The mass spectrum (FigII.2d) of the acid (166) shows the molecular ion peak at [M+H]+ as in the case of polycarboxylic acid. The important peaks are at m/z 172(1), 162(5), 145(60), 127(12), 116(38), 99(84), 88(100), 60(48), 55(28). A tentative fragmentation pattern is depicted in Scheme II.3.

Scheme II.3
II.3 General Experimental Details

All commercial solvents were distilled prior to use. Dry solvents and reagents 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). Dry THF was used as such received from Aldrich. Leaves/ calyces of Hibiscus sabdariffa (Mathipuli) and the leaves of Hibiscus sabdariffa, Hibiscus furcatus (Uppanacham) and Hibiscus cannabinus were collected from local area. All reactions which require anhydrous condition, were carried out under a positive flow of dry nitrogen. Dry 1-bromotoluene and 1-bromonaphthalene were prepared following the procedures described in “Vogel’s Textbook of Practical Organic Chemistry” (4th edition, pp700 and 633 respectively.) All Grignard reagents were prepared in situ. Molecular sieves was activated at 200°C for three hours before use. Titanium (IV) chloride and Titanium (IV) isopropoxide were used as received from Aldrich. Dichlorodiisopropoxy titanium and 3-((E)-2-butenoyl)-1,3-oxazolidin-2-one, the dienophile were prepared following reported procedures. N-Butyl lithium was used as received from Lancaster. Freshly distilled cyclopentadiene was used. Asymmetric Diels-Alder reactions were carried out following reported procedures in presence of activated powdered Linde 4A type molecular sieves. 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 spectra were recorded on a Brucker WM 300 MHz or Brucker Avance 300 or Jeol GSX 400 MHz or Brucker AMX 400 MHz NMR system and chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane as internal standard (0.00 ppm). $^{13}$C NMR were recorded on a
Brucker WM 300 (75.5 MHz) or Jeol GSX 400 (100.6 MHz) or Brucker AMX 400 (100.6 MHz) NMR system and chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane (0.00 ppm). HMBC spectrum was recorded on Brucker DRX 500 NMR system. Electron impact mass spectra were recorded on a Finnigan MAT MS 8230 or Jeol D-300 or Jeol SX-102 mass spectrometer. Specific rotations were recorded using Jasco DIP 370 or Jasco DIP 1000 digital polarimeter. Elemental analyses were carried out on a Carlo-Erba CHNS-O-EA 1108 elemental analyser.

II.4 Experimental

(2S, 3R)-Tetrahydro-3-hydroxy-5-oxo-2, 3-furandicarboxylic acid (166)

Hibiscus acid (166) is isolated from the leaves of Hibiscus furcatus/Hibiscus sabdariffa or from the calyces of Hibiscus sabdariffa following the procedure given below.

(a) Fresh leaves of Hibiscus furcatus/Hibiscus sabdariffa (1 kg) was extracted with water (1 Lt). The concentrated aqueous extract was washed with hexane. To the aqueous layer, acetone (1 Lt) was added to precipitate insoluble materials. After evaporating the acetone, NaOH solution (8N, 80 ml) was added to adjust the pH to 12.0. Addition of alcohol (1 Lt) resulted in the precipitation of Na salt. The pH was readjusted to 2.0 by the addition of HCl (2N). Concentrated to a syrupy mass and extracted with dry acetone (2X250 ml). The acetone extract was concentrated. This was further extracted with ether (3X150 ml), followed by concentration yielded 10 g of the lactone in the pure form.

(b) Fresh calyxes (1 kg) of Hibiscus sabdariffa was extracted following the procedure described above yielded 16 g of lactone in the optically pure form.
Melting point  :  180°C (decomp.)  Reported: 182-183°C (decomp.)

\([\alpha]_D\)  :  +111°(c=1.0,H_2O)  Reported:  +110°(c=1.37,H_2O)

IR (KBr)  :  3400(\text{OH},\text{broad}) 1790 (\gamma\text{-lactone}) & 1735(\text{carbonyl}) \text{cm}^{-1}

\(^1\text{H NMR}(\text{acetone-d}_6)\)  :  5.36(s,1H), 2.8(d, J=17.09 Hz,1H), 3.3(d, J=17.09 Hz,1H) ppm

\(^1\text{C NMR}(\text{acetone-d}_6)\)  :  173.2, 172.3, 167.1, 82.9, 78.4, & 42.2 ppm

Mass spectrum  :  m/z191(M\(^+\))(2), 172(1), 162(5), 145(60), 127(12),

116(38), 99(84), 88(100), 60(48), 55(28).

**Elemental analysis**

*Found*  :  C 37.23, H 2.73

*Calculated*  :  C 37.30, H 2.99

**Dimethyl(2S,3R)-tetrahydro-3-hydroxy-5-oxo-2,3-furandicarboxylate (168)**

A solution of 166 (1.0g, 5.25mmol in 25 ml ether) was treated with excess diazomethane in ether. The reaction mixture on concentration gave colourless crystals of 168.

Yield  :  1.2g

Melting point  :  129°C  Reported: 128-129°C

\([\alpha]_D\)  :  +118.95°(c=0.4952,CHCl_3) Reported:  +112°(c=0.49,MeOH)

IR (KBr)  :  3500(\text{OH},\text{broad}) 1795 (\gamma \text{-lactone}) & 1745(ester) \text{ cm}^{-1}

\(^1\text{H NMR}(\text{CDCl}_3)\)  :  5.31(s,1H), 3.95 (s,3H), 3.84 (s,3H),

3.1(d, J=20.3Hz, 1H), 2.87(d,J= 20.3Hz,1H ) ppm.

\(^1\text{C NMR}(\text{DMSO-d}_6)\)  :  8172.74, 170.80, 166.14, 81.96, 53.12, 53.03, 40.14 ppm.

Mass spectrum  :  m/z  219(M+1)(66), 191(2), 159(100), 141(10), 130(38),

99(100) & 74(25).
Elemental analysis

Found : C 44.71, H 4.59
Calculated : C 44.04, H 4.59

Dimethyl(2S,3R)-tetrahydro-3-oxo-[(methylthio)methoxy]-5-oxo-2,3-furandicarboxylate (169)

Acetic acid (1.5ml) in acetic anhydride (10ml) was added to a solution of 168 (1g, 4.6 mmol) in anhydrous DMSO (14ml). The mixture was allowed to stand for three days. The reaction mixture was poured into cold, saturated aqueous sodium bicarbonate solution (180ml) and stirred for one hour. It was extracted in chloroform (3X200ml) and the combined chloroform extracts was washed with saturated sodium bicarbonate solution (50ml) followed by water (50 ml). The chloroform extracts was dried and evaporated to get crude 169 (0.9g) and purified by column chromatography. [eluent: hexane-chloroform, (7:3)].

Yield : 0.50g

$[\alpha]_D$ : -147.65° (c= 0.98, CHCl$_3$)

IR (film) : 1800 (y-lactone ), 1745 (ester) cm$^{-1}$

$^1$H nmr (CDCl$_3$) : 5.10 (s, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.23 (d, J = 17.85 Hz, 1H), 2.97 (d, J = 17.86 Hz, 1H) 2.14 (s, 3H) ppm.

$^{13}$C nmr (CDCl$_3$) : δ 172.0, 168.0, 167.1, 85.0, 81.7, 71.4, 53.3, 53.1, 40.1, 14.5 ppm.

Mass spectrum : m/z 278 (M$^+$)(11.6), 262 (13.3), 259 (24.3), 220 (8.7), 219 (64.9), 191 (29.9), 159 (100), 141 (14.6), 131 (11.9), 113 (5.2), 99 (40.3), 90 (4.5), 69 (2.98), 59 (17.1), 44 (25.3).