Chapter One

Synthesis of Avenanthramides
Constituents of Oats (Avena sativa L)
Introduction

What are Avenanthramides?

Oats have been considered to be a good source of antioxidants for a long time\(^1\) and several compounds in oats are known to have this antioxidant activity. These compounds are flavonoids, Vitamin E (tocopherols and tocotrienols), phenolic acids in free form (derivatives of benzoic and cinnamic acids) and esterified forms\(^2\).

Recently\(^3\) a group of amides trivially named avenanthramides has been isolated from oats. Interestingly, it has been shown that this antioxidant activity and the fresh taste of oat products is mainly due to the presence of these avenanthramides\(^2\). In fact, avenanthramides are the major phenolic constituents\(^4\) occurring in relatively high concentrations, about 0.2-0.8 mg/g, in the outer regions of the oat kernel e.g. bran and sub-aleurone layers\(^5\).

\[
\begin{align*}
&\text{HO} \\
&\text{ON} \\
&\text{COON} \\
&\text{I} \\
&\text{—, OH} \\
\end{align*}
\]

avenanthramide

They are low molecular weight soluble phenolic compounds, a group of nitrogen-containing constituents, collectively named as avenanthramides which are not present in any other cereal grains\(^3\).

Chemically avenanthramides are substituted \(N\)-cinnamoylanthranilate derivatives\(^6\) i.e. they are substituted hydroxycinnamic acid amides with simple anthranilic acid or its 5- or 4-hydroxy derivatives.

These avenanthramides are phytoalexins produced when oat leaves are infected or inoculated with an incompatible race of crown rust fungus (\textit{Puccinia coronate f. sp. Avenae})\(^7,8\) or by the treatment of oat leaves with various elicitors.
including chitin fragments\textsuperscript{9}, a host specific toxin\textsuperscript{10} victorin C, heavy metal ions\textsuperscript{11} and Ca ionophore\textsuperscript{12}. Although avenanthramides have also been isolated from oat groats and hulls\textsuperscript{3}, none appear to be present in healthy leaves prior to inoculation with pathogens\textsuperscript{6}.

**What are Phytoalexins?**

Plants express a variety of resistance responses to parasites, including fungal infections through various defence reactions. One of these reactions is the production of inducible secondary metabolites, antifungal substances, such as phytoalexins\textsuperscript{13}. These phytoalexins are shown to be important in preventing the growth of micro organisms at the site of the infection or rejecting the pathogens on the basis of their toxicity.

Avenanthramides belong to a sub-class of hydroxycinnamic acid amides\textsuperscript{6}. These amides are suggested to play important role in reinforcement of cell walls. For instance hydroxycinnamic acid amide of tyramine found in solanaceous plants have been indicated to be a component of suberin that accumulates in mechanically damaged tissues\textsuperscript{14}.

![N-p-coumaroyltyramine](image)

Avenanthramides may have been recruited as phytoalexins from those compounds that function to reinforce cell walls because of their toxicity to pathogens and may still have dual role to play that is as phytoalexins in chemical defence and as substrates for the reinforcement of cell walls in physical defence\textsuperscript{15}. The degree of contribution to these roles is dependent on the species of avenanthramides. However, it may be noted that phytoalexins are not necessarily the end products of plant metabolism.
Source of Avenanthramides

Avenanthramides are unique to oats and are not present in any other cereal grains\textsuperscript{5}. Oat phytochemicals can be roughly divided\textsuperscript{4} into low molecular weight, readily soluble “free phenols” and high molecular weight covalently linked to complex, insoluble cell components “bound phenols”.

In addition to avenanthramides, the low molecular weight soluble oat phenolics include\textsuperscript{4} tocopherols, tocotrienols, flavonoids, hydroxycinnamates, ferulic acid, caffeic acid, vanillic acid, \textit{p}-hydroxyphenylactic acid, protocatechuic acid, syringic acid, \textit{p}-coumaric acid, sinapic acid, etc.

The “free phenols” appear to represent readily absorbed sources of antioxidants in the human diet, while the insoluble “bound phenols” such as lignin, cell wall polysaccharides, structural and/or storage proteins etc. present different challenges in the attempt to evaluate their long term efficacy since they require further metabolism before absorption from the gastrointestinal tract\textsuperscript{4}.

Characteristic Physicochemical Properties of Avenanthramides

The natural avenanthramides are pale yellow to yellow-green crystalline substances having comparatively high melting points. They are soluble in organic solvents like ethylacetate, diethyl ether and aqueous mixture with acetone or the lower alcohols but are relatively insoluble in chloroform, benzene or water\textsuperscript{3}. They are resistant to acid hydrolysis but are slowly hydrolysed with some decomposition in alkali to the corresponding substituted cinnamic and anthranilic acids\textsuperscript{3}. Avenanthramides have an intermediate lipophilicity and seem to be heat stable\textsuperscript{5}.

In daylight and UV light, they may easily undergo \textit{Z-E} rearrangement but without prior exposure to UV light, both \textit{E} and \textit{Z} isomers exist in solution as a photomediated interconvertible mixture\textsuperscript{3}. 
To find out whether the E isomer only or both isomers are naturally occurring in oats would require that extraction, purification and estimation be carried out in the absence of UV and daylight.

Detection, Identification and Isolation of Naturally Occurring Avenanthramides

The silica gel two dimensional TLC analysis of the extracts of oat groats and hulls based on chromatographic diagnostic colour responses and preliminary MS data has revealed that there are at least 40 chromatographically distinct avenanthramides in addition to other related phenolics. This diversity of closely related large number of phenolics coupled with their individual occurrence in very small quantities has made detection, isolation and identification of avenanthramides difficult. Therefore very few avenanthramides have been individually ‘isolated’ and characterized.

To our knowledge, till date, only 17 different avenanthramides isolated from oats are known in the literature with their structures and spectroscopic data. In fact, it is not proper to say that they have been isolated because except one i.e. avenanthramide A which was isolated by Miyagawa et al., as yellow amorphous powder (15.7 mg), all other remaining 16 avenanthramides have been detected in oat extracts and identified by comparison of the retention times and UV and or MS data of their individual peaks in the HPLC chromatograms with those of synthetic standards.

Avenanthramides were formerly called avenalumins (benzoxazin-4-one derivatives) by Mayama et al who first reported in 1981 the occurrence of nitrogen containing phytoalexins in oat leaves. They were the first to isolate 100 mg (from 10 kg of infected fresh oat leaves) of a major oat phytoalexin and
named it as avenalumin I followed by related minor components avenalumin II and III.

Later on in 1989, Collins carried out fractionation of the methanolic extracts of oat groats and hulls using ion exchange column chromatography followed by analytical and preparative TLC and showed for the first time the presence of a group of closely related oat phytoalexins as open amides and trivially named them avenanthramides.

He also observed that 2-aryl-1,3-benzoxazin-4H-ones readily undergo hydrolysis in aqueous media to give the corresponding (N-aroylamido)benzoic acids and anticipated that the major biologically active phytoalexin may be the open amide (avenanthramide) rather than the benzoxazinone derivative (avenalumin) as reported by Mayama et al.

It may be noted that avenalumins were isolated from oat leaves and not from oat grain. Experiments using non-aqueous extraction solvents and/or rapid identification procedures may be necessary to establish the presence of avenalumins in the oat grain.
However, in 1990 Crombie and Mistry\textsuperscript{8}, recorded spectroscopic data on avenalumins prepared by them and comparison of this data with that reported by Mayama et al\textsuperscript{7} clearly indicated that the natural phytoalexin from oat leaves isolated\textsuperscript{7} is in fact the avenanthramide A and not avenalumin I.

Further, Collins\textsuperscript{3} succeeded in separation, purification and identification of ten avenanthramides using $^1$H & $^{13}$C NMR, MS & UV and this constituted the first report on the isolation of avenanthramides from the oat extracts (Table 1).

**Table 1:** First report\textsuperscript{3} on isolation of avenanthramides from oats

<table>
<thead>
<tr>
<th>Avenanthramide A</th>
<th>Avenanthramide A-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Avenanthramide A" /></td>
<td><img src="image" alt="Avenanthramide A-1" /></td>
</tr>
<tr>
<td>Avenanthramide B</td>
<td>Avenanthramide B-1</td>
</tr>
<tr>
<td><img src="image" alt="Avenanthramide B" /></td>
<td><img src="image" alt="Avenanthramide B-1" /></td>
</tr>
<tr>
<td>Avenanthramide C</td>
<td>Avenanthramide C-1</td>
</tr>
<tr>
<td><img src="image" alt="Avenanthramide C" /></td>
<td><img src="image" alt="Avenanthramide C-1" /></td>
</tr>
<tr>
<td>Avenanthramide D</td>
<td>Avenanthramide D-1</td>
</tr>
<tr>
<td><img src="image" alt="Avenanthramide D" /></td>
<td><img src="image" alt="Avenanthramide D-1" /></td>
</tr>
</tbody>
</table>
The only avenanthramide having -OCH₃ and -OH groups at C₄ and C₅ positions of the anthranilic acid moiety named avenanthramide A-2 was detected and identified along with avenanthramide B from the extracts of oat bran and was shown to have the structure shown below⁵. Although the concentration of A-2 was too low to be estimated, it was found to have significantly higher antioxidant activity than that of caffeic acid, ferulic acid and vanillin.

While studying the action of avenanthramides in the modulation of the inflammatory process associated with the development of atherosclerosis, the following four avenanthramides G, H, K and P were detected⁴ by using analytical HPLC. Individual peaks were identified by comparison of their retention times and UV spectra with authentic standards.

Formation of avenanthramides G and L was observed⁶ during the study of the biosynthesis of avenanthramides by administering labelled putative precursors to oat leaves.
Recently a new dimerized avenanthramide named bisavenanthramide B was found to accumulate when oat leaves were treated with elicitors due to a reaction of avenanthramide B (an oat phytoalexin) with peroxidase in the presence of hydrogen peroxide.\textsuperscript{16}

\textbf{Biosynthesis of Avenanthramides}

The chemical structure of avenanthramides suggests that these compounds are biosynthesised by the condensation of cinnamic acid derivatives with anthranilates or hydroxyanthranilates.\textsuperscript{6} It has been demonstrated by administering labelled putative precursors to oat leaf segments that
avenanthramides are de novo synthesised from primary metabolites and phenylpropanoid metabolism is involved in their biosynthesis.

The biosynthesis of avenanthramides has been investigated with feeding experiments and measurement of enzyme activities. Feeding of elicited oat leaves with labelled L-phenylalanine and anthranilic acid revealed that avenanthramides are produced from these precursors. Anthranilic acid and L-phenylalanine are derived from chorismate biosynthesised via the shikimate pathway as shown below in a proposed biosynthetic pathway to avenanthramides.

![Scheme 1: Proposed biosynthetic pathway to avenanthramides](image)

Anthranilate synthase and chorismate mutase, which catalyse the first reactions leading to anthranilate and phenylalanine, respectively, have been shown to be regulated by elicitor treatment.

Moreover, hydroxycinnamoyl-CoA:hydroxyanthranilate N-hydroxycinnamoyl transferase (HHT), which catalyses the final condensation reaction,
was identified\textsuperscript{6} in oat leaves treated with oligo-\textit{N}-acetylchitooligosaccharides and victorin C.

All the putative precursors of a series of avenanthramide found in the elicited leaves, acted to a greater or lesser extent, as substrates, suggesting that each of the avenanthramide was individually synthesized by the condensation of the corresponding substituted anthranilic acid and the substituted cinnamoyl-CoA thioesters\textsuperscript{17}.

**Reported Methods of Synthesis of Avenanthramides**

Avenanthramides have been prepared in milligram quantities mainly for the purpose of detection and identification in oat extracts\textsuperscript{2,3}, structure-antioxidant activity studies\textsuperscript{2} and other bioactivity studies\textsuperscript{4,18}. They are also prepared for providing support to the structure assigned\textsuperscript{3}.

**First Synthesis of Avenalumin**

Mayama et al\textsuperscript{7} were the first to isolate in 1981 the major phytoalexin, avenalumin I from oat leaves and confirm its structure (2-styryl-1,3-benzoxazin-4\textit{H}-one) by its synthesis from 5-hydroxyanthranilic acid in 45% yield.
However, when Crombie and Mistry\textsuperscript{8} repeated this synthetic scheme of avenalumin I reported by Mayama et al\textsuperscript{7}, they obtained the open amide avenanthramide A instead of the reported avenalumin I. Of course they did succeed in preparing the cyclized products (avenalums) from the corresponding avenanthramides which were prepared by using Collin’s method\textsuperscript{3} described below. Spectroscopic data especially IR and $^{13}$C NMR recorded on both avenalums and avenanthramides indicated that the natural phytoalexin from oat leaves isolated and then synthesized by Mayama et al\textsuperscript{7} is in fact the avenanthramide A and not avenalumin I.

**First Synthesis of Avenanthramides**

Collins\textsuperscript{3} who first isolated the oat phytoalexins as open amide and named them ‘avenanthramides’, confirmed their structures by synthesis using a modification of the Bain and Smallley’s procedure\textsuperscript{19} in which suitably protected substituted cinnamoyl chlorides are condensed with anthranilic acid in presence of pyridine followed by treatment with mild alkali to remove the protecting group. However, these methods\textsuperscript{3,8} involve additional protection and deprotection steps and the yields varied from 55-83%.

The structure of avenanthramide G identified\textsuperscript{12} as a stress compound in oats, induced by victorin, a host specific toxin from *Helminthosporium victoriae* was confirmed by its synthesis using above mentioned Collin’s procedure\textsuperscript{3}. The required 4-hydroxyanthranilic acid was prepared from 2,4-dinitrobenzoic acid and condensed with 4-acetylcinnamoyl chloride prepared from 4-hydroxycinnamic acid.
Bratt et al. used a modified version of the method described by Mayama et al. and synthesized nine avenanthramides in comparatively low (≈ 40%) yields to identify them in oat extracts and to study the structure-activity relationship.

Thus there are only two methods reported in the literature for the synthesis of avenanthramides: one is Collins' modification of Bain and Smalley and the other is Bratt et al.'s modification of Mayama et al. Others have used either of these two methods for the synthesis of avenanthramides.

**Synthesis of Avenanthramides Using a Two-Step General Procedure**

Although avenanthramides have been synthesized in 40-80% yields, the only two methods reported utilize acid chlorides and additional protection-deprotection steps are involved which make the synthetic process non-economical. Therefore, a need of a general and efficient method was felt to prepare these naturally occurring oat phytoalexins, avenanthramides, in good yields so that they can be easily detected in oat extracts and further to have them in sufficient quantities to study their biological activity.

In our laboratory a simple two-step general procedure was developed for the synthesis of natural cinnamyl esters and their analogues in good yields by condensation of monomalonates with substituted benaldehyde derivatives.

These monomalonates were obtained in high yields by simply heating Meldrum’s acid with alcohols or phenols in benzene or toluene.
The salient advantage of this method is that it does not involve any type of chromatography for separation and purification. The intermediate monomalonates can be chemically separated and purified. As such, this method can be conveniently scaled up.

Therefore, we decided to extend this two-step method previously used for the preparation of cinnamyl esters, to prepare avenanthramides by replacing alcohols and phenols with amines as shown below.

**First step**

\[
\text{toluene reflux, 4h} \quad \text{Ar-CHO} + \overset{\text{R-0H}}{\text{towards}} \quad \overset{\text{Meldrum's acid}}{\text{1}} \quad \overset{\text{R1-O-C-OH}}{\text{monomalonate}} \quad \overset{\text{Ar-CHO}}{\text{3 cinnamyl ester}}
\]

**Second step**

Taking note of the possible nucleophilic attack by the \(-\text{NH}_2\) group of the amine at the carbonyl carbon in Meldrum's acid 1, we envisaged the formation of the half amide of malonic acid from 1. The reaction of anthranilic acid 2 with Meldrum's acid 1 was of particular interest as it would give the monomalonamic acid 3, a key-starting material for the synthesis of naturally occurring avenanthramides and their analogues. Indeed, the reaction did proceed as expected to give 3 in 90% yield and the details are discussed below. To our knowledge compound 3 and its preparation is being reported for the first time.
Meldrum’s acid 1 was prepared from malonic acid and acetone using the literature procedure\textsuperscript{23}.

To begin with we decided to use benzene as the solvent and maintain the temperature of the reaction around 65°C because it was observed\textsuperscript{20} during synthesis of monomalonates that higher temperatures leads to the formation of acetates by decarboxylation of the monomalonate being a $\beta$-keto acid.

\[
\text{\includegraphics[width=0.5\textwidth]{reaction1.png}}
\]

Therefore, an equimolar mixture of Meldrum’s acid 1 and anthranilic acid 2 in dry benzene was heated while maintaining the temperature between 60 to 70°C. The progress of the reaction was periodically monitored by TLC which indicated the presence of unreacted starting compounds even after heating for a period of 48 hrs. Hence the reaction temperature was raised to 80°C but we could get only 38% yield of a brown solid (m.p. 174°C).

However, when an equimolar mixture of Meldrum’s acid 1 and anthranilic acid 2 in dry toluene was refluxed (110°C) and monitored by TLC complete conversion took place in 4 hrs. On cooling to room temperature, a white solid separated out which was first purified chemically (by dissolving in NaHCO$_3$ solution and reprecipitating it by 1:1 HCl) followed by recrystallization from hot water to give white solid in 90% yield, m.p. 174°C.

\[
\text{\includegraphics[width=0.5\textwidth]{reaction2.png}}
\]

When acetonitrile was used as the solvent and heated under reflux (80°C), we obtained 61% yield of the white solid (m.p. 174°C) and it took 6 hrs to complete the reaction.
The IR spectrum of the white solid showed bands at 3118 (NH), 1720 (-CH$_2$COOH), 1685 (NHCO) and 1643 (Ar-COOH) cm$^{-1}$ as expected for the monomalonamic acid 3.

The molecular formula of the monomalonamic acid 3 was determined to be C$_{10}$H$_9$NO$_5$.1/4H$_2$O on the basis of its elemental analysis.

In the $^1$H NMR spectrum, the characteristic singlet at $\delta$ 3.44 integrated for 2 protons indicated the presence of the methylene group flanked by two carbonyls, one of the carboxyl and the other of the amide group supporting the formation of the expected 2-[(carboxyacetyl)amino]benzoic acid 3. The remaining four aromatic protons were observed as doublet of doublets between $\delta$ 7.08 to 8.56.

![Figure I: $^1$H NMR assignments for the various protons of 3](image)

The $^{13}$C NMR spectrum of 3 showed 9 signals for the 10 carbons present. The signal due to the methylene carbon was unexpectedly missing although the two methylene protons are vividly observed as a downfield singlet at $\delta$ 3.44 in the $^1$H NMR spectrum of 3 (see Figure I). The three carbonyl carbons, two of the carboxyl groups at $\delta$ 164.9 & 169.0 and one of the amide group at $\delta$ 169.6 were observed in the $^{13}$C NMR spectrum of 3.
The next step involved condensation of monomalonamic acid 3 with various aromatic substituted aldehydes using modified Knoevenagel condensation\textsuperscript{24} to give the different avenanthramides in a single step.

The selection of the avenanthramides to be synthesised was mainly based on the availability of the required aldehydes in the store. Of course we could prepare two of them using reported procedures. Our priority was to prepare some of the natural avenanthramides which can be obtained by condensation of monomalonamic acid 3 with \textit{p}-hydroxybenzaldehyde 4, 3,4-dihydroxybenzaldehyde (protocatechualdehyde) 5, 4-hydroxy-3-methoxybenzaldehyde (vanillin) 6 and 3,4-dimethoxybenzaldehyde (veratraldehyde) 7.

3,4-Dihydroxybenzaldehyde 5 was prepared\textsuperscript{25} by Lewis acid (AlCl$_3$) catalyzed demethylation of 4-hydroxy-3-methoxybenzaldehyde (vanillin) 6.
Synthesis of Natural Avenanthramides

To begin with we decided to carry out this condensation reaction using one single aldehyde (p-hydroxybenzaldehyde) 4 and optimize the reaction conditions so as to obtain maximum yield of the products (avenanthramides).

Thus condensation of monomalonamic acid 3 with purified p-hydroxybenzaldehyde 4 (equimolar amount) in the presence of dry pyridine and β-alanine as a cocatalyst using Verley-Doebner modification of Knoevenagel condensation24 followed by acidification with conc. HCl gave yellow solid in 85% yield. Recrystallization from hot water and acetone mixture gave pale yellow crystals having melting point 220°C. The reported3 melting point for the expected avenanthramide D 8 is 219°C, thus indicating its identity with the previously synthesized3 (83%) and the natural avenanthramide D which was further confirmed by comparison of its spectroscopic data (IR, UV and 1H NMR) with that reported for N-[4'-hydroxy-(E)-cinnamoyl]anthranilic acid 8.

In its IR spectrum the presence of diagnostic bands at 3120 cm⁻¹ (-NH-group) and 1665 cm⁻¹ (conjugated amide carbonyl) supported the formation of 8.

The 1H NMR spectrum of 8 showed the presence of two downfield 1H doublets at δ 6.61 and 7.51 (J = 15.6 Hz) characteristic of α,β-unsaturated olefinic protons of the trans-cinnamyl double bond. The two doublets integrated for 2H each at δ 6.81 and 7.54 (J = 8.4 Hz) confirmed the presence of p-substituted benzene ring of the cinnamyl moiety. The remaining four aromatic protons of the anthranilic acid moiety showed three doublets and a triplet integrating for one proton each at δ 7.61 (d, J = 8.1 Hz, C₄-H), 7.98 (d, J = 7.8 Hz, C₆-H), 8.55 (d, J = 8.1 Hz, C₃-H) and 7.16 (t, J = 7.5 Hz, C₅-H) respectively.
Similarly condensation of monomalonamic acid 3 with 3,4-dihydroxybenzaldehyde 5, 4-hydroxy-3-methoxybenzaldehyde (vanillin) 6, and 3,4-dimethoxybenzaldehyde (veratraldehyde) 7 in the presence of dry pyridine and β-alanine using Verley-Doebner modification of Knoevenagel condensation\textsuperscript{24} gave avenanthramides 9 to 11 in good yields ranging from 74-85%.

Table 2: Natural avenanthramides synthesized using our method

<table>
<thead>
<tr>
<th>Benzaldehyde derivatives</th>
<th>Natural avenanthramides</th>
<th>Yield</th>
<th>Nature &amp; m.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H OH</td>
<td><img src="image1" alt="Structure of avenanthramide D" /></td>
<td>85</td>
<td>Pale yellow crystals m.p. 220°C (Lit.\textsuperscript{3} 219°C)</td>
</tr>
<tr>
<td>H OH</td>
<td><img src="image2" alt="Structure of avenanthramide E" /></td>
<td>75</td>
<td>Yellow crystals m.p. 230-234°C (Lit.\textsuperscript{2} 221-230°C)</td>
</tr>
<tr>
<td>H OCH\textsubscript{3}</td>
<td><img src="image3" alt="Structure of avenanthramide E" /></td>
<td>85</td>
<td>Pale yellow crystals m.p. 212°C (Lit.\textsuperscript{3} 235°C)</td>
</tr>
<tr>
<td>H OCH\textsubscript{3}</td>
<td><img src="image4" alt="Structure of tranilast" /></td>
<td>74</td>
<td>Yellow crystals m.p. 184°C</td>
</tr>
</tbody>
</table>
In the UV spectra of natural avenanthramides 8 to 10, the \( \lambda_{\text{max}} \) was observed in a narrow range of 336-338 nm except the avenanthramide 11 showed \( \lambda_{\text{max}} \) at 208 nm.

In the IR spectra of all, the amide carbonyl frequency was invariably observed within a range of 1660 to 1687 cm\(^{-1}\).

Similarly in the \( ^1\text{H} \) NMR spectra of all the four avenanthramides 8 to 11, the characteristic \( \alpha,\beta \)-unsaturated olefinic cinnamyl protons appeared as two doublets between \( \delta 6.52 \) and 7.55 with \( J = 15.3 \) to 15.9 Hz.

The spectral data (UV, IR & \( ^1\text{H} \) NMR) recorded on our synthetic avenanthramides 8 to 10 was found to be identical in all respects with that reported in literature\(^2,3\) on the respective natural avenanthramides. The m.p. (212°C) of the avenanthramide E 10 recorded by us did not match with that reported\(^3\) (235°C).

The avenanthramide \( N-[3',4'-\text{dimethoxy-(E)-cinnamoyl}]\)anthranilic acid 11 is a natural anthranilic acid amide occurring in Chinese medicinal plant \( Nandina domestica \)\(^26\) and is the only natural avenanthramide which has not been detected in oat extracts so far.

It is marketed under the trade names of Tranilast and Rizaben and is used in case of allergic reactions, such as bronchial asthma, allergic rhinitis, allergic conjunctivitis, food allergies, urticaria or atopic dermatitis\(^3\).

Interestingly, the \( Z \)-isomer of 11 has been shown to possess over 10 times the antiallergic activity of the \( E \)-isomer\(^27\).
Recently 11 has been shown⁴ to prevent restenosis after percutaneous transluminal coronary angioplasty. Tranilast 11 when taken orally is rapidly absorbed, transported to the liver and demethylated at the 4'-position producing the active metabolite N-(4'-hydroxy-3'-methoxycinnamoyl)anthranilic acid 10 (avenanthramide E).

The avenanthramide 11 was described²⁷ in pharmaceutical patents much before this class of compounds were detected, isolated, synthesized and called avenanthramides by Collins in 1989. However, neither melting point nor spectroscopic data was available in the patented²⁶ and subsequent literature³⁴ on 11. However, we could find its m.p. (211-213°C) from internet. Therefore, we have recorded its UV, IR and ¹H NMR data and is included in the experimental section so as to have complete spectral data on these compounds at one place.

The assignments of signals for the various protons in the ¹H NMR spectrum of Tranilast 11 are shown below (see Figure-III).

![Figure III](image)

**Figure III:** ¹H NMR assignments for the various protons of 11
Application of the two-step General Procedure for the Synthesis of many more Reported and New Avenanthramides

It may be noted that several phenolics such as $p$-hydroxybenzoic, protocatechuic, vanillic, syringic, $p$-coumaric, caffeic, ferulic, sinapic, etc. are also isolated as free acids or their esters from the bran layer of oat grains\textsuperscript{28}. Moreover, the silica gel two dimensional TLC analysis of the mixture of avenanthramides isolated from oat groat extracts is found to contain at least 40 chromatographically distinct avenanthramides\textsuperscript{3} of which very few have been individually isolated and characterized as they are present in very small quantities. Our method can be efficiently used to prepare these remaining (not isolated but detected) avenanthramides in sufficient quantities and subsequently used to detect their presence in oat extracts and also study their bioactivity.

The generality of our method has been exemplified by preparing several more reported and new avenanthramides 12 to 21.

Thus condensation of monomalonamic acid 3 with 4-hydroxy-3,5-dimethoxy-benzaldehyde (syringaldehyde) 22, 3,4,5-trimethoxybenzaldehyde 23, 3,4-methylenedioxybenzaldehyde (piperonal) 24, 4-methoxybenzaldehyde (anisaldehyde) 25, 2,4-dimethoxybenzaldehyde 26, 2-hydroxybenzaldehyde (salicylaldehyde) 27, 3-hydroxybenzaldehyde 28, benzaldehyde 29, 4-chlorobenzaldehyde 30 and 2-chlorobenzaldehyde 31 in the presence of dry pyridine and $\beta$-alanine using Verley-Doebner modification of Knoevenagel condensation\textsuperscript{24} gave avenanthramides 12 to 21 (Table 3) in good to excellent yields.
**Table 3: Avenanthramides synthesized using our method**

<table>
<thead>
<tr>
<th>Benzaldehyde derivatives</th>
<th>Natural avenanthramides</th>
<th>Yield %</th>
<th>Nature &amp; m.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="" alt="Image of benzaldehyde derivative 22" /></td>
<td><img src="" alt="Image of natural avenanthramide 12" /></td>
<td>65</td>
<td>Bright yellow crystals m.p. 214°C (Lit. 1 199-200°C)</td>
</tr>
<tr>
<td><img src="" alt="Image of benzaldehyde derivative 23" /></td>
<td><img src="" alt="Image of natural avenanthramide 13" /></td>
<td>65</td>
<td>Yellow crystals m.p. 168°C</td>
</tr>
<tr>
<td><img src="" alt="Image of benzaldehyde derivative 24" /></td>
<td><img src="" alt="Image of natural avenanthramide 14" /></td>
<td>71</td>
<td>Pale yellow crystals m.p. 202°C (dec)</td>
</tr>
<tr>
<td><img src="" alt="Image of benzaldehyde derivative 25" /></td>
<td><img src="" alt="Image of natural avenanthramide 15" /></td>
<td>70</td>
<td>Pale yellow crystals m.p. 190°C</td>
</tr>
<tr>
<td><img src="" alt="Image of benzaldehyde derivative 26" /></td>
<td><img src="" alt="Image of natural avenanthramide 16" /></td>
<td>86</td>
<td>Orange crystals m.p. 194-98°C (dec)</td>
</tr>
<tr>
<td>Structure</td>
<td>Description</td>
<td>Yield</td>
<td>Melting Point</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>-------</td>
<td>---------------</td>
</tr>
<tr>
<td><img src="image" alt="Structure 27" /></td>
<td>White cottony threads</td>
<td>73</td>
<td>m.p. 290°C (dec)</td>
</tr>
<tr>
<td><img src="image" alt="Structure 17" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure 28" /></td>
<td>White shiny crystals</td>
<td>95</td>
<td>m.p. 242°C (dec)</td>
</tr>
<tr>
<td><img src="image" alt="Structure 18" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure 29" /></td>
<td>Light green flakes</td>
<td>66</td>
<td>m.p. 188°C</td>
</tr>
<tr>
<td><img src="image" alt="Structure 19" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure 30" /></td>
<td>White crystals</td>
<td>65</td>
<td>m.p. 200°C (dec)</td>
</tr>
<tr>
<td><img src="image" alt="Structure 20" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Structure 31" /></td>
<td>Pale yellow crystals</td>
<td>74</td>
<td>m.p. 220°C (dec)</td>
</tr>
<tr>
<td><img src="image" alt="Structure 21" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above ten avenanthramides 12 to 21 were obtained as crystalline solids having a wide range of colours ranging from white to pale yellow to bright yellow to orange to light green and in yields ranging from 65-95%.

In the UV spectra of avenanthramides 14, 16, 17, 18, 19, 20, 21 & 23, the $\lambda_{\text{max}}$ was observed in a narrow range of 207-214 nm except the avenanthramides 12, 13 & 15 showed $\lambda_{\text{max}}$ in range of 326-339 nm.
In the IR spectra of all, the amide carbonyl frequency was invariably observed within a range of 1660 to 1697 cm\(^{-1}\).

In the \(^1\)H NMR spectra of all the ten avenanthramides \(\text{12 to 21}\) the characteristic \(\alpha,\beta\)-unsaturated olefinic cinnamyl protons appeared as two doublets between \(\delta\) 6.52 and 7.93 with \(J = 15.5\) to 16.0 Hz except the avenanthramide 17 showed the \(\alpha,\beta\)-unsaturated olefinic cinnamyl protons at \(\delta\) 7.53 and 8.68 with \(J = 8.2\) Hz indicating it to be a Z-isomer.

Similarly in the \(^{13}\)C NMR spectra of all the ten avenanthramides \(\text{12 to 21}\) the characteristic signals due to the carboxyl carbonyl carbon (Ar-COOH) and the amide carbonyl carbon (Ar-NH-CO-) appeared between \(\delta\) 162.9 and 171.9 except the avenanthramide 17 showed the signals due to the carboxyl carbonyl carbon (Ar-COOH) at \(\delta\) 159.7 and the amide carbonyl carbon (Ar-NH-CO-) at \(\delta\) 167.6.

These avenanthramides \(\text{12 to 21}\) have not been detected in oat extracts nor have they been isolated from other natural sources so far. However, in view of the presence of several hydroxy and methoxy derivatives of cinnamic acids in the phenolic portion of oat extracts, the occurrence of at least some of them like \(\text{12 to 15}\) cannot be ruled out at this stage. For the same reason (to detect its presence in oat extracts) the avenanthramide \(\text{12}\) was previously prepared by Bratt et al\(^2\) using modified version of the method described by Mayama et al\(^7\).

The avenanthramide \(\text{12}\) on recrystallization from water and acetone mixture gave bright yellow crystals having m.p. 214°C (Lit.\(^2\) 199-200°C). Although the m.p. did not match its spectroscopic data (UV, IR and \(^1\)H NMR) matched well with that reported\(^2\) for N-[4'-hydroxy-3',5'-dimethoxy-(\(E\))-cinnamoyl]anthranilic acid \(\text{12}\).

Its methyl ether, N-[3',4',5'-trimethoxy-(\(E\))-cinnamoyl]anthranilic acid \(\text{13}\), prepared by condensation of monomalonamic acid \(\text{3}\) with 3,4,5-trimethoxy-benzaldehyde \(\text{23}\) displayed \(^1\)H NMR spectrum having pattern similar to that of avenanthramide \(\text{12}\) but was having an additional 3H singlet at \(\delta\) 3.89 due to the methoxy group at C\(_4\)-position which was further supported by a signal at \(\delta\) 60.1 (C\(_4\)-OCH\(_3\)) in the \(^{13}\)C NMR spectrum of \(\text{13}\).
Finally 13 being a new compound its structure was confirmed by its HRFABMS data which showed a peak at \( m/z \) 380.1113 \([M + Na]^+\) as expected and its molecular formula was determined to be \( C_{19}H_{19}NO_6 \).

The structure of each new avenanthramide was confirmed by \(^1\)H, \(^{13}\)C NMR spectroscopy and mass spectrometry.

The avenanthramide 14 obtained by condensation of monomalonamic acid 3 with piperonal 24 is a new compound. Therefore, we recorded its complete spectroscopic (UV, IR, \(^1\)H & \(^{13}\)C NMR and MS) data which is in perfect agreement with the assigned structure 14.

The molecular formula of 14 was determined to be \( C_{17}H_{13}NO_5 \) on the basis of its LCMS data which showed a peak at \( m/z \) 334.2902 for \([M + Na]^+\) as expected.

The \(^1\)H NMR assignments for the various protons of 14 are shown below in figure IV.

\[ \begin{array}{c}
\text{H} & \text{H} & \text{H} & \text{H} \\
7.1, \text{ d} & 8.0, \text{ d} & 7.4, \text{ d} & 6.1, \text{ s} \\
J = 7.2 \text{ Hz} & J = 8.0 \& 1.5 \text{ Hz} & J = 1.2 \text{ Hz} & J = 8.1 \text{ Hz} \\
\end{array} \]

\[ \begin{array}{c}
\text{H} & \text{H} & \text{H} & \text{H} \\
7.6, \text{ d} & 8.6, \text{ d} & 7.5, \text{ d} & 7.2, \text{ d} \\
J = 7.2 \text{ Hz} & J = 8.4 \text{ Hz} & J = 15.6 \text{ Hz} & J = 8.1 \text{ Hz} \\
\end{array} \]

\[ \begin{array}{c}
\text{COOH} & \text{NH} & \text{O} & \text{H} \\
6.7, \text{ d} & 11.3, \text{ s} & 6.1, \text{ s} & 6.9, \text{ d} \\
& J = 15.6 \text{ Hz} & & J = 8.1 \text{ Hz} \\
\end{array} \]

\[ \begin{array}{c}
\text{H} & \text{H} & \text{H} & \text{H} \\
6.7, \text{ d} & 7.2, \text{ d} & 7.4, \text{ d} & 6.1, \text{ s} \\
& J = 8.1 \text{ Hz} & J = 15.6 \text{ Hz} & J = 8.1 \text{ Hz} \\
\end{array} \]

**Figure IV:** \(^1\)H NMR assignments for the various protons of 14

The assignments of the \(^{13}\)C NMR signals for the various carbons of 14 are shown below in figure V.
Figure V: $^{13}$C NMR assignments for the various carbons of 14

The avenanthramide 15 is the methyl ether of the naturally occurring avenanthramide D 8 and was prepared by condensation of monomalonamic acid 3 with $p$-methoxybenzaldehyde (anisaldehyde) 25 in 70% yield. Recrystallization from acetone and water mixture gave pale yellow needles having m.p. 190°C.

Its structure was established to be $N$-(4'-methoxy-(E)-cinnamoyl)anthranilic acid 15 by comparison of its $^1$H and $^{13}$C NMR data with that of the avenanthramide D 8. In the $^1$H NMR spectrum of 15 the 3H singlet due to the methoxy group was not observed as it got buried under the DMSO signal however, the $^{13}$C NMR spectrum of 15 showed the presence of methoxy carbon signal at $\delta$ 56.2 (-OCH$_3$).

Condensation of monomalonamic acid 3 with 2,4-dimethoxybenzaldehyde 26 gave the avenanthramide 16 as orange coloured solid in 86% yield. Recrystallization from water-methanol mixture gave orange crystals having m.p. 194-98°C (dec). The required 2,4-dimethoxybenzaldehyde 26 was
prepared† in our lab by formylation of 1,3-dimethoxybenzene using
\(N,N\)-dimethylformamide and POCl₃.

Avenanthramide 16 is also a new compound and its structure was shown
to be \(N\)-(2',4'-dimethoxy-\((E)\)-cinnamoyl)anthranilic acid 16 mainly on the basis
of its \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR and MS data.

The \(^1\text{H}\) NMR spectrum of 16 showed two 3H singlets at \(\delta 3.89\) and 3.82
indicating the presence of two methoxy groups. The detail assignments of the
chemical shifts with \(J\) values for the various protons of 16 are presented below in
figure VI.

\[\text{Figure VI: } ^1\text{H} \text{ NMR assignments for the various protons of 16}\]

The \(^{13}\text{C}\) NMR spectrum showed in all 18 distinct signals as expected and
their assignments for all the 18 carbons of 16 are shown below in figure VII.

† We are thankful to Dr. Asha D’Souza for a sample of 2,4-dimethoxybenzaldehyde
The structure 16 was confirmed by recording its LCMS data which showed a peak at $m/z$ 350.6357 for \([M + Na]^+\) indicating its molecular formula to be $C_{18}H_{17}N_05$ as expected.

Condensation of monomalonamic acid 3 with 2-hydroxybenzaldehyde (salicylaldehyde) 27 and then with 3-hydroxybenzaldehyde$^+$ 28 gave the two avenanthramides 17 and 18 as white solids in 73 and 95% yields respectively.

These two avenanthramides being new compounds, their structures were established mainly on the basis of their $^1H$ NMR, $^{13}C$ NMR and MS data.

In the $^1H$ NMR spectrum of 17 the $\alpha,\beta$-unsaturated olefinic protons were observed at $\delta$ 7.53 and 8.68 with $J = 8.2$ Hz indicating $Z$ configuration for the cinnamyl double bond. Probably the phenolic $C_2$-OH is getting chelated with the amide carbonyl through hydrogen bonding forcing the cinnamyl double bond to remain in the $Z$ configuration.

The $^1H$ NMR assignments for the various protons of 17 are shown below in figure VIII.

$^+$ We are thankful to Dr. S. G. Tilve, Goa University, for providing a sample of 3-hydroxybenzaldehyde.
Figure VIII: $^1$H NMR assignments for the various protons of 17

The $^{13}$C NMR spectrum of the avenanthramide 17 showed in all 16 signals as expected and their assignments are shown below in figure IX which are consistent with the structure $N$-[2'-hydroxy-(Z)-cinnamoylanthranilic acid 17.

Figure IX: $^{13}$C NMR assignments for the various carbons of 17

The avenanthramide 18 was obtained as white shiny crystals (m.p. 242°C with decomp). Its molecular formula was determined to be C$_{16}$H$_{13}$NO$_4$ on the basis of its LCMS data which showed a peak at $m/z$ 306.0243 for [M + Na]$^+$ as expected.
Its $^1$H and $^{13}$C NMR data is fully consistent with the assigned structure N-[3'-hydroxy-(E)-cinnamoyl]anthranilic acid 18 and is given in the experimental section.

Condensation of monomalonamic acid 3 with simple benzaldehyde 29 gave the avenanthramide 19 as pale green solid in 66% yield. Recrystallization from hot water and acetone mixture gave light green flakes having m.p. 188°C.

\[ \text{19} \]

Its molecular formula was determined to be C$_{16}$H$_{13}$NO$_3$ on the basis of its HRFABMS data which showed a peak at $m/z$ 290.0791 for [M + Na]$^+$ as expected. Its structure was established to be N-(E)-cinnamoylanthranilic acid 19 on the basis of its $^1$H and $^{13}$C NMR data and is given in the experimental section.

The $^{13}$C NMR spectrum of 19 showed in all 14 distinct signals for 16 carbons as expected.

Although preparation of avenanthramides 10, 15 and 19 has been reported by Ashok Kumar and coworkers, their melting points and the $^1$H NMR data reported only on 10 clearly shows its non-identity with 10 prepared in the present study as well as with that reported in the literature. Moreover, our attempts to prepare 10 using the procedure of Ashok Kumar and coworkers did not work and instead gave the starting N-acetylanthranilic acid back with no trace of 10. No NMR data was reported on 15 and 19.

Condensation of the monomalonamic acid 3 with 4-chlorobenzaldehyde 30 and 2-chlorobenzaldehyde 31 gave avenanthramides 20 as pale yellow crystals m.p. 220°C (dec) and 21 as white crystals m.p. 200°C (dec) in 74 and 65% yields respectively.

§ We are thankful to Dr. S. G. Tilve, Goa University, for a sample of 2-chlorobenzaldehyde.
The study of their $^1$H NMR data clearly indicated the 1,4- and 1,2-substitution pattern in the cinnamyl moiety of 20 and 21 respectively.

The $^{13}$C NMR spectrum of 20 showed 14 distinct signals for 16 carbons while that of 21 showed 16 signals for 16 carbons as expected.

![Structures 20 and 21](image)

The molecular formula of both 20 and 21 was determined to be C$_{16}$H$_{12}$NO$_3$Cl on the basis of their LCMS data which gave a peak at $m/z$ 324.0379 [M + Na]$^+$ for 20 and at $m/z$ 324.0572 [M + Na]$^+$ for 21. Thus the structures of these two new chloroavenanthramides were confirmed to be N-[4'-chloro-(E)-cinnamoyl]anthranilic acid 20 and N-[2'-chloro-(E)-cinnamoyl]anthranilic acid 21 respectively.

Finally to establish the generality of this procedure we carried out one reaction of monomalonamic acid 3 with 2-furaldehyde** 32 and obtained a new compound 33 as greyish white flakes m.p. 184°C (dec) in 74% yield.

![Structures 32 and 33](image)

The structure of this compound was established on the basis of its spectroscopic (IR, $^1$H & $^{13}$C NMR and MS) data.

The assignments of the $^1$H NMR signals for the various protons of 33 are shown below in figure X.

** We are thankful to Dr. S. G. Tilve, Goa University, for a sample of 2-furaldehyde
Figure X: $^1$H NMR assignments for the various protons of 33

The $^{13}$C NMR spectrum of 33 showed 14 distinct signals for 14 carbons and the assignments of the signals to various carbons of 33 are shown below in figure XI.

Figure XI: $^{13}$C NMR assignments for the various carbons of 33

Its molecular formula was determined to be C$_{14}$H$_{11}$NO$_4$ on the basis of its LCMS data which showed a peak at m/z 280.0580 [M + Na]$^+$ as expected.
Salient Advantages of our Method

It’s a two-step general method and does not involve protection-deprotection steps as in reported methods.

Products are obtained in good to excellent yields (65 to 95%)

The major advantage of our method is that the intermediate monomalonamic acid 3 can be chemically separated and purified.

Secondly, this method does not involve any type of time and chemicals consuming chromatography for separation and purification.

We have carried out all these reactions on microscale using 40 to 100 mg quantities of the starting materials and the yields reported are the actual isolated yields for these microscale quantities.

However, the reactions can be conveniently scaled up to gram quantities without affecting either the yields or the quality of the products.

Drawbacks of this method

The reaction of 5-hydroxyanthranilic acid 34 with Meldrum’s acid 1 to give the corresponding monomalonamic acid did not work as we failed to isolate the expected product 35.

\[
\begin{align*}
\text{HO} & \quad \text{COOH} \\
\text{NH}_2 & \quad \text{COOH} \\
\text{34} & \quad \text{1} \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{COOH} \\
\text{NH} & \quad \text{COOH} \\
\text{35} & \\
\end{align*}
\]
Synthesis of monomalonamic acids

Introduction

Literature survey indicated that the β-dicarbonyl derivatives belonging to the monomalonamic acid family are very important compounds having interesting pharmacological properties, including antihypertensive, sedative, anticonvulsant, anti-inflammatory, analgesic and central nervous system stimulating activities.

N-arylmalonamic acids are formed from benzoxazolinone derivatives by fungal transformation and these acids have been shown to possess both plant growth regulatory and fungicidal activity.

The metabolism of 2-benzoxazolinone involves the cleavage of the amide linkage leading to the formation of α-aminophenol intermediate. The aminophenol is then converted into the corresponding malonamic acid by the enzyme N-malonyl transferase.
Reported methods for the preparation of $N$-arylmalonamic acids

$N$-arylmalonamic acids have been synthesized from malonic acid and its derivatives with or without using a solvent. Khetan et al$^{39}$ synthesized malonamic acids from appropriate aniline and malonic acid derivative using either pyridine or acetic acid as solvents at 100°C.

![Chemical structure of $N$-arylmalonamic acid](image)

$R = Br, I, OCH₃, OEt$

A convenient one-pot synthesis of $N$-arylmalonamic acid has been demonstrated based on the in-situ generation of malonyl monoacyl chloride, followed by reaction with aniline$^{40}$.

![Chemical reaction](image)

$N$-arylmalonamic acids have also been prepared by refluxing the appropriate aniline with malonic acid or with diethyl malonate$^{41}$ or with ethyl malonyl chloride$^{42}$ followed by alkaline hydrolysis of the ester group.

Alternatively the silylated aniline$^{43}$ is treated with Meldrum's acid 1 followed by hydrolysis of the malonic silyl ester to give $N$-arylmalonamic acid.

![Chemical reactions](image)

Matoba et al$^{44}$ demonstrated that when diazomethane ($CH₂N₂$) is added to a methanolic solution of Meldrum's acid 1, violent evolution of $N₂$ gas occurs.
with the formation of dimethyl malonate in quantitative yield. But when CH$_2$N$_2$ was added to a solution of 1 in piperidine, N-(methoxycarbonylacetyl) piperidine was obtained in 59% yield.

\[
\begin{align*}
\text{CH}_2\text{N}_2 + &\quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \text{I} \\
\rightarrow &\quad \begin{array}{c} \text{N} \\ \text{O} \end{array} \quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \\
&\quad \text{CH}_3\text{O} 
\end{align*}
\]

Hurd et al.$^{45}$, during investigation studies on Meldrum’s acid 1, prepared $N$-$p$-bromophenylmalonamic acid from $p$-bromoaniline and Meldrum’s acid 1 by heating them under reflux in acetonitrile solvent (yield was not reported).

\[
\begin{align*}
\text{Br} &\quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \text{I} \\
\text{NH}_2 \\
\rightarrow &\quad \begin{array}{c} \text{Br} \\ \text{O} \\ \text{O} \\ \text{O} \\ \text{O} \end{array} \\
&\quad \text{NH} \quad \text{OH} 
\end{align*}
\]

Hurd et al.$^{45}$ also made a statement in his article that “if Meldrum had performed his experiment with aniline using CH$_3$CN as the solvent, he would undoubtedly have obtained the $N$-phenylmalonamic acid 36 instead of acetanilide”.

\[
\begin{align*}
\uparrow &\quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \begin{array}{c} \text{O} \\ \text{O} \end{array} \quad \text{I} \\
\text{NH} &\quad \text{NH} \quad \text{O} \\
\rightarrow &\quad \begin{array}{c} \text{O} \\ \text{O} \\ \text{O} \end{array} \\
&\quad \text{NH} \quad \text{OH} 
\end{align*}
\]

Interestingly, this reaction of aniline with Meldrum’s acid 1 was neither carried out by Meldrum$^{46}$ nor by Hurd et al.$^{45}$. Hence we decided to investigate this reaction using our procedure and the results obtained are discussed below.
Present work

Our successful attempt to prepare\textsuperscript{20} half esters of malonic acid by the reaction of Meldrum's acid 1 with all types of alcohols and phenols led us to prepare successfully monomalonamic acid 3 by the reaction of Meldrum's acid 1 with anthranilic acid 2. We also found that compound 3 and its preparation is not reported previously.

Although the reaction of 5-hydroxyanthranilic acid 34 with Meldrum's acid 1 to give the corresponding monomalonamic acid 35 did not work as we failed to isolate the expected product, the results obtained by Hurd et al\textsuperscript{45} especially with \textit{p}-bromoaniline and 1 tempted us to investigate this reaction of 1 with several amines (including aniline) using our procedure involving benzene\textsuperscript{20} or toluene\textsuperscript{22} as the solvents.

Preparation of \textit{N}-phenylmalonamic acid 36

To begin with we carried out the reaction of aniline with Meldrum's acid 1 in anhydrous CH\textsubscript{3}CN using reaction conditions reported\textsuperscript{45} for \textit{p}-bromoaniline and obtained the expected product 36 (m.p. 130°C, Lit\textsuperscript{47} 132°C) in 40% yield.

When benzene was used as the solvent, the optimum yield of 36 was 60% if the temperature was maintained between 60-70°C. Refluxing temperature (80°C) of benzene gave < 60% yield due to the formation of acetanilide by decarboxylation.

However, when dry toluene was used as the solvent at its reflux temperature, acetanilide (m.p. 110°C, Lit\textsuperscript{48a} 113-115°C) was obtained exclusively with no trace of the expected \textit{N}-phenylmalonamic acid 36.

Preparation of 4-[(carboxyacetyl)amino]benzoic acid 37

Since the reaction of anthranilic acid 2 with Meldrum's acid 1 in refluxing toluene gave us 90% yield of the required monomalonamic acid 3, we decided to use \textit{p}-aminobenzoic acid (PABA) which is used in pharmaceutical preparations,
forms a part of the structure of folic acid (vitamin B<sub>9</sub>) and a precursor in the biosynthesis of folic acid.

An equimolar mixture of p-aminobenzoic acid and <i>1</i> when heated under reflux in dry toluene gave the expected 4-[(carboxyacetyl)amino]benzoic acid 37 as a white solid in 91% yield. Recrystallization from ethanol afforded white shiny crystals m.p. 260°C (with decomposition).

![Chemical structure of 37 and reaction scheme](image)

In the IR spectrum of 37 bands at 3273 (NH), 1720 (CH<sub>2</sub>COOH), 1678 (NHCO), 1664 (Ar-COOH) cm<sup>-1</sup> indicated the functional groups present.

The <sup>1</sup>H NMR spectrum of 37 showed only three signals, two <i>o</i>-coupled 2H doublets centred at δ 7.68 and 7.9 indicating the presence of <i>p</i>-anthranilic acid moiety and a 2H singlet at δ 3.4 due to the methylene group flanked by two carbonyls confirming the formation of 37.

This was further supported by the presence of 8 distinct signals for the 10 carbons in the <sup>13</sup>C NMR spectrum of 37.

**Figure XII:** Assignments of <sup>1</sup>H & <sup>13</sup>C NMR signals for the Hs & Cs of 37
Preparation of N-(1-naphthyl)malonamic acid 38

Reaction of 1-naphthylamine with Meldrum's acid 1 using toluene as the solvent gave the unwanted N-naphthylacetamide (m.p. 152°C, Lit\textsuperscript{48b} 160°C) in 77% yield.

\[
\text{toluene} \overset{\text{reflux, 4h}}{\longrightarrow} \text{N-naphthylacetamide} \quad \begin{array}{c}
\text{1-naphthylamine} \\
1
\end{array} \overset{\text{benzene}}{\rightarrow} \text{60-65°C, 24h} \quad \text{38}
\]

However, when benzene was used as the solvent and the temperature maintained between 60-65°C, the expected N-(1-naphthyl)malonamic acid 38 was obtained in 69% yield. Recrystallization from ethanol gave buff coloured needles having m.p. 144°C.

The IR spectrum of 38 showed bands at 3260 (NH), 1732 (CH\textsubscript{2}COOH) and 1710 (NHCO) cm\textsuperscript{-1} indicating the presence of the functional groups present.

The \textsuperscript{1}H NMR spectrum of 38 displayed the characteristic 2H singlet at \(\delta\) 3.55 for the two methylene protons flanked by carbonyl groups.

Its \textsuperscript{13}C NMR spectrum showed 13 signals (including two carbonyls) as expected.

In the reaction of the following four amines\textsuperscript{\dagger\dagger} with Meldrum's acid 1 either with toluene or benzene we failed to isolate the expected product but instead obtained their acetamide derivatives.

\[
p\text{-diaminobenzene} \quad 2,4\text{-dinitroaniline} \quad N,N\text{-diethylamine} \quad N,N\text{-diphenylamine}
\]

\textsuperscript{\dagger\dagger} We are thankful to Dr. B. R. Srinivasan, Goa University, for providing samples of 1,4-diaminobenzene and 1,2-diaminobenzene.
Surprisingly, the following four aromatic amines neither gave the expected product nor the acetate and the starting material was recovered back.

\[
\begin{array}{cccc}
\text{o-diaminobenzene} & \text{o-aminophenol} & \text{o-nitroaniline} & \text{p-nitroaniline} \\
\end{array}
\]

However, when benzylamine was condensed with 1, \(N\)-benzylmalonamic acid 39 was obtained as colourless solid in 13-40% yield depending upon the solvent used (details in the experimental section). The maximum yield of 40% of 39 was obtained when the reaction was carried out in pyridine and diethyl ether. Recrystallization from benzene gave colourless crystals (m.p. 86-88°C).

\[
\begin{array}{ccc}
\text{O} & \text{C} & \text{O} \\
\text{O} & \text{C} & \text{O} \\
\text{O} & \text{C} & \text{O} \\
\end{array}
\]

The \(N\)-benzylmalonamic acid 39 was characterized by the study of its IR, \(^1\)H NMR and \(^{13}\)C NMR data.

Its IR spectrum showed bands at 3287 (NH) and 1745 cm\(^{-1}\).

In the \(^1\)H NMR spectrum of 39 three signals were observed, a 2H singlet at \(\delta 3.32\), a 2H doublet at \(\delta 4.46 (J = 5.4 \text{ Hz})\) and a 5H multiplet between \(\delta 7.25\) to 7.37. Their assignments are shown below in figure XIII.
Figure XIII: $^1$H NMR assignments for the various protons of 39

In the $^{13}$C NMR spectrum of 39 only 7 signals were observed for 10 carbons. Three $sp^2$ carbons of the benzene ring are overlapping at $\delta$ 127.8 and their assignments are shown below in figure XIV.

Figure XIV: $^{13}$C NMR assignments for the various carbons of 39

To our knowledge the compounds 37, 38 and 39 as well as their preparations are being reported for the first time.
Fig. 1.01: $^1$H NMR spectrum of 3
Fig. 1.02: $^{13}$C NMR spectrum of 3
Fig. 1.03: $^1$H NMR spectrum of 13
Fig. 1.04: ${}^{13}$C NMR spectrum of 13
Fig. 1.05: HRFABMS spectrum of 13
Fig. 1.06: $^1$H NMR spectrum of 14
Fig. 1.07: $^{13}$C NMR spectrum of 14
Fig. 1.08: LCMS spectrum of 14
Fig. 1.09: \(^1\)H NMR spectrum of 15
Fig. 1.10: $^{13}$C NMR spectrum of 15
Fig. 1.11: HRFABMS spectrum of 15
Fig. 1.12: $^1$H NMR spectrum of 16
Fig. 1.13: $^{13}$C NMR spectrum of 16
Fig. 1.14: LCMS spectrum of 16
Fig. 1.15: $^1$H NMR spectrum of 17
Fig. 1.16: $^{13}$C NMR spectrum of 17
Fig. 1.17: $^1$H NMR spectrum of 18
Fig. 1.18: $^{13}$C NMR spectrum of 18
Fig. 1.19: LCMS spectrum of 18
Fig. 1.20: $^1$H NMR spectrum of 19
Fig. 1.21: $^{13}$C NMR spectrum of 19
Fig. 1.22: HRFABMS spectrum of 19
Fig. 1.23: $^1$H NMR spectrum of 20
Fig. 1.24: $^{13}$C NMR spectrum of 20
Fig. 1.25: LCMS spectrum of 20
Fig. 1.26: $^1$H NMR spectrum of 21
Fig. 1.27: $^{13}$C NMR spectrum of 21
Fig. 1.28: LCMS spectrum of 21
Fig. 1.29: $^1$H NMR spectrum of 33
Fig. 1.30: $^{13}$C NMR spectrum of 33
Fig. 1.31: LCMS spectrum of 33
Fig. 1.32: $^1$H NMR spectrum of 37
Fig. 1.33: $^{13}$C NMR spectrum of 37
Fig. 1.34: $^1$H NMR spectrum of 38
Fig. 1.35: $^{13}$C NMR spectrum of 38
Fig. 1.36: $^1$H NMR spectrum of 39
Fig. 1.37: $^{13}$C NMR spectrum of 39
Experimental

Preparation of Meldrum’s acid 1

Prepared according to the reported\textsuperscript{23} literature procedure.

Preparation of 2-[(carboxyacetyl)amino]benzoic acid 3

Using benzene as the solvent

An equimolar mixture of Meldrum’s acid 1 (1.44 g, 10 mmole) and anthranilic acid 2 (1.37 g, 10 mmole) in dry benzene (10 mL) was heated under reflux while maintaining the temperature between 60 to 70°C. The progress of the reaction was periodically monitored by TLC which indicated the presence of unreacted starting compounds even after 48 hrs. Hence the temperature was raised to 80°C. The reaction mixture was cooled to room temperature, extracted with saturated NaHCO\textsubscript{3} solution, regenerated using 1:1 HCl. Brown solid separated was filtered under suction, washed with water and dried at 100°C to give 3 (0.848 g, 38%), m.p. 174°C.

Using toluene as the solvent

An equimolar mixture of Meldrum’s acid 1 (1.44 g, 10 mmole) and anthranilic acid 2 (1.37 g, 10 mmole) in dry toluene (10 mL) was refluxed for 4 hrs. On cooling to room temperature, the malonamic acid 3 separated out as white solid which was filtered and washed with water. It was then chemically purified by
dissolving in saturated NaHCO₃ solution, regenerated using 1:1 HCl, filtered under suction, washed with water and dried at 100°C to give 3 (2.01 g, 90%). Recrystallization from hot water afforded white solid, m.p. 174°C.

IR νₘₐₓ (KBr): 3118 (NH), 1720 (CH₂COOH), 1685 (NHCO), 1643 (Ar-COOH), 1608, 1591, 1296 cm⁻¹.

¹H NMR (300 MHz, CDCl₃, MeOH in traces, Fig. 1.01): For assignments refer Figure I, pg 15.

¹³C NMR (75 MHz, CDCl₃, MeOH in traces, Fig. 1.02): For assignments refer Figure II, pg 16.

Elemental analysis: Calcd. for C₁₀H₉NO₅·1/₄H₂O: C, 52.96; H, 4.22; N, 6.27. Found: C, 52.74; H, 4.17; N, 6.15.

Using acetonitrile as the solvent

An equimolar mixture of Meldrum’s acid 1 (1.44 g, 10 mmole) and anthranilic acid 2 (1.37 g, 10 mmole) in dry CH₃CN (10 mL) was refluxed for 6 hrs. The reaction mixture was cooled to room temperature, extracted with saturated NaHCO₃ solution, regenerated using 1:1 HCl. White solid separated was filtered under suction, washed with water and dried at 100°C to give 3 (1.362 g, 61%) having m.p. 174°C.

3,4-Dihydroxybenzaldehyde (protocatechualdehyde) 5

![Chemical structure of 3,4-Dihydroxybenzaldehyde (protocatechualdehyde) 5](image)

Prepared according to the reported literature procedure.
General procedure for the preparation of avenanthramides

A mixture of 2-[(carboxyacetyl)amino]benzoic acid 3 (0.45 mmole), the corresponding benzaldehyde derivatives (0.45 mmole) and catalytic amount of β-alanine (10 mg) was refluxed in pyridine (0.5 mL) for 110 min. The reaction mass was cooled in ice and acidified with conc HCl (1.0 mL). The solid product that separated out was filtered, washed with water and recrystallized using hot water-acetone mixture to give the respective avenanthramide.

For the hydroxybenzaldehydes, the reaction mixtures were just kept in dark at room temperature in loosely stoppered Erlenmeyer flasks for a period of two weeks and then worked up as given above.

Avenanthramide D 8 N-4'-Hydroxy-(E)-cinnamoylanthranilic acid

![Chemical Structure](image)

Reaction of monomalonamic acid 3 (0.10 g, 0.45 mmole) and 4-hydroxybenzaldehyde 4 (0.055 g, 0.45 mmole) gave avenanthramide D 8 as yellow solid (0.110 g, 85%). Recrystallization from water-acetone mixture gave pale yellow crystals, m.p. 220°C, Lit.3 219°C.

UV $\lambda_{max}$ (MeOH): 328, 211 nm.

IR $\nu_{max}$ (KBr): 3120, 1665 (NHCO), 1610 cm$^{-1}$.

$1^H$ NMR (300 MHz, DMSO-$d_6$): δ 6.61 (d, $J = 15.6$ Hz, 1H, C$_8$-H), 6.81 (d, $J = 8.4$ Hz, 2H, C$_{3,5}$-H), 7.16 (t, $J = 7.5$ Hz, 1H, C$_5$-H), 7.51 (d, $J = 15.6$ Hz, 1H, C$_7$-H), 7.54 (d, $J = 8.4$ Hz, 2H, C$_{2,6}$-H), 7.61 (d, $J = 8.1$ Hz, 1H, C$_4$-H), 7.98 (d, $J = 7.8$ Hz, 1H, C$_6$-H), 8.55 (d, $J = 8.1$ Hz, 1H, C$_3$-H), 10.15 (s, 1H, OH), 11.26 (s, 1H, COOH).
Avenanthramide 9 N-[3',4'-dihydroxy-(E)-cinnamoyl]anthranilic acid

\[
\begin{align*}
\text{C} & \quad \text{COOH} \\
\text{NH} & \quad \text{O} \\
\text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

Reaction of 3 (0.10 g, 0.45 mmole) and 3,4-dihydroxybenzaldehyde 5 (0.062 g, 0.45 mmole) gave avenanthramide 9 as yellow solid (0.10 g, 75%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 230-234°C, Lit.² 221-230°C.

UV \( \lambda_{\max} \) (MeOH): 338, 211 nm.

IR \( \nu_{\max} \) (KBr): 3260, 1665 (N=O), 1600, 1590, 1270 cm\(^{-1}\).

\(^1\)H NMR (300 MHz, DMSO-\text{d}_6): \delta 6.52 (d, \( J = 15.3 \) Hz, 1H, C8-H), 6.79 (d, \( J = 8.1 \) Hz, 1H, C5-H), 7.00 (d, \( J = 8.1 \) Hz, 1H, C6-H), 7.10 (s, 1H, C2-H), 7.18 (t, \( J = 7.8 \) Hz, 1H, C5-H), 7.44 (d, \( J = 15.6 \) Hz, 1H, C7-H), 7.61 (t, \( J = 7.8 \) Hz, 1H, C4-H), 8.00 (d, \( J = 8.1 \) Hz, 1H, C6-H), 8.55 (d, \( J = 8.1 \) Hz, 1H, C3-H), 9.34 (s, 1H, OH), 9.68 (s, 1H, OH), 11.29 (s, 1H, COOH).

Avenanthramide 10 N-[4'-hydroxy-3'-methoxy-(E)-cinnamoyl]anthranilic acid

\[
\begin{align*}
\text{C} & \quad \text{COOH} \\
\text{NH} & \quad \text{O} \\
\text{OCH}_3 & \quad \text{OH}
\end{align*}
\]

Reaction of 3 (0.10 g, 0.45 mmole) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) 6 (0.069 g, 0.45 mmole) gave avenanthramide 10 as yellow solid (0.12 g, 85%). Recrystallization from water-acetone mixture gave pale yellow crystals, m.p. 212°C, Lit.³ 235°C.

UV \( \lambda_{\max} \) (MeOH): 336, 211 nm.
IR $\nu_{\text{max}}$ (KBr): 3515, 1660 (NHCO), 1600, 1520, 1270 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.68 (d, $J = 15.6$ Hz, 1H, C$_8$-H), 6.81 (d, $J = 8.1$ Hz, 1H, C$_5$-H), 7.12 (d, $J = 8.4$ Hz, 1H, C$_6$-H), 7.17 (d, $J = 7.5$ Hz, 1H, C$_7$-H), 7.30 (s, 1H, C$_2$-H), 7.51 (d, $J = 15.6$ Hz, 1H, C$_7$-H), 7.60 (t, $J = 7.8$ Hz, 1H, C$_4$-H), 8.00 (d, $J = 7.8$ Hz, 1H, C$_6$-H), 8.56 (d, $J = 8.4$ Hz, 1H, C$_3$-H), 9.71 (s, 1H, OH), 11.25 (s, 1H, COOH).

**Avenanthramide 11 (Tranilast)** $N$-[3',4'-dimethoxy-(E)-cinnamoyl]anthranilic acid

\[
\begin{align*}
\text{OH} & \quad \text{COOH} \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{C}_6 & \quad \text{C}_5 & \quad \text{C}_3 & \quad \text{C}_2 & \quad \text{C}_4 & \quad \text{C}_7
\end{align*}
\]

Reaction of 3 (0.10 g, 0.45 mmole) and 3,4-dimethoxybenzaldehyde 7 (0.074 g, 0.45 mmole) gave avenanthramide 11 as yellow solid (0.109 g, 74%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 184$^\circ$C.

UV $\lambda_{\text{max}}$ (MeOH): 336, 208 nm.

IR $\nu_{\text{max}}$ (KBr): 3200, 1687 (NHCO), 1597, 1260, 1192 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.77 (d, $J = 15.9$ Hz, 1H, C$_8$-H), 7.00 (d, $J = 8.4$Hz, 1H, C$_5$-H), 7.17 (d, $J = 7.5$ Hz, 1H, C$_4$-H), 7.23 (d, $J = 7.5$ Hz, 1H, C$_6$-H), 7.34 (s, 1H, C$_2$-H), 7.55 (d, $J = 15.9$ Hz, 1H, C$_7$-H), 7.63 (d, $J = 8.4$ Hz, 1H, C$_3$-H), 8.00 (d, $J = 7.2$ Hz, 1H, C$_6$-H), 8.58 (d, $J = 8.4$ Hz, 1H, C$_3$-H), 11.28 (s, 1H, COOH).
Avenanthramide 12 \textit{N-\[4'-hydroxy-3',5'-dimethoxy-(E)-cinnamoyl\]anthranilic acid}

\begin{center}
\includegraphics[width=0.2\textwidth]{avenanthramide12}
\end{center}

Reaction of 3 (0.10 g, 0.45 mmole) and 4-hydroxy-3,5-dimethoxybenzaldehyde 22 (0.082 g, 0.45 mmole) gave avenanthramide 12 as yellow solid (0.10 g, 65%). Recrystallization from water-acetone mixture gave bright yellow crystals, m.p. 214°C, Lit.\textsuperscript{2} 199-200°C.

\textbf{UV }\lambda_{\text{max}} (\text{MeOH}): 339, 211 \text{ nm}.

\textbf{IR }\nu_{\text{max}} (\text{KBr}): 3503, 1665 (\text{NHCO}), 1609, 1270 \text{ cm}^{-1}.

\textbf{\textsuperscript{1}H NMR} (300 MHz, DMSO-d\textsubscript{6}): \delta 6.71 (d, \textit{J} = 15.5 \text{ Hz}, 1H, C\textsubscript{8}-H), 6.98 (s, 2H, C\textsubscript{2',6'}-H), 7.14 (t, \textit{J} = 7.5 \text{ Hz}, 1H, C\textsubscript{5}-H), 7.50 (d, \textit{J} = 15.5 \text{ Hz}, 1H, C\textsubscript{7}-H), 7.58 (t, \textit{J} = 7.85 \text{ Hz}, 1H, C\textsubscript{4}-H), 7.97 (d, \textit{J} = 7.0 \text{ Hz}, 1H, C\textsubscript{6}-H), 8.55 (d, \textit{J} = 8.4 \text{ Hz}, 1H, C\textsubscript{3}-H), 8.90 (s, 1H, OH), 11.23 (s, 1H, COOH).

Avenanthramide 13 \textit{N-[3',4',5'-trimethoxy-(E)-cinnamoyl]anthranilic acid}

\begin{center}
\includegraphics[width=0.2\textwidth]{avenanthramide13}
\end{center}

Reaction of 3 (0.10 g, 0.45 mmole) and 3,4,5-trimethoxybenzaldehyde 23 (0.088 g, 0.45 mmole) gave avenanthramide 13 as yellow solid (0.104 g, 65%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 168°C.

\textbf{UV }\lambda_{\text{max}} (\text{MeOH}): 339, 211 \text{ nm}.
IR $v_{\text{max}}$ (KBr): 3580, 1660 (NHCO), 1600, 1583, 1267 cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$, Fig. 1.03): $\delta$ 3.89 (s, 3H, C$_4$-OCH$_3$), 3.92 (s, 6H, C$_{3,5}$-OCH$_3$), 6.52 (d, $J$ = 15.6 Hz, 1H, C$_8$-H), 6.81 (s, 2H, C$_{2,6}$-H), 7.15 (t, $J$ = 7.8 Hz, 1H, C$_3$-H), 7.65 (t, $J$ = 4.5 Hz, 1H, C$_4$-H), 7.69 (d, $J$ = 15.6 Hz, 1H, C$_7$-H), 8.15 (d, $J$ = 7.8 Hz, 1H, C$_6$-H), 8.90 (d, $J$ = 8.7 Hz, 1H, C$_3$-H), 11.25 (s, 1H, COON).

$^{13}$C NMR (75 MHz, CDCl$_3$, Fig. 1.04): $\delta$ 56.1 (3',5'-OCH$_3$), 60.1 (4'-OCH$_3$), 105.3 (C-2',6'), 115.3 (C-1), 120.3 (C-8'), 121.0 (C-3), 122.6 (C-5), 130.2 (C-1'), 131.5 (C-6), 134.6 (C-4), 139.8 (C-2), 141.8 (C-3',5'), 142.2 (C-7'), 153.3 (C-4'), 164.7 (C-7), 171.0 (C-9').

HRFABMS (Fig. 1.05): $m/z$ [M + Na]$^+$ calcd for C$_{19}$H$_{19}$NO$_6$Na 380.1110, Found 380.1113.

*Avenanthramide 14*  $N$-[3',4'-methylenedioxy-(E)-cinnamoyl]anthranilic acid

![Chemical Structure of Avenanthramide 14](image)

Reaction of 3 (0.10 g, 0.45 mmole) and 3,4-methylenedioxybenzaldehyde (piperonal) 24 (0.067 g, 0.45 mmole) gave avenanthramide 14 as yellow solid (0.22 g, 71%). Recrystallization from water-methanol mixture gave pale yellow crystals, m.p. 202°C (dec).

UV $\lambda_{\text{max}}$ (MeOH): 337, 207 nm.

IR $v_{\text{max}}$ (KBr): 1691 (NHCO), 1600, 1450, 1211 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-d$_6$, Fig. 1.06): For assignments refer Figure IV, pg 25.
$^{13}$C NMR (75 MHz, DMSO-$d_6$, Fig. 1.07): For assignments refer Figure V, pg 26.

LCMS (Fig. 1.08): $m/z$ [M + Na]$^+$ calcd for C$_{17}$H$_{11}$NO$_4$Na 334.2832, Found 334.2902.

*Avenanthramide 15 N-[4'-methoxy-(E)-cinnamoyl]anthranilic acid*

![Structure of Avenanthramide 15]

Reaction of 3 (0.10 g, 0.45 mmole) and 4-methoxybenzaldehyde (anisaldehyde) 25 (0.061 g, 0.45 mmole) gave avenanthramide 15 as yellow solid (0.093 g, 70%). Recrystallization from water-acetone gave pale yellow crystals, m.p. 190°C.

UV $\lambda_{\text{max}}$ (MeOH): 326, 211 nm.

IR $\nu_{\text{max}}$ (KBr): 3310, 1670 (NHCO), 1600, 1255 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-$d_6$, Fig. 1.09): $\delta$ 6.70 (d, $J = 16$ Hz, 1H, C$_8$-H), 6.98 (d, $J = 8.4$ Hz, 2H, C$_{3',5'}$-H), 7.16 (t, $J = 7.5$ Hz, 1H, C$_3$-H), 7.56 (d, $J = 16$ Hz, 1H, C$_7$-H), 7.62 (d, $J = 8.1$ Hz, 2H, C$_{2',6'}$-H), 7.67 (d, $J = 8.1$ Hz, 1H, C$_4$-H), 8.00 (d, $J = 7.5$ Hz, 1H, C$_6$-H), 8.58 (d, $J = 8.7$ Hz, 1H, C$_3$-H), 11.28 (s, 1H, COOH).

$^{13}$C NMR (75 MHz, CDCl$_3$, Fig. 1.10): $\delta$ 56.2 (4'-OCH$_3$), 114.2 (C-3',5'), 115.4 (C-1), 119.3 (C-8'), 120.2 (C-3), 122.4 (C-5), 127.3 (C-1'), 129.5 (C-2',6'), 131.4 (C-6), 134.3 (C-4), 141.6 (C-2), 141.8 (C-7'), 161.1 (C-4'), 165.2 (C-7), 170.4 (C-9').

HRFABMS (Fig. 1.11): $m/z$ [M + Na]$^+$ calcd for C$_{17}$H$_{13}$NO$_4$Na 320.0899, Found 320.0898.

86
Avenanthramide 16 *N*-[*2',4'-dimethoxy-(E)-cinnamoyl]*anthranilic acid

![Chemical Structure](image)

Reaction of 3 (0.10 g, 0.45 mmole) and 2,4-dimethoxybenzaldehyde 26 (0.075 g, 0.45 mmole) gave avenanthramide 16 as orange solid (0.281 g, 86%). Recrystallization from water-methanol mixture gave orange crystals, m.p. 194-98°C (dec).

**UV** \( \lambda_{\text{max}} \) (MeOH): 339, 208 nm.

**IR** \( v_{\text{max}} \) (KBr): 1693 (NHCO), 1660, 1598, 1288 cm\(^{-1}\).

\(^1\)H NMR (300 MHz, DMSO-\(d_6\), Fig. 1.12): For assignments refer Figure VI, pg 27.

\(^13\)C NMR (75 MHz, DMSO-\(d_6\), Fig. 1.13): For assignments refer Figure VII, pg 28.

**LCMS** (Fig. 1.14): \( m/z \) [M + Na]\(^+\) calcd for C\(_{18}\)H\(_{17}\)NO\(_5\)Na 350.3258, Found 350.6357.

Avenanthramide 17 *N*-[*2'-hydroxy-(Z)-cinnamoyl]*anthranilic acid

![Chemical Structure](image)

Reaction of 3 (0.10 g, 0.45 mmole) and 2-hydroxybenzaldehyde 27 (0.055 g, 0.45 mmole) gave avenanthramide 17 as a white solid (0.206 g, 73%). Recrystallization from water-acetone mixture gave white cotton like threads, m.p. 290°C (dec).
UV $\lambda_{\text{max}}$ (MeOH): 207 nm.

IR $\nu_{\text{max}}$ (KBr): 3074 (br), 1726, 1693 (NHCO), 1674, 1608, 1531, 1261 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-d$_6$, Fig. 1.15): For assignments refer Figure VIII, pg 29.

$^{13}$C NMR (75 MHz, DMSO-d$_6$, Fig. 1.16): For assignments refer Figure IX, pg 29.

**Avenanthramide 18** N-[3'-hydroxy-(E)-cinnamoyl]anthranilic acid

![Avenanthramide 18](image)

Reaction of 3 (0.10 g, 0.45 mmole) and 3-hydroxybenzaldehyde 28 (0.055 g, 0.45 mmole) gave avenanthramide 18 as white solid (0.269 g, 95%). Recrystallization from water-acetone mixture gave white shiny crystals, m.p. 242°C (dec).

UV $\lambda_{\text{max}}$ (MeOH): 322, 207 nm.

IR $\nu_{\text{max}}$ (KBr): 3169, 1693 (NHCO), 1612, 1514, 1288 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-d$_6$, Fig. 1.17): $\delta$ 6.76 (d, $J = 15.7$ Hz, 1H, C$_8$-H), 6.83 (dd, $J = 7.9$ & 1.6 Hz, 1H, C$_5$-H), 7.06 (s, 1H, C$_2$-H), 7.12-7.26 (m, 3H, C$_{4,5,6}$-H), 7.52 (d, $J = 15.7$ Hz, 1H, C$_7$-H), 7.62 (dt, $J = 7.9$ & 1.5 Hz, 1H, C$_4$-H), 8.00 (dd, $J = 7.9$ & 1.6 Hz, 1H, C$_6$-H), 8.56 (d, $J = 8.4$ Hz, 1H, C$_3$-H), 11.30 (s, 1H, COOH).

$^{13}$C NMR (75 MHz, DMSO-d$_6$, Fig. 1.18): $\delta$ 113.9 (C-2'), 116.6 (C-1), 116.8 (C-4'), 118.7 (C-5'), 120.0 (C-8'), 121.7 (C-3), 122.5 (C-5), 129.5 (C-6'), 130.6 (C-1'), 133.5 (C-6), 135.2 (C-4), 140.2 (C-2), 141.0 (C-7'), 157.2 (C-3'), 163.3 (C-7), 168.9 (C-9').
LCMS (Fig. 1.19): \( m/z \ [M + Na]^+ \) calcd for \( C_{16}H_{13}NO_4Na \) 306.2728, Found 306.0243.

\textit{Avenanthramide 19} \textit{N-(E)-cinnamoylanthranilic acid}

![Chemical structure of Avenanthramide 19](image)

Reaction of 3 (0.10 g, 0.45 mmole) and benzaldehyde 29 (0.048 g, 0.45 mmole) gave avenanthramide 19 as light green solid (0.080 g, 66%). Recrystallization from water-acetone gave light green flakes m.p. 188°C.

UV \( \lambda_{\text{max}} \) (MeOH): 311, 211 nm.

IR \( v_{\text{max}} \) (KBr): 3141, 1668 (NHCO), 1611, 1548, 1223 cm\(^{-1}\).

\(^1\text{H NMR}\) (300 MHz, DMSO-\(d_6\), Fig. 1.20): \( \delta \) 6.63 (d, \( J = 15.6 \) Hz, 1H, C\(_8\)-H), 7.16 (t, \( J = 7.5 \) Hz, 1H, C\(_5\)-H), 7.40-7.64 (m, 5H, Ar-H), 7.66 (t, \( J = 7.8 \) Hz, 1H, C\(_4\)-H), 7.80 (d, \( J = 15.6 \) Hz, 1H, C\(_7\)-H), 8.12 (d, \( J = 8.1 \) Hz, 1H, C\(_6\)-H), 8.91 (d, \( J = 8.4 \) Hz, 1H, C\(_3\)-H), 11.23 (s, 1H, COOH).

\(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\), Fig. 1.21): \( \delta \) 114.1 (C-1), 120.9 (C-8'), 121.8 (C-3), 122.9 (C-5), 128.2 (C-2',6'), 128.9 (C-3',5'), 130.2 (C-4'), 131.9 (C-1'), 134.7 (C-6), 135.7 (C-4), 142.3 (C-2), 142.8 (C-7), 164.9 (C-7), 171.9 (C-9').

HRFABMS (Fig. 1.22): \( m/z \ [M + Na]^+ \) calcd for \( C_{16}H_{13}NO_3Na \) 290.0793, Found 290.0791.
Reaction of 3 (0.10 g, 0.45 mmole) and 4-chlorobenzaldehyde 30 (0.063 g, 0.45 mmole) gave chloroavenanthramide 20 as pale yellow solid (0.223 g, 74%). Recrystallization from water-methanol mixture gave pale yellow crystals, m.p. 220°C (dec).

**UV** \( \lambda_{\text{max}} \) (MeOH): 317, 208 nm.

**IR** \( \nu_{\text{max}} \) (KBr): 3329, 1672 (NH\(_2\)), 1608, 1531, 1259 cm\(^{-1}\).

**\( ^1H \) NMR** (300 MHz, DMSO-\( d_6 \), Fig. 1.23): \( \delta \) 6.83 (d, \( J = 15.6 \) Hz, 1H, C\(_8\)-H), 7.14 (t, \( J = 8.4 \) Hz, 1H, C\(_5\)-H), 7.43 (d, \( J = 8.4 \) Hz, 2H, C\(_3:5\)-H), 7.57 (t, \( J = 8.0 \) Hz, 1H, C\(_4\)-H), 7.60 (d, \( J = 15.6 \) Hz, 1H, C\(_7\)-H), 7.71 (d, \( J = 8.4 \) Hz, 2H, C\(_2:6\)-H), 8.00 (d, \( J = 7.8 \) Hz, 1H, C\(_6\)-H), 8.61 (d, \( J = 8.4 \) Hz, 1H, C\(_3\)-H), 11.44 (s, 1H, COOH).

**\( ^{13}C \) NMR** (75 MHz, DMSO-\( d_6 \), Fig. 1.24): \( \delta \) 115.9 (C-1), 119.6 (C-8'), 122.1 (C-3), 122.5 (C-5), 128.3 (C-2',6'), 129.1 (C-3',5'), 130.5 (C-6), 132.7 (C-1'), 133.3 (C-4'), 134.0 (C-4), 139.3 (C-7'), 140.4 (C-2), 162.9 (C-7), 169.0 (C-9').

**LCMS** (Fig. 1.25): \( m/z \) [M + Na]\(^+\) calcd for C\(_{16}\)H\(_{12}\)NO\(_3\)ClNa 324.7185, Found 324.0379.
Avenanthramide 21 N-[2'-chloro-(E)-cinnamoyl]anthranilic acid

![Chemical Structure](image)

Reaction of 3 (0.10 g, 0.45 mmole) and 2-chlorobenzaldehyde 31 (0.063 g, 0.45 mmole) gave chloroavenanthramide 21 as white solid (0.196 g, 65%). Recrystallization from water-methanol mixture gave white crystals, m.p. 200°C (dec).

**UV** \( \lambda_{\text{max}} \) (MeOH): 318, 214 nm.

**IR** \( \nu_{\text{max}} \) (KBr): 3064, 1678 (NHCO), 1608, 1531, 1228 cm\(^{-1}\).

**\(^1\)H NMR** (300 MHz, DMSO-d\(_6\), Fig. 1.26): \( \delta \) 6.95 (d, \( J = 16.0 \) Hz, 1H, C\(_8\)-H), 7.21 (t, \( J = 8.0 \) Hz, 1H, C\(_5\)-H), 7.43 (t, \( J = 8.0 \) Hz, 2H, C\(_4\),\(_5\)-H), 7.54 (d, \( J = 8.0 \) Hz, 1H, C\(_6\)-H), 7.64 (t, \( J = 8.0 \) Hz, 1H, C\(_3\)-H), 7.93 (d, \( J = 16.0 \) Hz, 1H, C\(_7\)-H), 8.00 (d, \( J = 8.0 \) Hz, 1H, C\(_4\)-H), 8.01 (d, \( J = 8.0 \) Hz, 1H, C\(_6\)-H), 8.60 (d, \( J = 8.0 \) Hz, 1H, C\(_3\)-H), 11.43 (s, 1H, COOH).

**\(^{13}\)C NMR** (75 MHz, DMSO-d\(_6\), Fig. 1.27): \( \delta \) 117.3 (C-1), 120.7 (C-8'), 123.3 (C-3), 125.7 (C-5), 127.9 (C-5'), 128.4 (C-6'), 130.2 (C-6), 131.4 (C-4'), 131.7 (C-3'), 132.3 (C-1'), 133.9 (C-2'), 134.2 (C-4), 136.3 (C-7'), 140.8 (C-2), 163.5 (C-7), 169.7 (C-9').

**LCMS** (Fig. 1.28): \( m/z \) [M + Na]\(^+\) calcd for C\(_{16}\)H\(_{12}\)NO\(_3\)ClNa 324.7185, Found 324.0572.
Reaction of 3 (0.10 g, 0.45 mmole) and 2-furaldehyde 32 (0.043 g, 0.45 mmole) gave 33 as greyish white solid (0.0885 g, 74%). Recrystallization from water-acetone mixture gave grey flakes, m.p. 184°C (dec).

UV $\lambda_{\text{max}}$ (MeOH): 325, 208 nm.

IR $v_{\text{max}}$ (KBr): 1697 (NHCO), 1658, 1604, 1531, 1217 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-d$_6$, Fig. 1.29): For assignments refer Figure X, pg 32.

$^{13}$C NMR (75 MHz, DMSO-d$_6$, Fig. 1.30): For assignments refer Figure XI, pg 32.

LCMS (Fig. 1.31): $m/z$ [M + H]$^+$ calcd for C$_{14}$H$_{12}$NO$_4$ 258.0760, Found 258.1111; [M + Na]$^+$ calcd for C$_{14}$H$_{13}$NO$_4$Na 280.0580, Found 280.0802.

Preparation of Avenanthramide 10 N-[4'-hydroxy-3-methoxy-(E)-cinnamoyl]anthranilic acid using procedure reported by Ashok Kumar et al

N-Acetyl anthranilic acid

Prepared according to the reported literature procedure.

Reaction of N-acetyl anthranilic acid with vanillin 6

To a solution of N-acetyl anthranilic acid (0.25 g, 1.4 mmole) in methanol (8 mL) was added vanillin 6 (0.175 g, 1.4 mmole) in aqueous NaOH (2%, 5 mL) and the reaction mixture was stirred at room temperature for 10 hrs and then heated under reflux for 6 hrs. Methanol was distilled off and ice cold water was added to the
residue. Acidification of the aqueous layer gave N-acetyl anthranilic acid (identified by comparison of m.p. & IR) as yellow solid which was filtered, washed with water and dried.

**Conclusion**

Under the reaction conditions reported\(^\text{29}\) by Ashok Kumar et al for the preparation of 10, the starting material N-acetyl anthranilic acid was recovered unchanged.

**N-Phenylmalonamic acid 36**

![Chemical Structure](image)

**Using acetonitrile as the solvent**

An equimolar mixture of Meldrum's acid 1 (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry acetonitrile (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a CaCl\(_2\) guard tube. The reaction mixture was heated under reflux and the progress of the reaction was monitored by TLC. After 6 hrs, when no more change in TLC was observed, the reaction mixture was cooled to room temperature, extracted with saturated NaHCO\(_3\) solution and regenerated using 1:1 HCl. The solid separated was filtered, washed with water and dried at 100°C to give N-phenylmalonamic acid 36 (0.075 g, 40%). Recrystallization from ethanol afforded white shiny crystals, m.p. 130°C, Lit.\(^\text{47}\) 132°C.

**Using benzene as the solvent**

An equimolar mixture of Meldrum's acid 1 (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry benzene (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a CaCl\(_2\) guard tube. The reaction
mixture was heated to 60-65°C and the progress of the reaction was monitored by TLC. After 24 hrs, when no more change in TLC was observed, the reaction mixture was cooled to room temperature, extracted with saturated NaHCO₃ solution and regenerated using 1:1 HCl. The solid separated was filtered, washed with water and dried at 100°C to give N-phenylmalonamic acid 36 (0.113 g, 60%). Recrystallization from ethanol afforded white shiny crystals, m.p. 130°C, Lit.⁴⁷ 132°C.

IR νmax (KBr): 3118 (NH), 1720 (CH₂COOH), 1685 (NHCO), 1643 (Ar-COOH), 1608, 1591, 1296 cm⁻¹.

Using toluene as the solvent

An equimolar mixture of Meldrum’s acid 1 (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry toluene (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a CaCl₂ guard tube. The reaction mixture was heated under reflux. TLC of the reaction mixture indicated formation of acetanilide. The reaction mixture was worked up as before to afford acetanilide as white solid (0.0924 g, 65%), m.p. 110°C, Lit ⁴⁷ 113-115°C.

4-[(Carboxyacetyl)amino]benzoic acid 37

An equimolar mixture of Meldrum’s acid 1 (0.288 g, 2 mmole) and p-amino-benzoic acid (0.274 g, 2 mmole) in dry toluene (3 mL) was heated under reflux and the progress of the reaction monitored by TLC. After 4 hrs on cooling the reaction mixture to room temperature a white solid separated out, this was filtered and washed with water. It was then chemically purified by dissolving in saturated NaHCO₃ solution, regenerated using 1:1 HCl, filtered under suction,
washed with water and dried at 100°C to give a white solid (0.407 g, 91.25%). Recrystallization from ethanol afforded white shiny crystals of 4-[(carboxyacetamido]benzoic acid 37, m.p. 260°C (decomp).

**IR** $\nu_{\text{max}}$ (KBr): 3273 (NH), 1720 (CH$_2$COOH), 1678 (NHCO), 1664 (Ar-COOH), 1537, 1176, 933 cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$, MeOH in traces, **Fig. 1.32**): For assignments refer **Figure XII**, pg 38.

$^{13}$C NMR (75 MHz, CDCl$_3$, MeOH in traces, **Fig. 1.33**): For assignments refer **Figure XII**, pg 38.

**Reaction of Meldrum’s acid 1 with 1-naphthylamine**

**Formation of N-naphthylacetamide**

An equimolar mixture of Meldrum’s acid 1 (0.216 g, 1.5 mmole) and 1-naphthyl amine (0.2 g, 1.4 mmole) was heated to reflux in dry toluene (5 mL) for 4 hrs. On cooling to room temperature, white solid separated out which was filtered, washed with water and dried at 100°C to give N-(1-naphthyl)acetamide as white solid (0.2 g, 77.29%). Recrystallization from CHCl$_3$-MeOH afforded colourless crystals m.p. 152°C, Lit.$^{48b}$ 160°C.

**N-(1-Naphthyl)malonamic acid 38**

![38](image)

An equimolar mixture of Meldrum’s acid 1 (0.216 g, 1.5 mmole) and 1-naphthyl amine (0.2 g, 1.4 mmole) in dry benzene (5 mL) was heated to 60-65°C for 24 hrs. On cooling to room temperature, the half amide separated out as white solid which was filtered and washed with water. It was then chemically purified by
dissolving in saturated NaHCO₃ solution, regenerated using 1:1 HCl, filtered under suction, washed with water and dried at 100°C to give white solid (0.22 g, 68.75%). Recrystallization from benzene afforded colourless crystals m.p. 144°C (decomp) of N-(1-naphthyl)malonamic acid 38.

IR v max (KBr): 3260 (NH), 1732 (CH₂COOH), 1710 (NHCO), 1550, 792 cm⁻¹.

¹H NMR (300 MHz, CDCl₃, MeOH in traces, Fig. 1.34): δ 3.55 (s, 2H, C₂-H), 7.47-7.56 (m, 3H), 7.68 (d, J = 6.9 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 4.8 Hz, 1H), 8.13 (d, J = 5.1 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃, MeOH in traces, Fig. 1.35): δ 42.93 (C-2'), 121.09, 122.19, 125.03, 125.12, 125.46, 125.63, 127.25, 127.69, 132.82, 133.25, 164.94 (C-1'), 169.17 (C-3').

**Reaction of Meldrum’s acid 1 with a) p-phenylenediamine, b) 2,4-dinitroaniline, c) N,N-diethylamine and d) N,N-diphenylamine.**

**Formation of respective N-acetamide derivatives.**

An equimolar mixture of Meldrum’s acid 1 (1 mmole) and the respective amine (1 mmole) in dry toluene (3 mL) was refluxed for 4 hrs. On cooling to room temperature the reaction product was worked up as before to give the respective acetamide derivatives: (a) N,N′-benzene-1,4-diylacetamide as pale brown solid m.p. 302°C, Lit.⁴⁷ 301-303°C; (b) N-(2,4-dinitrophenyl)acetamide as yellow crystals, m.p. 116°C, Lit.⁴⁷ 121°C; (c) N,N-diethylacetamide as yellow oil; (d) N,N-diphenylacetamide as colourless plates m.p. 100°C, Lit.⁴⁷ 100°C.
N-Benzylmalonamic acid 39

\[
\begin{array}{cccc}
\text{Solvent} & \text{Temperature °C} & \text{Yield %} \\
\hline
\text{Toluene} & \text{Reflux} & \text{Nil} \\
\text{C}_6\text{H}_6 & \text{R.T.} & 13 \\
\text{C}_6\text{H}_6 & 40-50 & 16 \\
\text{C}_6\text{H}_6 & 60-70 & 20 \\
\text{CH}_3\text{CN} & 80 & 23 \\
\text{Ether} & \text{Reflux} & 20 \\
\text{Pyridine/ether} & \text{Reflux} & 40 \\
\end{array}
\]

An equimolar mixture of Meldrum’s acid 1 (0.36 g, 2.5 mmole) and benzylamine (0.265 g, 2.5 mmole) in appropriate solvent (5 mL) was refluxed for 24 hrs as indicated in the table.

On cooling to room temperature, the organic layer was extracted with saturated NaHCO₃ (3 × 3 mL). The NaHCO₃ extract was acidified with conc. HCl and extracted with ether (3 × 3 mL). The combined organic extracts were washed with H₂O (2 × 3 mL), dried over Na₂SO₄ and evaporated to give white solid (0.188 g, 53%). Recrystallization from benzene gave colourless crystals (m.p. 86-88°C) of N-benzylmalonamic acid 39.

\text{IR } \nu_{\text{max}} (\text{KBr}): 3287 (\text{NH}), 1745 (\text{CH}_2\text{COOH}), 1632 (\text{NHCO}), 1604, 1566, 1193 cm\(^{-1}\).

\text{¹H NMR} (300 MHz, CDCl₃, MeOH in traces, Fig. 1.36): For assignments refer Figure XIII, pg 41.

\text{¹³C NMR} (75 MHz, CDCl₃, MeOH in traces, Fig. 1.37): For assignments refer Figure XIV, pg 41.
References

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