CHAPTER III

"An Investigation Directed Towards The Synthesis Of

Suksdorfin Type Coumarin"
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An Investigation Directed Towards The Synthesis Of Suksdorfin Type Coumarin:

Introduction:

After the discovery of Suksdorfin 1, a coumarin derivative, as a potent HIV-1 protease inhibitor, researchers had been trying to synthesise its different analogues in order to get better anti-HIV activity.

Keeping in view of the above aspect we had ventured out to synthesise suksdorfin type coumarin derivatives. To achieve this goal, we prepared 4-methyl-7-hydroxy coumarin 2 starting from resorcinol and ethylacetocacetate and decided to proceed according to the retrosynthetic scheme shown below.

The basic skeleton 4-methyl-7-hydroxy coumarin 2 was prepared by following the
Von-Pechman reaction\(^4\) as shown in scheme-1. Here, resorcinol was condensed with ethylacetoacetate at low temperature under strong acidic condition. The product was characterised by spectroscopic analysis as well as from the melting point.

\[
\begin{align*}
\text{Resorcinol} & \quad + \quad \text{Ethylacetoacetate} \quad \xrightarrow{\text{a}} \quad \text{Product} \\
\end{align*}
\]

\(a: \text{Conc. H}_2\text{SO}_4, \, 0^\circ\text{C}, \, 10\% \text{ NaOH solution.}\)

Scheme-1

An IR absorption peak at 3433 cm\(^{-1}\) was indicative of an aromatic hydroxyl group. The peaks at 1160,1350,1450,1656 and 1700 cm\(^{-1}\) were the characteristic for eoumarin skeleton. In \(^1\text{H NMR}\), a singlet at \(\delta 5.03\) ppm integrating to one proton was the characteristic of the proton at C-3 position (\(\alpha\) to the carbonyl). The singlet at \(1.80\) ppm integrating to three protons indicated the existence of one methyl group on the double bond. Two doublets and a singlet in the aromatic region is indicative of the hydroxy group at 7 position. Further support for this structure came from the MS where M\(^+\) was at \(m/z\) 176 along with the usual fragmentation peaks.

From the NMR spectrum and the mechanism of the reaction it was established
that the position of the methyl group was at C-4 of coumarin 2. The mechanism of the reaction is shown above.

4-Methyl-7-hydroxy coumarin 2 was treated with acetic anhydride/pyridine reagent system at room temperature to get the corresponding acetate which was recrystallised from 95% ethanol. An additional strong absorption band in IR spectrum at 1720 cm\(^{-1}\) is indicative of an acetate group which was further confirmed by a singlet at $\delta$ 2.36 ppm integrating to three protons in \(^1\)H NMR spectrum. The mass spectral fragmentations with molecular ion peak at m/z 218 revealed the product as 4-methyl-7-acetoxy coumarin 3. Melting point (150 -151°C) of the product was also identical with the reported one.\(^4\)

Performing Fries rearrangement\(^5\) using anhydrous AlCl\(_3\) on the compound 3, we obtained a product in 72% yield. A sharp IR peak at 1733 cm\(^{-1}\) indicated the presence of a keto group adjacent to the aromatic ring. Appearance of an IR absorption peak at 3394 cm\(^{-1}\) indicated the existence of hydrogen bonded hydroxyl group which was further established by the one proton singlet at $\delta$ 12.38 ppm in \(^1\)H NMR spectrum. Shifting of
the acetate peak from δ 2.36 ppm to δ 2.30 ppm even in deuterated acetone and disappearance of the singlet at δ 6.05 ppm confirmed the rearrangement of the acetate group to 8 position. Mass spectral fragmentation with molecular ion peak at m/z 218 was also in agreement with the assigned structure 4. The product showed an extra band at 1750 cm⁻¹ in its IR spectrum with simultaneous disappearance of the band at 3395 cm⁻¹.

Since the compound 4 contains α-hydroxy arylketone moiety, attempts were made to cyclise it under Kostanecki-Robinson condition i.e. treating the compound with a mild base like NaOAc in acetic anhydride under reflux to form a pyron ring of the type 4-A as was conceived in the retrosynthetic scheme. On completion of the reaction a crystalline product with melting point 202 °C was obtained.

\[
\text{\begin{align*}
\text{\includegraphics[width=0.3\textwidth]{5.png}}
\end{align*}}
\]

In the \textsuperscript{1}H NMR spectrum, appearance of a new three proton singlet at δ 2.45 ppm vis a vis disappearance of the -OH peak at δ 12.38 ppm confirmed the introduction of an acetate group in the molecule. Display of molecular ion peak at m/z 260 in the mass spectrum was a further confirmation for the said product to assigned the structure as 5 not the 4-A. Confirmation for the said product 5 was further deduced by comparing its spectral data with that of its authentic material prepared by acetylation of 4 with acetic anhydride and pyridine.

\[
\text{\begin{align*}
\text{\includegraphics[width=0.3\textwidth]{4-A.png}}
\end{align*}}
\]
These observations inferred that anhydrous sodium acetate was not a strong enough as a base to enolise the arylketone moiety for possible cyclisation. Attempts to cyclise the acetate 5 to 4-A were also a failed under similar reaction conditions. Use of stronger base was restricted due to its effect on the lactone ring. Attempts to enolise the aryl methyl ketone moiety with triethylamine and p-toluenesulphonic acid were also failed.

Since the proposed route to the desired molecule failed in the cyclisation step, therefore we decided to proceed through another route by making propionate analogue where enolisation of the aryl ketone moiety might be possible because of the additional alkyl group in the moiety.

A propionyl group was attached to the substrate 2 by the treatment of propionic anhydride in dry pyridine at room temperature. In its spectral analysis, absence of IR absorption peak at 3433 cm$^{-1}$ indicated the absence of hydroxyl group. Peak at 1735 cm$^{-1}$ was a stronger evidence for the presence of the propionate group. $^1$H NMR spectrum showed a quartet at $\delta$ 2.4 ppm integrating to two protons and an adjacent triplet ($J=7$Hz) at $\delta$1.03 ppm integrating to three protons which further supported the presence of the propionyl group. Mass spectrum showed molecular ion peak at m/z 232. From all these data, the structure of the product was assigned as 6.

Fries rearrangement of the compound 6 using anhydrous AlCl$_3$ afforded a crystalline product in 70% yield. Appearance of IR absorption peak at 3390 cm$^{-1}$ and 1725 cm$^{-1}$ indicated the presence of hydrogen bonded hydroxyl group and keto group respectively. This indicated the formation of Fries rearrangement product. $^1$H NMR
peaks at δ 7.46 ppm (d, J=8Hz) and δ 6.73 ppm (d, J=8Hz) integrating to one proton each indicated the presence of 5-H and 6-H respectively. Also absence of the singlet at δ 6.80 ppm (one proton) confirmed the shifting of propionyl moiety to C-8 position. Therefore, the structure of the product was assigned as 4-methyl-7-hydroxy-8-propionyl coumarin.7.

Treatment of the compound 7 with acetic anhydride/pyridine reagent system afforded a crystalline product. Disappearance of IR peak at 3390 cm\(^{-1}\) and appearance of a new peak at 1760 cm\(^{-1}\) along with \(^1\)H NMR singlet at δ 2.28 ppm integrating to three protons indicated the conversion of the phenolic hydroxy to acetoxy group. Along with this, molecular ion peak at m/z 274 suggested the structure of the product obtained as 8.

Attempts to cyclise the compound 8 to form the 'C' ring with sodium acetate in acetic anhydride gave the desired product, chromone 9 which was in full agreement with all the spectroscopic data. The changes in the acetate moiety was clearly indicated by the absence of IR peak at 1760 cm\(^{-1}\). In its \(^1\)H NMR spectrum, two sharp singlets at δ 2.46 ppm and δ 2.40 ppm integrating to three protons indicated the existence of two methyl groups on the double bond. This was also supported by the fact that the peaks at
δ 2.98 ppm (quartet, two protons) and δ 1.21 ppm (triplet, three protons) disappeared in this product. Molecular ion peak m/z 256 in its mass spectrum also supported the structure of the compound as 9–

Attempts to reduce the keto group of the γ-pyron ring with several different reducing agents including sodium borohydride in ethanol failed. However, the double bond α to the keto group in the γ-pyrone ring of the chromone was reported to be quite resistant to reduction.
Step Towards the Synthesis of Suksdorfin type Compound from Resorcinol:

1. Preparation of 4-methyl-7-hydroxy coumarin 2:

\[
\begin{align*}
&\text{OH} \quad + \quad \text{CO} \quad \text{CO} \quad \text{Et} \\
&\text{OH} \\
a : H^+; b : \text{NaOH.}
\end{align*}
\]

In a 250 ml two necked round bottom flask fitted with a dropping funnel was placed 18 ml of conc. H$_2$SO$_4$ and cooled in an ice bath. When the temperature reached below 10 °C a solution of resorcinol (2g, 18.1 mmol) in freshly distilled ethylacetoacetate (1.29 ml, 18.1 mmol) was added dropwise. The mixture was stirred with a magnetic needle and the temperature was kept below 10 °C by means of ice and salt (exothermic reaction). When the addition was complete, the mixture was allowed to stand at r.t. for overnight. The mixture was poured in an ice cold water (about 100 ml) with vigorous stirring. The precipitated formed was filtered through buchner funnel and washed with cold water. The crude product so obtained was dissolved in 30 ml of 5% aqueous sodium hydroxide solution. The solution was filtered and the organic compound was reprecipitated from the filtrate by slow addition of dilute H$_2$SO$_4$ (1:1, 10 ml) with stirring until the solution was acidic to litmus. The product was collected on a buchner funnel, washed with cold water and dried. The product was recrystallised from 95% ethanol to get 2 as colourless needles.

Yield : 2.6 g (83%)

M.P. : 185 - 186 °C

IR (KBr) : 3433, 3050, 1700, 1656, 1600, 1450, 1392, 1350, 1281, 1260, 1167, 1150, 1080, 860 cm$^{-1}$.

$^1$H NMR, (CD$_3$)$_2$CO, $\delta$ ppm : 6.80 (d, $J$=8Hz, 1H), 6.10 (d, $J$=8Hz, 1H), 6.00 (s, 1H), 5.03 (s, 1H), 1.80 (s, 3H).

MS, m/z : 176 (M+, 59%), 148 (100%).
2. Preparation of 4-methyl-7-acetoxy coumarin, 3 :

Freshly distilled acetic anhydride (16.95 mmol, 1.6 ml) along with 0.2 ml of dry pyridine was added to 2 g (11.36 mmol) of 4-methyl-7-hydroxy coumarin 2 while stirring in a 250 ml r.b. flask at ambient temperature. The flask was tightly stoppered and kept at r.t. for 3 hrs. To this mixture a cold dilute solution (100 ml) of HCl was added followed by extraction with CHCl₃. The organic layer was washed with water (3 x 50 ml), dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The product was recrystallized from 95% EtOH (1 g of compound to 5 ml of ethanol) to get almost colourless fibrous needles of acetate 3.

Yield : 2378 mg (95%)

M.P. : 150-151 °C.

IR (CHCl₃) : 2950, 1720, 1700, 1617, 1375, 1275, 1272, 1233, 1142, 1085, 1036, 1000, 925, 900, 875 cm⁻¹.

¹H NMR (CDCl₃), δ ppm : 7.37 (d, J=8 Hz, 1H), 6.90 (s, 2H), 6.73 (d, J=8 Hz, 1H), 6.05 (s, 1H), 2.36 (s, 3H), 2.26 (s, 3H).

MS, (m/z) : 218 (M⁺)

3. Preparation of 4-Methyl-7-Hydroxy-8-Acetyl Coumarin 4 :

...
In a 250 ml two necked dry r.b. flask were placed 1g (4.5 mmol) of dry powdered 4-methyl-7-acyloxy coumarin 3 and 2.265 g (16.9 mmol) of technical grade anhydrous AlCl₃ under nitrogen atmosphere. The flask was stoppered and shaken vigorously for proper mixing (for about 10 minutes). The stopper was removed and the flask attached to a reflux condenser fitted with CaCl₂ guard tube. The flask was placed in an oil bath, the temperature of which was raised quickly to 125 °C and then slowly (over a period of 2 hours) to 170 °C. This temperature was kept constant for one hour. The flask was removed from the oil bath, allowed to cool to 0 °C by immersing in an ice bath. To the cold mixture 10 ml of ice cooled water was added followed by slow addition of 13 ml of dilute HCl (1:7). The mixture was heated with vigorous stirring. It was filtered and ppt washed with cold water and sucked dry. The crude product was recrystallised by dissolving in 20 ml of hot 95% ethanol, filtering the hot solution and chilling the filtrate to get 4.

Yield : 720 mg (72%)
M.P. : 162-163 °C.
IR, (KBr) : 3393, 1733, 1700, 1633, 1611, 1569, 1486, 1394, 1383, 1319, 1247, 1183, 1106, 1075, 989, 922, 894, 875 cm⁻¹.

¹H NMR,(CD₃)₂CO,δ ppm : 12.38 (s, 1H), 7.15 (d, J=9Hz, 1H), 6.20 (d, J=9Hz, 3H), 5.50 (s, 1H), 2.30 (s, 3H), 1.80 (s, 3H).

MS, (m/z) : 218 (M+, 72%), 203, (92%), 175.

4. Reaction Of Compound 3 with NaOAc/Ac₂O :

A mixture of 218mg (1 mmol) of 3 and 65 mg of sodium acetate (1 mmol) in acetic
anhydride was refluxed for five hours. The mixture was cooled and poured into 50 ml water. The product was extracted with CHCl₃ and the organic layer was again washed with dilute NaHCO₃ solution and water respectively. The extract was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to get 230 mg of the product 5.

Yield: 230 mg (86%)
M.P.: 202 °C

IR (CHCl₃): 3025, 2950, 1750, 1690, 1486, 1392, 1369, 1286, 1250, 1211, 1067, 931, 911, 886 cm⁻¹.

¹H NMR [(CDCl₃) + (CD₃)₂ CO] δ ppm: 7.50 (d, J=8 Hz, 1H), 6.80 (d, J=8Hz, 1H), 6.03 (s,1H) 2.45 (s, 3H), 2.33 (s, 3H), 2.10 (s, 3H).

MS, (m/z): 260 (M+, 5%), 218 (98%), 203 (100%), 190 (48%).

5. Preparation of 4-Methyl-7-Acetoxy-8-Acetyl Coumarin 5:

Freshly distilled acetic anhydride (10.6 mmol, 1 ml) alongwith 0.2 ml of dry pyridine was added to 700 mg (3.2 mmol) of 4-methyl-7-hydroxy coumarin 4 with stirring in a 250 ml r.b. flask at r.t.. The flask was tightly stoppered and kept for three hours. Usual work up and evaporation of the organic layer under reduced pressure yielded a product which was recrystallized from 95% EtOH (1g of compound to 5 ml of solvent) to get almost a colourless compound 5 as needles.

Yield: 768 mg (92%).
M.P.: 202 °C.
6. Reaction of Coumarin 5 with NaOAc/Ac₂O:

In a 50 ml r.b. flask 260 mg (1 mmol) of 4-methyl-7-acetoxy-8-acyl coumarin was refluxed for five hours with anhydrous sodium acetate (82 mg, 1 mmol) in acetic anhydride as described earlier. On usual work up the starting compound 5 was recovered quantitatively.

7. Treatment of 4-Methyl-7-Acetoxy-8-AcyI coumarin with Et₃N:

To a 50 ml r.b. flask containing 150 mg (0.5 mmol) of 4-methyl-7-acetoxy-8-acyl coumarin 5 was added 0.3 ml of Et₃N (2.16 mmol). The mixture was stirred for overnight at r.t.. No change was observed in TLC. The mixture was poured into water, extracted with CHCl₃. The organic layer was washed several times with water, dried over anhydrous Na₂SO₄ and distilled off to get the starting material back.
8. Preparation of Propionate of 4-methyl-7-hydroxy coumarin 2:

![Reaction Diagram]

Freshly distilled propionic anhydride (11.36 mmol, 1.5 ml) alongwith 0.5 ml of dry pyridine was added at r.t. to 2 g (11.36 mmol) of dry 4-methyl-7-hydroxy coumarin 2 while stirring in a 250 ml r.b. flask. The flask was tightly stoppered and left at r.t. for one hr. To this mixture a cold dilute solution (100 ml) of HCl was added followed by extraction with CHCl₃. The organic layer was washed with water (3 x 50 ml) and dried over anhydrous Na₂SO₄. The crude product obtained after removal of solvent was recrystallised from 95% EtOH (1g of compound to 5 ml of solvent) to get the white fibrous needles of 6.

Yield : 2.372 g (90%)
M.P. : 139 °C
IR (CHCl₃) : 3050,1735,1700,1656,1620,1470,1425,1275,1170,900 cm⁻¹.

¹H NMR (CDCl₃),δ ppm : 7.3 (d, J=10Hz, 1H), 6.8 (s, 1H), 6.7 (d, J=10Hz, 1H), 5.93 (s, 1H), 2.4 (q, J=7Hz, 2H), 2.2 (s, 3H), 1.03 (t, J=7Hz, 3H).

MS, (m/z) : 232 (M⁺)
9. Preparation of 4-methyl-7-hydroxy-8-propionyl coumarin 7 from 6:

In a 250 ml dry two necked r.b. flask were placed 2 g (8.62 mmol) of dry powdered 4-methyl-7-propionyloxy coumarin 6 and 3.9 g (29.3 mmol) of technical grade androus AlCl₃ under nitrogen atmosphere. The reaction was carried out as described earlier in case of compound 4 and usual work up yielded a product which on recrystallisation from 95 % ethanol afforded crystalline product 4-Methyl-7-hydroxy-8-propionyl coumarin 7.

Yield : 1.4 g (70%)
M.P. : 198 °C
IR, KBr : 3390, 3050, 1725, 1700, 1620, 1470, 1425 cm⁻¹.
¹H NMR, (CDCl₃), δ ppm : 11.43 (s, 1H), 7.46 (d, J=8Hz, 1H), 6.73 (d, J=8Hz, 1H), 6.0 (s, 1H), 3.3 (q, J=7Hz, 2H), 2.36 (s, 3H), 1.2 (t, J=7Hz, 3H).
MS, (m/z) : 232 (M⁺)

10. Preparation of 4-Methyl-7-Acetoxy-8-Propionyl Coumarin 8 from 7 through acetylation:

[Diagram of the reaction]
Acetylation of 1 g (4.3 mmol) of 4-methyl-7-hydroxy-8-propionyl coumarin 7 with Ac₂O/py as described earlier yielded 1.004 g (85%) of the corresponding acetate 8.

Yield : 1.004 g (85%)
M.P. : 144 °C
IR (CHCl₃) : 1760, 1725, 1700, 1600, 1292, 1375, 1281, 1214, 1094, 989, 925, 886 cm⁻¹.

¹H NMR, (CDCl₃), δ ppm : 7.66 (d, J=9Hz, 1H), 7.1 (d, J=9Hz, 1H), 6.28 (s, 1H), 2.98 (q, J=7Hz, 2H), 2.45 (s, 3H), 2.28 (s, 3H), 1.21 (t, J=7Hz, 3H).

MS (m/z) : 274 (M⁺)

11. Cyclization of 4-Methyl-7-Acetoxy-8-Propionyl coumarin 8:

A mixture of 1 g (3.65 mmol) of coumarin 8 and 410 mg (5 mmol) of anhydrous sodium acetate in acetic anhydride (10 ml) was refluxed for five hours in a 100 ml r.b. flask fitted with a reflux condenser and a CaCl₂ guard tube. The reaction was monitored in TLC (25% hexane in ethylacetate as mobile phase) which indicated completion of the reaction after five hours.

The reaction mixture was cooled and poured into a separating funnel containing 150 ml distilled water and extracted with chloroform. The chloroform layer was further washed with a dilute sodium bicarbonate solution followed by water for complete removal of acetic anhydride. The organic layer was separated, dried over anhydrous sodium sulphate and evaporated under reduced pressure to get a colourless crystalline product 9.

Yield : 672 mg (72%).
M.P. : 205 °C
IR (CHCl₃) : 1714, 1625, 1425, 1392, 1364, 1339, 1225, 1183, 1147, 1083, 875 cm⁻¹.
¹H NMR (CDCl₃), δ ppm : 7.85 (d, J=9Hz, 1H), 7.10 (d, J=9Hz, 1H), 6.15 (s, 1H), 2.46 (s, 3H), 2.40 (s, 3H), 2.03 (s, 3H).
MS, (m/z) : 256 (M+)
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3. Chapter-1 of this Thesis.

