Chapter 3
Chapter 3

An attempt towards the synthesis of griseolic acid

3.1. Introduction:
Griseolic acids are family of complex nucleosides isolated from the cultured broths of *Streptomyces griseoaurantiacus* SANK 63479. The structure of griseolic acid A, B and C were assigned on the basis of spectroscopic and crystallographic analysis. Griseolic acids A, B and C are nucleosides having an adenine base, a bicyclic ring in its sugar moiety and two carboxylic acid groups. In griseolic acid A 259 and griseolic acid B 260 there is a double bond in one of the furanose ring while in griseolic acid C 261 carbon-carbon double bond is absent (Figure 93).

![Griseolic acids, complex bicyclic nucleosides](image)

Figure 93. Griseolic acids, complex bicyclic nucleosides

Griseolic acids are strong competitive inhibitor of guanosine 3', 5'-cyclic monophosphate (cGMP) phosphodiesterase (PDE). Griseolic acids are also used as template to discover more selective inhibitors to use as antihypertensive agents. In the search for structure-activity relationship, a few analogues of griseolic acids were synthesized and analyzed for their activity and specificity in spite of the difficulty in constructing the bicyclic skeleton.
Total synthesis of griseolic acid is restricted mainly due to the stereochemical and structural complications which include (a) preparation of strained 1,5-dioxabicyclo ring system-[3.3.0]-oct-3-ene and (b) stereochemical problem in the diastereoselective formation of a quaternary carbon (C-6) atom with –COOH and –CH₂COOH substituents. Due to these difficulties, only four synthetic strategies are known so far for the synthesis of griseolic acid and its analogues. A brief account of literature reports on the synthesis of griseolic acids and related compounds is described herein.

3.2. Reported methods:

Method due to Tulshain and coworkers

The first total synthesis of griseolic acid and its analogues is reported by Tulshain and coworkers (Scheme 57). The D-glucose derived α-D-pentodialdose 262 derived from D-glucose on the Wittig olefination yielded α,β-unsaturated ester 263 which on hydrogenation afforded hydroxyl ester 264. Reaction of hydroxyl ester 264 with LDA and bromine afforded a C-6 epimeric mixture of bicyclic system 265. This reaction probably follows in situ formation of α-bromoester and concomitant cyclization to give 1,6-dioxabicyclo[3.3.0]octane 265. Further treatment of 265 with LDA and ethylidooacetate afforded two epimeric products 266a and 266b in the ratio 4:1. The nOe and X-ray crystallographic studies established the stereochemistry at C-6 carbon with major isomer having required stereochemistry. Acetolysis of 266a gave C-1 anomeric mixture of 267a while acetolysis of 266b yielded that of 267b which was further subjected to Vorbruggen glycosylation to yield 268a and 268b, respectively. The glycosylated product on base induced ester hydrolysis afforded corresponding griseolic acid analogues 269a and 269b.

The same analogy was extended by Tulshian and coworkers for the synthesis of other two analogues of griseolic acid 270a/b starting from α-D-gulo-pentodialdose (Scheme 58).
Scheme 57. Reagents and conditions: (a) Ph₃P=CHCOOEt; (b) H₂, 10% Pd-C; (c) LDA, Br₂; (d) LDA, ICH₂COOEt; (e) Ac₂O, AcOH; (f) Silylated N²-acetylguanine, TMSOTf, CICH₂CH₂Cl; (g) i) NaOH, ii) HCl.

Scheme 58. Reagents and conditions: (a) Ph₃P=CHCOOEt; (b) H₂, 10% Pd-C; (c) LDA, Br₂; (d) LDA, ICH₂COOEt; (e) Ac₂O, AcOH; (f) Silylated N²-acetylguanine, TMSOTf, CICH₂CH₂Cl; (g) i) NaOH, ii) HCl.
Method due to Tulshian and coworkers

After the successful synthesis of griseolic acid analogues 269a/b and 270a/b, Tulshian and coworkers in 1995 reported the first successful synthesis of griseolic acid A from 3,6-anhydrosugar derivative 271 obtained from D-gulo pentodialdose. As shown in Scheme 59, phenylsulfide functionality was introduced at C-6 position of 271 followed by ethanolysis (cleavage of 1,2-acetonide functionality in the presence of ethanol and H$_2$SO$_4$) to afford 272. Reaction of 272 with m-CPBA followed by thermolysis afforded vinylether 273. Treatment of 273 with ethyloxalylchloride in the presence of pyridine yielded C-2-O-ethyloxalate 274.

Scheme 59. Reagents and conditions: (a) LDA, PhS$_2$Ph, -78 to 0 °C; (b) H$_2$SO$_4$, EtOH; (c) m-CPBA, toluene, reflux; (d) EtO$_2$CCOCl, Py, rt; (e) PhSNa, 0 to 30 °C; (f) Ac$_2$O, Py; (g) i) NaBH$_4$, ii) Ac$_2$O, Py, 30 °C; (h) Bis(trimethylsilyl)-N-benzoyladenine, TMSOTf; (i) m-CPBA, toluene reflux; (j) NaOH, 30 °C
Conjugate addition of sodiumphenylsulfide to 274 followed by in situ nucleophilic attack at carbonyl group of ethyloxalate afforded a mixture of C-6 epimeric compounds, in the ratio 10:1, with required stereochemistry of -CH$_2$COOEt as a major product 275. The C-2 hydroxyl group was acetylated followed by reduction of α-ketonyl functionality and further acetylation to yield 276. The diacete 276 on Vörbruggen glycosylation afforded 277. Oxidation of 277 followed by thermolysis gave required carbon-carbon double bond that on hydrolysis led to the griseolic acid A 259.

Method due to Knapp and coworkers

The synthesis of griseolic acid B is reported by Knapp and coworkers$^7$ in the year 2001 using α-d-ribo-furanopentodaldose 278 (Scheme 60). The C-3 hydroxyl group in 278 was protected with TBDMSCl to afford 279. The compound 279 was reacted with hydrazine hydrate to afford corresponding hydrazone that was directly subjected to iodine, tetramethylguanidine and refluxed with DBU in toluene to afford vinyl iodide 280. Cleavage of OTBDMS in 280 and conjugate reaction with diethylacetylenedicarboxylate yielded 281. The key step in the reaction sequence is the reaction of 281 with Bu$_3$SnH, which gave the required bicyclic ring skeleton with desired stereochemistry at C-6 carbon as minor product 282 (33%) along with the major product 283 (49%). The radical assisted sulfenation at C-5, acetolysis and Vörbruggen glycosylation$^6$ of 282 gave 284. Oxidation of 284 to set phenylsulfoxy compound followed by thermal treatment afforded griseolic acid B 270.
Method due to Nair and Pickering

Bicyclic nucleoside analogue of griseolic acid was synthesized and reported by Nair and Pickering\textsuperscript{4b} using isosorbitol 285 as the starting material (Scheme 61). The C-5 hydroxyl functionality in 285 was selectively converted to benzoate ester and C-2 hydroxyl functionality was activated with triflic anhydride to afford triflate 286. The nucleophilic displacement of triflate in 286 with lithium azide followed by hydrogenation obtained amine 287. Treatment of 287 with pyrimidine derivative gave 288 which on reaction with triethylorthoformate in acidic medium followed by deprotection gave bicyclic nucleoside 289.
Scheme 61. Reagents and Conditions: (a) i) Bz₂O, PbO, CH₂Cl₂; ii) Tf₂O, Et₃N, CH₂Cl₂; (b) i) LiN₃, DMF; ii) H₂, Pd-C; (c) Pyrimidine, Et₃N, n-butanol; (d) i) CH(OEt)₃, conc. HCl; ii) NH₃, MeOH.

Method due to Dhavale and coworkers

Synthesis of griseolic acid analogue is reported by Dhavale and coworkers⁶d in the year 2003 (Scheme 62). The synthesis centered on the construction of bicyclic ring skeleton 292 along with formation of quaternary carbon atom C-6 with proper stereochemistry of -COOEt and -CH₂COOEt using rhodium (II) carbenoid chemistry. Thus, D-glucose derived β-keto ester 290 on reaction with mezy lazide and triethylamine afforded the C-3-O-alkylated diazo compound 291. The diazo compound 291 on treatment with catalytic amount of rhodium acetate resulted in the exclusive formation of bicyclic compound 292 via oxonium ylide followed by [1,2]-migration. The reduction of ketone functionality in bicyclic compound 292 using sodium borohydride gave 293 as the only diastereomer. Acetolysis of 293 afforded 294 that on Vörbruggen glycosylation followed by ester hydrolysis under basic condition and further acidification yielded 295 as a griseolic acid analogue.

The bicyclic alcohol 293 on reductive removal of hydroxyl functionality under Barton's protocol afforded the deoxy compound 296, a precursor for the synthesis of C-5 deoxy griseolic acid analogue of 295.
Scheme 62. Reagents and conditions: (a) i) 10% H₂SO₄, MeOH-H₂O; ii) NaIO₄, acetone-water; (b) Ethyldiazoacetate (EDA), BF₃·OEt₂, CH₂Cl₂; (c) MsN₃, Et₃N, CH₃CN; (d) Rh₂(OAc)₄, Benzene; (e) NaBH₄, MeOH-H₂O; (f) Ac₂O, AcOH, H₂SO₄; (g) i) Bis(trimethylsilyl)-N-benzoyladenine (1.5 equiv.), TBDSOTf, CH₃CN; (h) i) NaOH, ii) HCl; (i) NaH, CS₂, Mel, imidazole; (j) n-Bu₃SnH, AIBN (cat.), toluene, reflux.

3.3. Present work:
The limited number of publications on the synthesis of griseolic acid or their analogues, finger out the difficulty of constructing the bicyclic oxygen containing heterocyclic skeleton with the proper stereochemical orientation of -CH₂COOH and -COOH functionalities at the C-6 carbon. A closer look at the reported strategies revealed that reactive organic intermediates such as free radical or carbene are being used to construct the bicyclic ring. In this respect, our earlier report on the synthesis of griseolic acid analogue made use of D-glucose derived α-diazo β-ketoester 291 in the synthesis of bicyclic system 292. This reaction of 291 follows the formation of bicyclic compound intramolecularly by rhodium (II) catalyzed decomposition of α-diazo β-ketoester and subsequent [1,2]-migration (Steven’s rearrangement) to give the bicyclic compound with
the required stereochemistry as shown in Scheme 62. Inspired with this success we thought that rhodium carbenoid chemistry can be well utilized for the synthesis of naturally occurring griseolic acid B as well as griseolic acid C. While analyzing the sugar furanose skeleton in the griseolic acid framework we realized that the dioxabicyclic frame work could be constructed from the catalytic decomposition of D-gulose derived α-diazo β-ketoester (Y) which inturn could be obtained from D-glucose. The rhodium acetate catalyzed decomposition of α-diazo β-keto ester with D-gulose configuration will lead to the bicyclic 1,5-dioxabicyclic system (X) with [1,2]-migration of C3-O-alkylated functionality (Scheme 63). The outcome of the oxonium ylide mediated [1,2]-migration will decide the stereochemistry at the C-6 position. Our attempts towards the synthesis of griseolic acid is described.

As the chemistry involved in the synthetic sequence is the rhodium catalyst induced extrusion of nitrogen from diazo carbonyl compound, it is apt to have a brief description on the synthetic utility of rhodium (II) carbenoids in organic synthesis. The rhodium (II) catalyzed reactions of α-diazo carbonyl compounds and their applications in the selective formation of polycyclic systems have received much attention over the years. In general, rhodium carbenoids have been utilized in three major reaction pathways as shown in
Scheme 64. This include (a) insertion reactions (b) addition reactions (olefin cyclopropanation, cyclopropenation) and Buchner reaction (c) reactions through ylide formation followed by $[1,2]$-, $[1,4]$- migration as well as $[3,3]$-sigmatropic rearrangement.

\[
\begin{align*}
Z &= \text{alkyl, aryl, H, OR}, NR^2 \\
R &= \text{alkyl, aryl, H, COZ, SO}_2R, \text{CN, NO}_2 \\
M\text{Ln} (-N_2) & \rightarrow \text{C=C (cyclopropanation)} \\
to \text{C=C (cyclopropanation)} & \rightarrow \text{Addition} \\
to \text{ArH (aromatic cycloaddition)} & \rightarrow \text{Ylide formation} \\
to O-H, S-H, N-H, C-H, Si-H & \rightarrow \text{[1,2]-insertion (Stevens rearrangement)} \\
& \rightarrow \text{[2,3]-sigmatropic rearrangement} \\
& \rightarrow \text{Dipolar cycloaddition}
\end{align*}
\]

**Scheme 64. Reactions of rhodium (II) carbenoids**

Thus, for a given substrate several distinct possibilities are available and the chemoselectivity of such processes is dependent on steric, conformational, electronic factors, and the nature of both substrate and the catalyst.

(a) **Insertion reactions:**

This type of reaction is one of the most explored reactions of carbenoid chemistry. The most common insertion reactions involve i) C-H insertion$^{10}$ ii) N-H insertion$^{11}$ iii) S-H insertion$^{12}$ iv) O-H insertion$^{13}$ and v) Si-H insertion$^{14}$ (Scheme 65). Intramolecular C-H insertion reactions were first reported by McKervey \textit{et al.}$^{9d}$ The importance of such a reaction is greatly enhanced when the process is done stereo-/regio-selectively while constructing carbon frame works. For example, the intramolecular insertion of keto carbene into unactivated C-H bonds allows carbon-carbon bond formation with high stereoselectivity which would be otherwise difficult to achieve. It has been observed that carbene insertion reaction favours the formation of five membered ring over six/four membered rings even though it is easy to construct six as well as strained four membered rings. Size of the ring formed during the reaction is mainly controlled by the type of the
diazo function, degree of substitution on the carbon where insertion takes place, steric and electronic effects.

(b) Addition reactions:

α-Diazo carbonyl compounds react effectively with π-bond both intra-as well as intermolecular fashion (Scheme 66). This type of reaction is the first among the reaction used in organic synthesis to prepare strain packed cyclopropane, cyclopropene derivatives which act as suitable reaction intermediates to fulfil specific stereochemical requirement while synthesizing complex natural products. Interestingly, carbenoids are also found to exhibit reaction with aromatic systems to yield the Buchner product, named after the Buchner who has commenced the study of reactivity of ethyl diazoacetate after the Curtius first reported EDA in 1893.
Scheme 66. Addition reactions involving rhodium carbenoid chemistry

(c) Ylide formation:

The decomposition of α-diazo carbonyl compounds with rhodium acetate or copper acetate lead to metal carbenoids which exhibit high electrophilic properties. As a result they react with available lone pair of electrons on hetero atoms resulting in the formation of ylide species. The different types of ylides are (i) nitronium ylides (ii) sulfonium ylides (iii) carbonyl and thiocarbonyl ylides and (iv) oxonium ylides. The intra as well as intermolecular formation of ylides subsequently undergoes different types of chemical transformations such as [3,3]-sigmatropic rearrangements, cycloaddition, [2,3]-sigmatropic rearrangement, [1,2]-migration (Stevens rearrangement) etc.

(i) Nitronium ylide:

Nitronium ylides (azomethine ylides) are very useful intermediate while constructing nitrogen heterocycles and are thus useful in the synthesis of alkaloids.¹⁶
(ii) Sulfonium ylide:

Similar to the case of nitrogen in nitronium ylide, carbenoid also reacts with the lone pair of electrons on sulphur to form sulphonium ylide. The thionium ylide can further initiate [3,3]-sigmatropic rearrangement (Thio-Claisen rearrangement) as well as [2,3]-sigmatropic rearrangement.\(^{17}\)

\[
\begin{array}{c}
\text{Sulfonium ylide} \\
\text{Similar to the case of nitrogen in nitronium ylide, carbenoid also reacts with the lone pair of electrons on sulphur to form sulphonium ylide. The thionium ylide can further initiate [3,3]-sigmatropic rearrangement (Thio-Claisen rearrangement) as well as [2,3]-sigmatropic rearrangement.}^{17}
\end{array}
\]

(iii) Carbonyl and thiocarbonyl ylides:

Studies on carbonyl ylide are mainly due to Padwa and coworkers.\(^{18}\) This high profile reaction intermediate is usually generated by reacting carbenes/carbonoids with carbonyl/thiocarbonyl compounds.

\[
\begin{array}{c}
\text{Carbonyl and thiocarbonyl ylides} \\
\text{Studies on carbonyl ylide are mainly due to Padwa and coworkers.}^{18} \text{ This high profile reaction intermediate is usually generated by reacting carbenes/carbonoids with carbonyl/thiocarbonyl compounds.}
\end{array}
\]
(iv) Oxonium ylide:

Amongst rhodium (II) catalyzed oxonium ylide formation reactions, the product derived from either [2,3]-sigmatropic rearrangement or [1,2]-migration is routinely observed. Thejis and Zwanenburg\textsuperscript{19} had first proposed an intramolecular formation of four membered oxonium ylide, in the reaction of \(\alpha,\beta\)-epoxy diazomethyl ketones with activated copper power in hydroxyl solvents, to produce alkene oxoacetals (eq 1).

\[
\begin{align*}
\text{CHN}_2
\begin{array}{c}
\text{CHN}_2
\end{array}
\overset{\text{Cu}}{\longrightarrow}
\rightarrow
\end{align*}
\]

\[
\begin{align*}
\text{EtOH}
\end{align*}
\]

In 1984, Doyle and coworkers reported the formation of product in the reaction of acrolein dimethyl acetal and ethyl diazoacetate via the intermediacy of the oxonium ylide.\textsuperscript{19b} Soon after, Roskamp and Johnson noticed the formation of [1,2]-rearrangement product in the intramolecularly generated oxonium ylide from \(\alpha\)-diazoketone bearing a cyclic acetal (eq 2).\textsuperscript{20}

\[
\begin{align*}
\text{O}
\begin{array}{c}
\text{O}
\end{array}
\overset{\text{Rh}_2(\text{OAc})_4}{\longrightarrow}
\rightarrow
\end{align*}
\]

Asymmetric diazo decomposition to afford chiral nonracemic [2,3]-sigmatropic rearrangement products by formation of oxonium ylides was first reported by McKervey and McCann (eq 3).\textsuperscript{21} Even though there is no direct evidence for the formation of oxonium ylide the reaction is better understood through this assumed intermediate.
The Stevens rearrangement ([1,2]-insertion),\(^{22}\) although defined as stereoelectronically disfavoured transformation, has been used in several systems to provide ring expansion products with high levels of enantiocontrol.

Besides these usual reaction pathways, Pirrung and coworkers in 1991 first observed a case of [1,4]-migration in the rhodium (II) catalyzed decomposition of an \(\alpha\)-diazo \(\beta\)-keto ester (eq 3).\(^{9h}\) Subsequently, Dhavale and coworkers\(^{8c}\) firmly established that the formation of the [1,4]-migration product, in the rhodium carbenoid generated bicyclic oxonium ylides, is a prominent process in addition to the usual [1,2]-migration. It is proposed that the migratory aptitude of the migrating group is one of the deciding factors in such competitive processes. The effect of different electrophilic rhodium catalysts, by changing the ligands, on the product selectivity and the mechanistic aspect involving radical approach is also studied.

Among the different catalysts that have been tried to effect the decomposition of diazo compounds, the dirhodium (II) tetraacetate discovered by Teyssie and coworkers\(^{23}\) was found to be highly active, (Figure 94). Influence of stereoelectronic factors on the reactivity of rhodium complexes have been well documented.
From this brief literature survey it is clear that carbenoid chemistry now hold a unique position in the synthetic organic chemistry because of the inherent flexibility it posses to get transformed into complex structural frameworks that are necessary in natural product synthesis.21 In the continuation of our interest in the synthesis of griseolic acid we now thought of exploring the rhodium catalyzed reaction of D-gulose derived α-diazo β-keto ester. We assume that the reaction will follow oxonium ion formation and [1,2]-migration thereby giving an access to the 1,5-dioxabicyclic system with quaternary carbon atom having -COOR and -CH\textsubscript{2}COOR functionalities-a key intermediate to the synthesis of griseolic acid. Our attempts in this direction are discussed below.

3.2.1. Synthesis of 1,2:5,6-di-C\textsubscript{2}H\textsubscript{5}-isopropylidene α-D-gulo-1,4-furanose (300):

1,2:5,6-di-O-isopropylidene-D-gulo-furanose was prepared according to the literature procedure.24 Thus, 1,2:5,6-di-O-isopropylidene-α-D-gluco-furanose was treated with pyridinium chlorochromate (PCC), and molecular sieves in CH\textsubscript{2}Cl\textsubscript{2} to afford C-3 keto derivative 297 (Scheme 67). The C-3 keto compound 297 was then reacted with acetic anhydride in pyridine DMAP (cat.) to afford enolacetate 298 as a white solid. Spectral and analytical data were in agreement with the reported data. In the next step, enolacetate was first reacted with sodiumborohydride as per the reported method which resulted in the isolation of only 15% of gulose 300 (reported yield 20%). In another method, the reaction of 298 with NaBH\textsubscript{3}CN led to the recovery of starting compound. As an alternative, the
enolacetate 298 was subjected to hydrogenation in the presence of palladium black that led to 299 in 35% yield. The spectral data of 299 matched with the literature report.\textsuperscript{24}

![Diagram of chemical reactions](image)

**Scheme 67. Reagents and conditions:** (a) PCC, 4A Molecular sieves, CH\textsubscript{2}Cl\textsubscript{2}, 98%; (b) Ac\textsubscript{2}O, Py, DMAP(cat.), 80%; (c) NaBH\textsubscript{4}, EtOH, 0-20 °C, 3 h; (d) H\textsubscript{2}(80 psi), Pd-black, 12 h; (e) H\textsubscript{2}(balloon pressure), 10% Pd-C, EtOH, 30 °C, 1 h, 80%; (f) LAH, THF, 0-25 °C, 2 h.

In an attempt to improve the yield, the hydrogenation was performed with 10% Pd-C at 80 psi (30 °C) for 24 h. This resulted in the recovery of starting material. However, hydrogenation of 298 at balloon pressure (40 °C) in the presence of 10% Pd-C afforded 299 in 80% yield.

In another attempt, the reaction of enolacetate 298 with HCOONH\textsubscript{4} and 10% Pd-C in methanol at reflux afforded a white solid in 73% yield.

![Diagram of chemical reactions](image)

In the IR spectrum the absence of peak at 1730 cm\textsuperscript{-1} indicated the absence of acetate functionality.

The \textsuperscript{1}H NMR spectrum of the compound (Figure 95) showed following signals:
\[ \delta_{H} \text{ NMR (300 MHz, CDCl}_3) \ 1.32 \ (3H, s, \ CH_3), \ 1.37 \ (3H, s, \ CH_3), \ 1.44 \ (3H, s, \ CH_3), \ 1.57 \ (3H, s, \ CH_3), \ 1.82 \ (1H, dd, \ J = 14.0 \ and \ 3.5 \ Hz, \ H-3), \ 2.21 \ (1H, ddd, \ J = 14.0, \ 8.2 \ and \ 6.3 \ Hz, \ H-3), \ 3.60 \ (1H, t, \ J = 7.1 \ Hz, \ H-6), \ 4.01-4.15 \ (2H, m, \ H-2, \ H-4), \ 4.42 \ (1H, m, \ H-6), \ 4.68-4.75 \ (1H, m, \ H-5), \ 5.79 \ (1H, d, \ J = 3.5 \ Hz, \ H-1). \]

The appearance of high field signals at \( \delta 1.82 \) with \( J = 14.0 \) and 3.5 Hz and another ddd with \( J = 14.0, 8.2 \) and 6.3 Hz indicated the formation of deoxy compound. A doublet at \( \delta 5.79 \) with \( J = 3.5 \) Hz corresponding to H-1 indicated the presence of 1,2-acetonide.

The \(^1^3^C\) NMR spectrum showed following signals (Figure 96):

\[ \delta_{C} \text{ NMR (75 MHz, CDCl}_3) \ 25.1 \ (CH_3), \ 26.1 \ (CH_3), \ 26.5 \ (CH_3), \ 27.1 \ (CH_3), \ 33.3 \ (C-3), \ 65.1 \ (C-6), \ 75.4 \ (C-5), \ 80.1 \ (C-2), \ 82.3 \ (C-4), \ 106.2 \ (C-1), \ 109.1 \ (OCO), \ 112.6 \ (OCO). \]

A strong signal at \( \delta 33.3 \) was spurious and accounted for methylene carbon using DEPT study. The compound was analyzed for molecular formula C\(_{12}\)H\(_{20}\)O\(_{5}\). Based on the spectral and analytical data the compound was found to be 3-deoxy-D-gulose 301. The synthesis of 301 is also known by other method.\(^{25}\)

The formation of 3-deoxy D-gulose 301 is anomalous and could be explained on the following mechanistic pathway (Scheme 68).

![Scheme 68. Plausible mechanism for the formation of C-3 deoxy gulose 301](image)

It is proposed that after syn hydrogenation the H-4 and the C-3 OAc functionality takes an anti orientation, a criterion for elimination, which further undergoes elimination reaction to yield an olefinic intermediate which was concomitantly hydrogenated from β-face to yield the product 301. The ammonia formed during the course of the reaction might be acting as a base and is the plausible driving force for the reaction.
Figure 95: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 301
Figure 96: $^{13}$C NMR (75 MHz, CDCl$_3$) spectrum of compound 301
With the C3-O-acetyl gulose 299 in hand we thought of the conversion of acetate group to C3-O-CH₂COOEt group via the intermediacy of C3-OH functionality. Thus, C3-O-acetyl gulose 299 was treated with NaOMe-MeOH that led to the complex mixture of products. As an alternative we thought of reduction of ester with LAH. The treatment of acetyl gulose 299 with LAH in THF at 0 °C yielded gulose 300 in 88% yield.

3.2.2. Synthesis of 3-O-carbethoxymethylene α-diazo β-keto esters (306):

In the next step, C3-OH in 300 was treated with ethylbromoacetate and sodium hydride in THF to yield a white solid (mp 84 °C).

In the IR spectrum, a strong band at 1745 cm⁻¹ indicated the presence of COOEt group.

In the ¹H NMR spectrum (Figure 97), a three proton triplet at δ 1.29 with J = 6.8 Hz and a multiplet at δ 4.04-4.31 accounting for seven protons due to methylene group of ester as well as alkyl part along with the two H-6 and H-3 protons indicated the alkylation of C3-OH. This fact was supported by ¹³C NMR data (Figure 98), wherein a signal at δ 169.3 was due to the ester carbonyl. Based on the spectral and analytical data the structure of the compound was assigned as 3-O-carbethoxymethylene-1,2:5,6-di-O-isopropylidene-α-D-gulo-1,4-furanose 302.

The next step was the deprotection of 5,6-isopropylidene functionality selectively. In the subsequent step, 5,6-acetonide deprotection was performed using usual protocol.
Figure 97: $^1$H NMR (300 MHz, CDCl$_3$) spectrum of compound 302
The reaction of 302 in the presence of 10% H₂SO₄ in ethanol at 0 to 15 °C for 7 h gave a thick liquid in only 15% yield (37% starting material recovered).

The IR spectrum of the compound showed signals at 3100-3600 (broad), 1728 cm⁻¹ indicating the presence of hydroxyl and carboxylate functionality, respectively.

In the ¹H NMR spectrum (Figure 99) the presence of two broad singlets at δ 2.59 and 3.07 were assigned to -OH group. The presence of only two isopropylidene methyl groups (and not four) indicated the selective deprotection of 5,6- acetonide group. This fact was supported by the ¹³C NMR spectrum (Figure 100) suggesting structure 303 to the product.

In an attempt to improve the yield of 303 the hydrolysis C3-O- alkylated diacetone D-gulose 302 was performed under different reaction conditions (Table 8).

**Table 8: Controlled hydrolysis of C-3-O-alklated gulose 302**

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Reaction conditions</th>
<th>Product (303)</th>
<th>Starting material (302)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10% H₂SO₄, EtOH, 0-15 °C, 7 h.</td>
<td>15%</td>
<td>35%</td>
</tr>
<tr>
<td>2.</td>
<td>5% H₂SO₄, EtOH, 0-10 °C, 15 h.</td>
<td>10%</td>
<td>40%</td>
</tr>
<tr>
<td>3.</td>
<td>CuCl₂.2H₂O, THF, 0 °C, 5 h.</td>
<td>7%</td>
<td>10%</td>
</tr>
<tr>
<td>4.</td>
<td>Acidic resin(Indion 140), THF-H₂O, 0-20 °C, 6 h.</td>
<td>30%</td>
<td>20%</td>
</tr>
<tr>
<td>5.</td>
<td>30% HClO₄, THF, 15 min.</td>
<td>3%</td>
<td>---</td>
</tr>
<tr>
<td>6.</td>
<td>20% TFA-H₂O, CH₂Cl₂, 0-15 °C, 8 h.</td>
<td>68%</td>
<td>6%</td>
</tr>
</tbody>
</table>

The use of 5% H₂SO₄ in ethanol for prolonged time did not afford good yield of the product (entry 2). The reaction with mild reagents like CuCl₂.2H₂O (entry 3) was also found to be sluggish with complex mixture of product. The use of 30% perchloric acid at 0 °C for short period of time did not afford the product (entry 5). The reaction in the presence of acidic resin (Indion 140, entry 4) afforded 30% of diol 303.
Figure 99. $^1$H-NMR (300 MHz, CDCl$_3$) spectrum of compound 303.
The better result was however obtained when reaction was performed in the presence of 20% TFA-H2O in CH2Cl2 to give diol in 68% yield (entry 6).

In the next step, the diol 303 was reacted with sodium metaperiodate in the presence of acetone-water to obtain a thick liquid.

\[
\begin{align*}
\text{HO} & \quad \text{NaIO}_4, \text{acetone-water} \\
\text{EtOOC} & \quad (8:3), 0 \degree C \text{ to } 15 \degree C
\end{align*}
\]

The IR spectrum of the compound showed peaks at 2720 and 1743(broad) cm\(^{-1}\) showing the presence of aldehydic and carboxylate functionality. In the \(^1\)H NMR spectrum (Figure 101) a doublet at \(\delta 9.89\) with \(J = 1.9\) Hz clearly indicated the presence of aldehydic proton.

The \(^{13}\)C NMR spectrum (Figure 102) showed a peak at \(\delta 199.7\) supporting the presence of aldehydic functionality. Based on the spectral and analytical data of the compound the structure was assigned as 304.

In the subsequent steps, aldehyde 304 was reacted ethyl diazoacetate in the presence of catalytic BF3.OEt2 in CH2Cl2 to yield a thick liquid in 20% yield.

\[
\begin{align*}
\text{HO} & \quad \text{EDA, BF}_3, \text{OEt}_2, \text{CH}_2\text{Cl}_2 \\
\text{EtOOC} & \quad 0 \degree C \text{ to } 15 \degree C, 15 \text{ min.}
\end{align*}
\]

The IR spectrum showed broad peaks at 3223 and 1743 cm\(^{-1}\). The \(^1\)H NMR and \(^{13}\)C NMR was found to be very complicated and difficult to interpret. This could be due to the existence of keto-enol tautomerism in the compound. It is also likely that the presence of \(\alpha\)-oriented \(-\text{CHO}, -\text{OCH}_2\text{COOEt}\) and 1,2-acetonide groups makes the aldehyde 304 unstable to the reaction condition.
Our attempt to improve the yield by performing the reaction at different reaction conditions such as low temperature (−20 °C), use of catalytic amount of BF$_3$.OEt$_2$ and use of other Lewis acids like SnCl$_4$, TiCl$_4$, at −40 °C did not improve the yield. Due to the low yield of the β-keto ester 305 and the difficulties associated in characterizing the compound we have abandoned the project at this stage. However work is in progress to optimise and characterize the D-gulose derived β-keto ester and pursue the synthesis of griseolic acid as depicted in the Scheme 69.

![Scheme 69: Synthesis of griseolic acid](image)

3.4. Conclusion:

In conclusion, we have attempted the synthesis of griseolic acid using D-gulose derived β-keto ester.
3.5. Experimental:

Expt.No.3.5.1. Preparation of 1,2:5,6-di-O-isopropylidene α-D-glucopyranose 1,4-furan 3-one (297).

Pyridinium chlorochromate (20.00 g, 92.31 mmol) was added to a solution of diacetone D-glucose 129 (4.0 g, 15.38 mmol) molecular sieves (4 A, 13.20 g) in CH$_2$Cl$_2$ (100 mL). After stirring the reaction for 24 h, the reaction was diluted by adding diethyl ether (50 mL). The reaction mixture was then directly poured on silica column and the eluents evaporated to afford the ketone 297 (3.9 g, 98%).

Expt.No.3.5.2. Preparation of 3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-erythrose 1,4-furan-3-one (298).

To a solution of ketone 297 (10.00 g, 38.7 mmol) in pyridine (25 mL) at 0 °C was added acetic anhydride (20 mL) and catalytic amount of DMAP (0.028 g, 0.19 mmol) and stirred for 24 h at 30 °C. The reaction mixture was concentrated, and purified using column chromatography afforded enol acetate 298 as a white solid (9.40 g) in 80% yield.

mp 78 °C;
$R_f = 0.56$ (20% ethyl acetate-n-hexane);
$[\alpha]_D^{25} = +33.3$ (c 0.12, CHCl$_3$);
$\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1766, 1377;
δ\textsubscript{H} NMR (300 MHz, CDCl\textsubscript{3}) 1.36 (3H, s, CH\textsubscript{3}), 1.42 (3H, s, CH\textsubscript{3}), 1.44 (3H, s, CH\textsubscript{3}), 1.55 (3H, s, CH\textsubscript{3}), 2.11 (3H, s, COCH\textsubscript{3}), 4.07 (2H, ddd, J = 12.1, 8.5 and 6.6 Hz, H-6a, H-6b), 4.69 (1H, t, J = 6.6 Hz, H-5), 5.38 (1H, d, J = 5.5 Hz, H-2), 6.02 (1H, d, J = 5.5 Hz, H-1);
δ\textsubscript{C} NMR (75 MHz, CDCl\textsubscript{3}) 20.4 (COCH\textsubscript{3}), 25.5 (CH\textsubscript{3}), 25.7 (CH\textsubscript{3}), 27.8 (CH\textsubscript{3}), 65.7 (C-6), 68.4 (C-5), 80.6 (C-2), 103.8 (C-1), 110.1 (OCO), 113.1 (OCO), 128.7 (C-3), 144.9 (C-4), 168.5 (COCH\textsubscript{3});

Anal. Calcd. For C\textsubscript{14}H\textsubscript{20}O\textsubscript{7}: C, 55.99 H, 6.71; Found: C, 55.41 H, 6.66.

Expt.No.3.5.3. Preparation of 3-O-acetyl-1\textsubscript{2}:5,6-di-0-isopropylidene-\alpha\textsubscript{-d}-gulo-furanose (299).

\[
\text{\begin{array}{c}
\text{Enolacetate 298} \quad 1.00 \text{ g, 1.33 mmol} \\
\text{Pd-C (20 mg), 1 h, 40 } ^\circ \text{C} \\
\end{array}}
\]

To a solution of enolacetate 298 (1.00 g, 1.33 mmol) in dry ethanol at 40 °C was added Pd-C (20 mg) and hydrogenated at 20 psi. The reaction mixture was filtered through celite 540 and purified using column chromatography to afford gulose acetate 299 as a white solid (0.81 g, 80%).

mp 74 °C;

R\textsubscript{f} = 0.36 (20% ethyl acetate-n-hexane);

[\alpha]\textsubscript{D}\textsuperscript{25} = +60.0 (c 0.10, CHCl\textsubscript{3});

ν\textsubscript{max}(KBr)/cm\textsuperscript{-1} 1733, 1247.9;

δ\textsubscript{H} NMR (300 MHz, CDCl\textsubscript{3}) 1.35 (3H, s, CH\textsubscript{3}), 1.39 (3H, s, CH\textsubscript{3}), 1.45 (3H, s, CH\textsubscript{3}), 1.59 (3H, s, CH\textsubscript{3}), 2.13 (3H, s, COCH\textsubscript{3}), 4.07 (1H, t, J = 6.8 Hz, H-6a), 4.10 (1H, dd, J = 8.8 and 6.3 Hz, H-4), 4.62 (2H, m, H-5, H-6), 4.80 (1H, dd, J = 5.4 and 4.1 Hz, H-2), 5.06 (1H, dd, J = 6.3 and 5.4 Hz, H-3), 5.81 (1H, d, J = 4.1 Hz, H-1);
δc NMR (75 MHz, CDCl3) 20.4 (COCH3), 25.1 (CH3), 26.5 (CH3), 26.6 (CH3), 26.7 (CH3), 66.1 (C-6), 71.5 (C-5), 74.9 (C-4), 78.3 (C-2), 81.0 (C-3), 104.7 (C-1), 108.9 (OCO), 114.0 (OCO), 169.1 (COCH3);

Anal. Calcd. For C14H22O7: C, 55.68 H, 7.33; Found: C, 55.75 H, 7.28.

Expt. No. 3.5.4. Preparation of 1,2:5,6-di-O-isopropylidene α-D-diacetone D-gulose (300).

To a slurry of lithium aluminiumhydride (1.27 g, 33.16 mmol) in dry THF at 0 °C was added gulose acetate 299 (4.00 g, 13.22 mmol) in THF drop wise. The reaction mixture was stirred to room temperature for 2 h. Reaction was neutralized using ethyl acetate (10 mL) and ammonium chloride (2 mL). Filtered, concentrated and purified using column chromatography. Elution using hexane-ethyl acetate (2/8) afforded to afford gulose 300 as a white solid (3.29 g, 96%);

mp 103 °C;

Rf = 0.30 (50% ethyl acetate-n-hexane);

[α]$_D^{25}$ = +6.3 (c 0.94, CHCl3);

ν$_{max}$(KBr)/cm$^{-1}$ 3340;

δh NMR (300 MHz, CDCl3) 1.38 (3H, s, CH3), 1.43 (3H, s, CH3), 1.45 (3H, s, CH3), 1.63 (3H, s, CH3), 2.67 (1H, bs, (exchangeable with D$_2$O), OH), 3.72 (1H, dd, J = 8.6 and 7.3 Hz, H-4), 3.89 (1H, dd, J = 8.6 and 6.1 Hz, H-3), 4.22 (2H, m, H-5, H-6), 4.48 (1H, dt, J = 8.5 and 6.7 Hz, H-6), 4.66 (1H, dd, J = 6.1 and 4.1 Hz, H-2), 5.79 (1H, d, J = 4.1 Hz, H-1);

δc NMR (75 MHz, CDCl3) 25.2 (CH3), 26.7 (CH3), 27.2 (CH3), 27.3 (CH3), 66.3 (C-6), 69.6 (C-3), 75.5 (C-5), 79.8 (C-2), 84.2 (C-4), 105.2 (C-1), 109.1 (OCO), 114.9 (OCO);
Expt.No.3.5.5. Preparation of 3-Deoxy-1,2:5,6-di-O-isopropylidene-α-D-gulo-furanose (301).

To a methanolic solution of enolacetate 298 (0.20 g, 0.26 mmol) at reflux was added a mixture of ammonium formate and 10% Pd-C and stirred for 15 min. The reaction mixture was filtered over celite and purified over column chromatography to afford a white solid 301 (0.14 g, 73%).

mp 81 °C;

$R_f = 0.40$ (25% ethyl acetate-n-hexane);

$[\alpha]_D^{25} = -28.4$ (c 0.10, CHCl$_3$);

$\nu_{\text{max}}$(KBr)/cm$^{-1}$ 1247.9;

$\delta$H NMR (300 MHz, CDCl$_3$) 1.32 (3H, s, CH$_3$), 1.37 (3H, s, CH$_3$), 1.44 (3H, s, CH$_3$), 1.57 (3H, s, CH$_3$), 1.82 (1H, dd, $J = 14.0$, 3.5 Hz, H-3), 2.21 (1H, m, H-3), 3.60 (1H, t, $J = 7.1$ Hz, H-6), 4.01-4.15 (2H, m, H-2, H-4), 4.42 (1H, m, H-6), 4.68-4.75 (1H, m, H-5), 5.79 (1H, d, $J = 3.5$ Hz, H-1);

$\delta$C NMR (75 MHz, CDCl$_3$) 25.1 (CH$_3$), 26.0 (CH$_3$), 26.5 (CH$_3$), 27.1 (CH$_3$), 33.3 (C-3), 65.1 (C-6), 77.4 (C-5), 80.1 (C-2), 81.3 (C-4), 106.2 (C-1), 109.1 (OCO), 112.6 (OCO);

Anal. Calcd. For C$_{12}$H$_{20}$O$_5$: C, 59.00 H, 8.25; Found: C, 58.78 H, 8.32.

Expt.No.3.5.6. Preparation of 3-O-Carbethoxymethylene-1,2:5,6-di-O-isopropylidene-α-D-gulo-1,4-furanose (302).
To a slurry of 60% NaH (0.23 g, 5.76 mmol) in dry THF at 0 °C a solution of gulose 300 in THF was added dropwise and stirred well for 5 minutes. Ethyl bromoacetate and TBAI was added sequentially to the reaction mixture and stirred to 30 °C for 6 h. Reaction mixture was concentrated, extracted using CHCl₃ (3 X 15 mL), concentrated and purified on column chromatography to afford C-3-O-alkylated gulose 302 as a white solid (1.19 g) in 90% yield.

mp 84 °C;

$R_f = 0.64$ (30% ethyl acetate-n-hexane);

$[\alpha]_D^{25} = +43.3$ (c 3.74, CHCl₃);

$\nu_{\text{max}}$(KBr)/cm⁻¹ 1745, 1215;

$\delta_H$ NMR (300 MHz, CDCl₃) 1.29 (3H, t, $J = 6.8$ Hz, OCH₂CH₃), 1.36 (6H, s, 2 X CH₃), 1.44 (3H, s, CH₃), 1.58 (3H, s, CH₃), 3.70 (1H, dd, $J = 8.8$ and 7.1 Hz, H-4), 4.04-4.31 (7H, m, 2 X H-6, H-3, OCH₂CH₃, OCH₂CO), 4.60-4.65 (2H, m, H-5, H-2), 5.77 (1H, d, $J = 3.8$ Hz, H-1);

$\delta_C$ NMR (75 MHz, CDCl₃) 14.1 (OCH₂CH₃), 25.1 (CH₃), 26.5 (CH₃), 26.6 (CH₃), 26.7 (CH₃), 61.1 (OCH₂CH₃), 66.7 (C-6), 67.4 (C-5), 75.1 (C-3), 78.2 (C-4, OCH₂CO), 78.3 (C-2), 104.7 (C-1), 108.3 (OCO), 114.0 (OCO), 169.3 (COOEt);

Anal. Calcd. For C₁₆H₂₆O₈: C, 55.44 H, 7.51; Found: C, 55.28 H, 7.36.

Expt.No.3.5.7.Preparation of 3-O-Carbethoxymethylene-1,2-O-isopropylidene-α-D-gulo-1,4-furanose (303).
To a chilled solution of alkylated gulose 302 (0.35 g, 0.10 mmol) in dry CH₂Cl₂ at 0 °C 20% TFA-H₂O (1.60 mL) was added drop wise over a period of 6 h. The reaction mixture was neutralized using strong basic resin, filtered, concentrated, purified using column chromatography to yield gulose diol 303 (0.22 g) in 68% yield.

\( R_f = 0.54 \) (ethyl acetate);

\( \left[\alpha\right]^{25}_{D} = +43.3 \) (c 3.74, CHCl₃);

\( \nu_{max} \) (Neat)/cm\(^{-1}\) 3100-3600 (broad), 1745, 1215;

\( \delta_H \) NMR (300 MHz, CDCl₃) 1.29 (3H, t, \( J = 7.1 \) Hz, OCH₂CH₃), 1.36 (3H, s, CH₃), 1.60 (3H, s, CH₂), 2.59 (1H, bs, exchangeable with D₂O, OH), 3.07 (1H, bs, exchangeable with D₂O, OH), 3.70-3.78 (2H, m, H-3, H-4), 4.05-4.38 (7H, m, 2 X H-6, H-5, OCH₂CH₃, OCH₂CO), 4.71 (1H, t, \( J = 4.5 \) Hz, H-2), 5.75 (1H, d, \( J = 4.4 \) Hz, H-1);

\( \delta_C \) NMR (75 MHz, CDCl₃) 14.0 (OCH₂CH₃), 26.2 (CH₃), 26.4 (CH₃), 61.0 (OCH₂CH₃), 63.1 (OCH₂CO), 67.5 (C-6), 69.5 (C-5), 78.5 (C-3), 78.7 (C-2), 78.8 (C-4), 104.3 (C-1), 114.2 (OCO), 169.7 (COOEt);

Anal. Calcd. For C₁₃H₂₂O₈: C, 50.97 H, 7.24; Found: C, 50.94 H, 7.3.

Expt.No.3.5.8.Preparation of 3-O-Carboxethoxymethylene-1,2-O-isopropylidene-\( \alpha \)-D-1,4-furanose (304).

Expt.No.3.5.8. Preparation of 3-O-Carboxethoxymethylene-1,2-O-isopropylidene-\( \alpha \)-D-1,4-furanose (304).
To a solution of diol 303 (0.93 g, 3.03 mmol) in acetone-water (15 mL, 10:3), NaIO₄ (0.78 g, 3.68 mmol) was added at 0 °C and stirred for 30 min at 15 °C. The excess of NaIO₄ was decomposed using ethylene glycol (0.2 mL), concentrated, extracted using CHCl₃ (3 X 15 mL). Combined organic layer was concentrated and purified on column chromatography to yield aldehyde 304 (0.64 g) in 78% yield as a thick liquid.

\[ R_f = 0.60 \text{ (50\% ethyl acetate-n-hexane)}; \]

\[ [\alpha]_{D}^{25} = -23.3 \text{ (c 1.8, CHCl₃)}; \]

\[ \nu_{\text{max}} \text{(Neat)/cm}^{-1} \text{2374, 1743;} \]

δ\text{H} \text{NMR (300 MHz, CDCl₃) 1.27 (3H, t, } J = 7.1 \text{ Hz, OCH}_2\text{CH}_3), 1.35 (3H, s, CH}_3\text{), 1.58 (3H, s, CH}_3\text{), 4.23 (2H, q, COOCH}_2\text{CH}_3\text{), 4.28 (2H, s, OCH}_2\text{COOEt), 4.47 (1H, dd, } J = 8.2 \text{ and 1.9 Hz, } H-4\text{), 4.53 (1H, dd, } J = 8.2 \text{ and 4.1 Hz, } H-3\text{), 4.75 (1H, dd, } J = 4.1 \text{ and 3.5 Hz, } H-2\text{), 5.84 (1H, d, } J = 3.5 \text{ Hz, } H-1\text{), 9.89 (1H, d, } J = 1.9 \text{ Hz, } H\text{CO);} \]

δ\text{C} \text{NMR (75 MHz, CDCl₃) 14.0 (OCH}_2\text{CH}_3\text{), 25.8 (CH}_3\text{), 26.1 (CH}_3\text{), 61.1 (OCH}_2\text{CH}_3\text{), 67.9 (OCH}_2\text{CO}, 77.3 (C-3), 81.1 (C-4), 81.7 (C-2), 104.8 (C-1), 114.1 (OCO), 169.5 (COCH}_3\text{), 199.7 (CHO);} \]


Expt.No.3.5.9. Preparation of Ethyl-6-deoxy-1,2-\text{O}-isopropylidene-3-\text{O}-carbethoxymethylene-\text{-\alpha-D-hept-5-ulo-1,4-furanuronate (305).}
To a solution of gulose aldehyde 304 (0.9 g, 3.28 mmol) in dry CH₂Cl₂ at 0 °C EDA (ethyldiazoacetate) (0.74 g, 7.56 mmol) in CH₂Cl₂ was added and made a homogenous solution. To the reaction mixture BF₃.OEt₂ (0.21 mL, 1.64 mmol) in CH₂Cl₂ was added drop wise over a period of 15 min. The reaction mixture was neutralized using saturated solution of sodium bicarbonate (5 mL), extracted using CHCl₃ (3 X 15 mL). The combined organic layer was dried over sodium sulphate and purified on column chromatography to yield β-keto ester 305 (0.32 g) as a thick liquid in 20% yield.

\[ R_f = 0.57 \text{(40% ethyl acetate-n-hexane)}; \]

\[ [\alpha]_D^{25} = -25.1 (c 2.1, CHCl₃); \]

\[ \nu_{\text{max}}(\text{Neat})/\text{cm}^{-1} 1743, 1733, 1719; \]

\[ \delta_H \text{ NMR (300 MHz, CDCl₃)} \text{ and } \delta_C \text{ NMR (75 MHz, CDCl₃)} \text{ showed doubling of signals as a result of keto-enol tautomerism}; \]
References


Abstract of the thesis entitled “Synthesis and glycosidase inhibitory activity studies of monocyclic and bicyclic iminosugars from D-glucose and an attempt towards the synthesis of griseolic acid using rhodium (II) carbenoid chemistry” to be submitted to the University of Pune for the Degree of Doctor of Philosophy in Chemistry by Mr. Ajish Kumar K. S under the guidance of Prof. Dilip D. Dhavale, Department of Chemistry, University of Pune, Pune.

The thesis is divided into three chapters

Chapter 1: Synthesis and Study of sugar derived Aziridine carboxylate and its application in the synthesis of Piperidine and pyrrolidine Analogues.

Introduction

Part A: Study of Sugar Derived Aziridine Carboxylates

The aziridine carboxylic esters of type A (R<sup>1</sup> = alkyl, aryl) play a vital role in the synthetic sequence due to their inherent capability to undergo nucleophilic ring opening either at C-2 or C-3 giving an access to differentially substituted β- or α-amino esters, respectively (Figure 1). The weak electrophilic nature of the aziridine carboxylate A is overpowered either by incorporation of an electron-withdrawing group (e.g. R<sup>3</sup> = sulphonyl) on the aziridinic nitrogen atom and/or by the use of acidic reaction conditions. In general, nucleophiles such as alcohols, Wittig reagents, thiols, indoles and amines attack at the C-3 carbon leading to the aziridine ring opening in the usual conjugate addition pathway, yielding α-amino esters.

Figure 1

A few examples are known wherein organocuprates, malonates and some indole derivatives react at both C-3 and C-2 of A to give a mixture of products. However, azidotrimethylsilane and lithium dimethylcuprate undergo regioselective aziridine ring opening at the C-2-N bond to give β-amino esters. In this context, we have studied the formation of sugar aziridine carboxylate A (R<sup>1</sup> = sugar) and aziridine ring opening with different nucleophiles. Thus, Wittig olefination of sugar aldehyde 1 derived from D-glucose using Ph<sub>3</sub>P=CBrCOOEt (Scheme 1) in dichloromethane afforded bromoenose 2 which on reaction with benzylamine (15 equiv.) afforded aziridine carboxylic ester 3 in good yield. The trans-aziridine geometry of 3 was established by <sup>1</sup>H NMR analysis and single crystal X-ray analysis.
Part B: Synthesis of Piperidine alkaloids

Nitrogen incorporated polyhydroxylated carbocyclic ring systems, commonly known as azasugars or iminosugars, are of great importance as they exhibit significant glycosidase inhibitory activity. Among these, the poly hydroxylated piperidine (e.g. 1-deoxynojirimycin 5a, homonojirimycin 5b, fagomine 5c) (Figure 2) alkaloids selectively inhibit glycosidase that modify glycoconjugates by hydrolyzing glycosidic linkages and are therefore potential candidates as antiviral, antibacterial or, antimitastatic agents.

Figure 2. Nojirimycin and analogues

In the search for structure-activity relationship, a number of six and five membered azasugar analogues were synthesized and evaluated for their biological activities. We thought of exploiting aziridine carboxylate 3 in the synthesis of 1-deoxy-L-idonojirimycin 6 and 7. Thus, reaction of 4 (Scheme 2) with LAH followed by amine protection, oxidative cleavage of diol, and sodium borohydride reduction yielded primary alcohol 8. The hydrolysis of 1,2-acetonide functionality in 8 with TFA-water followed by treatment with ammonium formate with 10% Pd/C in methanol gave 1-deoxy-L-ido-nojirimycin 6 as a thick liquid. The utility of α-hydroxy-β-aminoester 4 was also demonstrated by the synthesis of the new piperidine analogue 7. Thus, reduction of the ester functionality in 4 with LAH in THF followed by treatment with ammonium formate in the presence of 10% Pd/C, in methanol, afforded an aminotriol that was reacted with benzylchloroformate to give the N-Cbz protected triol 9. The cleavage of 1,2-acetonide group and hydrogenation afforded 1,5-dideoxy-1,5-imino-6-hydroxy-β-L-glycero-L-ido-heptitol 7 as a thick oil.

Part C: Synthesis of Pyrrolidine alkaloids
We have also successfully transformed α-hydroxy-β-aminoester 4 into the pyrrolidine alkaloids (Scheme 3 and 4). Thus, cleavage of 1,2-acetonide functionality in 8 by TFA-water and treatment with sodium metaperiodate afforded the one carbon degraded aminal that was directly subjected to hydrogenation with 10% Pd/C in methanol to give 1,4-dideoxy-1,4-imino-L-xylitol 10. The reaction of 10 with MeOH.HCl afforded hydrochloride salt of 10 as a sticky solid. The trihydroxy intermediate 9 was (Scheme 4) also exploited in the synthesis of pyrrolidine alkaloid 11. Thus, N-Cbz protected triol 9 was reacted with acetic anhydride in pyridine to give the tri-acetylated compound 12.

Cleavage of 1,2-acetonide functionality in 12, oxidative cleavage of C-1-C-2 bond and treatment with ammonium formate, 10% Pd/C in methanol yielded a product which on treatment with sodium methoxide in methanol (to remove acetate) at 0 °C afforded 1,4-dideoxy-1,4-imino-5-hydroxy-L-iditol 11 as a crystalline white solid.

Part D: Glycosidase inhibitory activity of piperidine and pyrrolidine alkaloids

The biological screening of piperidine 6, 7 and pyrrolidine 10, 11 alkaloids were studied and the compounds show inhibitory activity in millimolar range.

Chapter 2: Synthesis and glycosidase inhibitory activity studies of Uniflorine A, Lentigenosine, Swainswonine analogues, and Quinolizidine alkaloids

Introduction:
Polyhydroxylated indolizidine alkaloids have received a considerable attention in recent years because of their inherent ability to inhibit a specific class of hydrolytic enzymes that cleave different types of glycosidic linkages. For example, castanospermine—a tetrahydroxy indolizidine alkaloid, isolated from *Castanospermum australe* is found to be a potent and selective inhibitor of β-glucosidases with IC\(_{50}\) of 8 μM so is used in the treatment of viral infection like AIDS. Other promising indolizidine iminosugars include Swainswonine and Lentigenosine. A number of analogues of indolizidine are known among those, the recently isolated Uniflorine A—a pentahydroxylated indolizidine alkaloid showed activity toward maltase and sucrase with IC\(_{50}\) values of 12.0 and 3.5 μM. In the literature there is ambiguity in the structure of Uniflorine A as reported by Pyne and Mariano. We have attempted the synthesis of new pentahydroxy indolizidine alkaloid using sugar derived aziridine carboxylate as a ‘chiral synthon’.
Part A: Synthesis of Uniflorine A analogues
As shown in Scheme 5 the Wittig olefination of aminal 13 with Ph$_3$P=CHCOOEt afforded 14. Asymmetric dihydroxylation of 14 using AD mix-α and AD mix-β afforded a diastereomeric mixture of vicinal diols 15a and 15b. Compound 15b was obtained as a colorless solid and the single crystal X-ray analysis established the absolute configurations at the newly generated stereocenters as 6S and 7S. The dihydroxylated product 15a was subjected to hydrogenolysis (10% Pd-C and ammonium formate in methanol reflux) to give γ-lactam 16a. Reduction of lactam functionality in 16a with LAH in THF gave pyrrolidine, which on reaction with CbzCl gave 17a. In subsequent steps, opening of 1,2-acetonide group followed by hydrogenation and purification by chromatography afforded 18 as a white solid. The same reaction sequence was repeated for 20. The target molecules were further peracetylated to yield 19 and 21. The physical and spectral data as well as the optical rotation for 18 and 20 were not in agreement with the isolated compound Uniflorine A.

Part B: Synthesis of 8a-epi-homocastanospermine and 1-deoxy-8a-epi-homocastanospermine alkaloid
Synthesis of 8a-epi-homocastanospermine
Aziridine ester 3 (Scheme 6) was selectively reduced using DIBAL-H, to give aldehyde 22 which was subjected to two carbon homologation using the Wittig olefination resulting in the formation of E and Z isomer 23 in the ratio 9:1. The resultant aziridine conjugated ester 23 was then subjected to aziridine ring opening reaction using water as the nucleophile to get γ-hydroxy-δ-amino-α,β-unsaturated ester 24 with 100% regioselective ring opening. The amino ester 24 on hydrogenolysis produced six membered lactam 25 that on reduction, protection followed by TFA-water treatment and cyclization resulted in the formation of 8a-epi-homocastanospermine 26.
Synthesis of 1-deoxy-8a-epi-homocastanospermine alkaloid
We realized that the aziridine-conjugated ester 23 could also be used for the synthesis 1-deoxy-8a-epi-homocastanospermine. Thus, hydrogenolysis of 23 under 80 psi (Scheme 7) afforded an aminoester 27 which was in situ subjected to sodium ethoxide treatment to afford lactam 28 in quantitative yield. The lactam on reduction, protection, TFA-water treatment and hydrogenation afforded 1-deoxy-8a-epi-homocastanospermine 29.

Part C: Synthesis of Swainswonine and Lentiginosine analogue
Lactam 25 and 28 (Scheme 8) were individually subjected to benzyl protection to afford the respective benzyl protected lactam 30 and 32. Treatment of 30 and 32 separately with TFA-H2O, metaperiodate cleavage and hydrogenation afforded the Swainswonine 31 and Lentiginosine 33 analogues respectively.

Part D: Glycosidase inhibitory activity studies
The glycosidase inhibitory studies revealed that except 1-deoxy-8a epi-castanospermine, all the other compounds showed moderate to good inhibitory activity towards the glycosidases tested.
Chapter 3: An attempt towards the synthesis of Griseolic acid

Introduction

Griseolic acids are a family of complex nucleosides isolated from the cultured broths of *Streptomyces griseoaurantiacus*. They are known to have inhibitory activity against cyclic adenosine 3', 5' monophosphate phosphodiesterase. The core structure of griseolic acid i.e. the bicyclic [3,3,0] oct-3-ene ring with quaternary carbon having -COOH and -CH2COOH substituent was constructed using Rh(II) catalyzed reaction of D-gulose derived α-diazo-β-keto ester. Thus, diacetone D-glucose 34 was converted to 1,2,5,6-diacetone-3-O-acetyl-α-D-gulose 35 using (Scheme 9) standard literature procedures. Acetate 35 on reduction followed by alkylation and hydrolysis afforded -OCH2COOEt (alkylated) gulose diol 36 in moderate yield. Diol 36 on periodate oxidation followed by ethyl diazo acetate treatment resulted in β-keto ester 37 in 20% yield. We are trying to improve the yield and transform the ketoester 37 into diazo compound. Our efforts in the direction of the synthesis of Griseolic Acid 42 will be discussed in detail.

![Diagram](image_url)

**Scheme 9.** Reagents and conditions: (a) i) LAH, THF, 0 °C to rt; ii) EtBrOAc, NaH, THF, 0 °C to rt; iii) hydrolysis; (b) i) NaIO4, Acetone- water, 10 °C; ii) EDA, BF3.OEt2, CH2Cl2, 10 °C; (c) MsN3, Et3N, CH3CN, 15 °C to rt; (d) Rh2(OAc)4, benzene, reflux; (e) NaBH4, EtOH, 15 °C; (f) dehydration.

Prof. Dilip D. Dhavale  
(Research Guide)  
Ajish Kumar K. S  
(Research student)

Efficient synthesis of 1,8,8a-tri-epi-swainsonine, (+)-1,2-Di-epi-lentiginosine, (+)-9a-epi-homocastanospermine and (-)-9-Deoxy-9a-epi-homocastanospermine and study of their glycosidase inhibitory activities. K. S. Ajish Kumar, Vinod D. Chaudhari, and Dilip D. Dhavale. *Organic and Biomolecular Chemistry*, 2007 (Accepted)