CHAPTER 3
DISCUSSION
Chemical constituents of **Rhus alata**

*(Anacardiaceae)*

The family **Anacardiaceae** consists of 73 genera and about 600 species. The genus **Rhus** one of the largest genera (about 50 species) of trees, shrubs and climbers is chiefly distributed in the warm temperate region of both the hemispheres extending in the tropical and cold temperate region. About a dozen species occur in India\(^1\), find extensive use in folk medicines for the treatment as spasmodic, antiviral and anticancer\(^2\). Some species may cause dermatitis in sensitive people\(^3\). **Rhus** species have been reviewed by Khan\(^4\).

Earlier report from this plant is the isolation and characterization of biflavones\(^5\) and triterpenes\(^6\) from the leaves.

The present discussion deals with the isolation and characterization of the following compounds from the bark of **Rhus alata**

1) Dimethylester of terephthalic acid.
2) \(\beta\)-Amyrin
3) Friedelin
4) Lupeol
5) \(\beta\)-Sitosterol
6) Oleanolic acid
7) Taraxerone
8) Ethyl gallate
9) \((2E)-3-(4-hydroxy-5,7\text{-dimethylbenzo}[3,4-b]furan-6-yloxy)\)-prop-2-enoic acid.

The barks of **Rhus alata** were collected from Pucchunga University College, Aziaul, Mizoram. After defatting with petroleum ether (40-60°C), they were refluxed exhaustively with benzene, chloroform and ethylacetate respectively. Benzene and chloroform extracts responded positively to terpenoid test\(^7\) and showed similar spots on TLC plates. Hence, they were mixed together. After removal of the solvents under reduced pressure, a gummy mass was obtained. The gummy mass was chromatographed over silica gel column, using different solvent systems namely, petrol, petrol-benzene (9:1-1:1), benzene, benzene-chloroform (9:1-1:1) and ethylacetate
respectively. The fractions obtained from petrol-benzene (9:1-1:1) showed four spots on TLC plates with some minor impurities. Repeated column chromatography followed by fractional crystallization with chloroform-methanol gave four compounds labelled as RA-1, RA-2, RA-3 and RA-4. Benzene and benzene-chloroform (9:1) elutes were found to contains two same substances, which was separated by repeated column chromatography followed by fractional crystallization and labelled as RA-5 and RA-6. Benzene-chloroform elutes (8:2-1:1) were found to be a mixture of three compounds which were separated by repeated column chromatography and labelled as RA-7, RA-8 and RA-9.

**RA-1**

RA-1 was obtained from the column with petroleum ether-benzene (9:1) mixture. It was crystallized from chloroform-petroleum ether as white needles (100 mg), m.p. 138°C. The elemental analysis agreed with molecular formula C_{10}H_{10}O_{4}, further supported by the molecular ion peak [M^+] at m/z 194.

The ir spectrum of the compound RA-1 showed a carbonyl band at 1720 cm\(^{-1}\) along with bands at 1500, 1435 cm\(^{-1}\) characteristic of an aromatic compound. The uv spectrum showed maxima absorption at 265 nm along with weak absorption at 340nm.

The \(^1H\)-nmr spectrum showed a sharp singlet at 8.397 integrating for six protons, corresponded to two methoxy groups. A sharp singlet at 8.13 integrated for four protons was assigned to H-2, H-3, H-5 and H-6 of phthalic acid.

The mass spectrum was quite typical for aromatic methyl esters. The base peak was observed at m/z 163 [M^+-OCH\(_3\)] and other fragments were observed at m/z 179 [M^+-CH\(_3\)], 164 [M^+-2 x CH\(_3\)], 135 [M^+-COOCH\(_3\)] and 76 [M^+-2 x COOCH\(_3\)] respectively.

On the basis of above results, the RA-1 was identified as dimethylester of terephthalic acid. (I).
**RA-2**

RA-2 was eluted from column with petroleum ether-benzene (7:3) mixture and crystallized with chloroform-methanol as white needles (190 mg), m.p. 198 °C, \([\alpha]_D^{19} +88.4^\circ\) (CDCl$_3$). It gave positive Leibermann-Burchard test$^2$. The ir spectrum showed the bands at 3360 cm$^{-1}$ (OH), 2960 cm$^{-1}$, 2880 cm$^{-1}$, 1650 cm$^{-1}$, 1465 cm$^{-1}$, (C=C) 1040 cm$^{-1}$ and 980 cm$^{-1}$ indicating the presence of hydroxyl group and olefinic bond. The molecular ion peak of RA-2 at m/z 426 along with elemental analysis agreed with molecular formula C$_{30}$H$_{50}$O. The $^1$H-nmr data are given in (Table-1).

From the above data and their direct comparison with an authentic sample, RA-2 was identified as $\beta$-Amyrin$^8$ (II).

![Chemical Structure](image)

**Table-1**

$^1$H-nmr data of RA-2, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$8 \times$ CH$_3$</td>
<td>24</td>
<td>0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 6H), 0.95 (s, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.14 (s, 3H).</td>
</tr>
<tr>
<td>-CH$_2$ and -CH protons of</td>
<td>23</td>
<td>1.83, 2.01, 3.01 (dd, $J=9.0$ Hz and 7.0 Hz)</td>
</tr>
<tr>
<td>cyclic system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$- to OH</td>
<td>1</td>
<td>4.88 (s, br)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.21 (m)</td>
</tr>
</tbody>
</table>

$s$= singlet, $d$= doublet, $m$= multiplet, spectrum run at 100 MHz, using TMS as internal standard.
RA-3

RA-3 was obtained from the column with petroleum ether-benzene (6:4) mixture and crystallized with chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 262-64 °C, [α]$_D^{23}$ $-29.4^\circ$ (CDCl$_3$). It gave positive Leibermann-Burchard test$^7$ and Nollers test$^8$ indicating RA-3 to be a triterpene. The elemental analysis and molecular ion peak at m/z 426 agreed with molecular formula C$_{30}$H$_{50}$O. Its identity as freeelin (III) was established by comparison of its spectral data including ($^1$H-nmr data (Table-2), ir and mass spectra) and co-chromatography, m.p. and m.m.p. with that of authentic sample$^{10-12}$ of Friedelin.

![Diagram of compound RA-3](image)

### Table-2

$^1$H-nmr data of RA-3 values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.72</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.87</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.89</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>0.92</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>(6H, s)</td>
<td>0.95</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>1.05</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>(3H, s)</td>
<td>1.18</td>
</tr>
<tr>
<td>-CH$_2$ and -CH protons</td>
<td>(23H, m)</td>
<td>1.25, 1.34, 1.45, 1.52, 1.58</td>
</tr>
<tr>
<td>C$_2$- 2H and C$_4$- 1H</td>
<td>(3H, m)</td>
<td>2.26- 2.41</td>
</tr>
</tbody>
</table>

$s=$ singlet, $m=$ multiplet, spectrum run at 200 MHz in CDCl$_3$, using TMSi as internal standard
RA-4

RA-4 was obtained from the column with petroleum ether-benzene (1:1) mixture and crystallized from methanol-chloroform (170 mg), m.p. 214-15 °C, [α]D +23.64° (CHCl₃). It gave positive Leibermann-Burchard test⁷, Nollers test⁹ and gave yellow colour with tetranitromethane. The elemental analysis agreed with molecular formula C₃₀H₅₀O. IR showed bands at 3360 cm⁻¹ and 1030 cm⁻¹ (OH), 1645 cm⁻¹ (C=C) and 1385 cm⁻¹ (geminal dimethyl), 885 cm⁻¹ (terminal methylene). Mass spectrum of the triterpene alcohol gave [M⁺] m/z 426 (11.0%) with other principal ions at m/z 411 [M-CH₃] (6.0%), 207 (34.0%), 189 (77.0%) and a base peak at m/z 95. It afforded an acetate m.p. 218-20 °C. IR spectrum of the acetate revealed the presence of terminal methylene by a band at 875 cm⁻¹. Some other important bands were observed at 1245 cm⁻¹ (acetate), 1640 cm⁻¹ (C=C) and 1730 cm⁻¹ (C=O). ¹H-nmr spectrum gave the signals at δ 0.82, 0.87, 0.94, 1.04 (CH₃-protons), 1.27, 1.41, 1.46, 1.47 (CH₂-protons), δ 2.03 (OCOCH₃) and multiplets in the range of δ 4.28-4.77 (>CHOAc), δ 4.59-8 4.67 (J=8.0 Hz, >C=CH₂).

On the basis of the above physico-chemical data for RA-4 and its derivatives, RA-4 was identified as Lupeol¹³ (IV).

![IV](image)

RA-5

RA-5 was obtained from the column with benzene only and crystallized from chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 136-37°C, [α]D -32.1° (CDCl₃). It gave positive Leibermann-Burchard test⁷ and responded to tetranitromethane colour test. The ir spectrum showed the presence of gem-dimethyl groups, hydroxyl group and olefinic double bond and showed bands at 3340 cm⁻¹ (OH), 1055 cm⁻¹, 1655 cm⁻¹, 1460 cm⁻¹, 840 cm⁻¹ (C=C), 1375 cm⁻¹ (C-Me₂). RA-5 gave an acetate with acetic
anhydride and pyridine, m.p. 114-16 °C, $[\alpha]_{D}^{25} = -48.5^\circ$ (CHCl₃) and benzoate m.p. 145-46 °C. The $^1$H-nmr data are given in (Table-3). The mass spectrum showed the molecular ion peak at m/z 414.

From the above data and direct comparison with an authentic sample⁸, RA-5 was characterized as β-Sitosterol (V).

![Chemical Structure of RA-5](image)

**Table-3**

$^1$H-nmr data on RA-5, values on δ-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-CH₃</td>
<td>0.70 (s, 3H)</td>
</tr>
<tr>
<td>29-CH₃</td>
<td>0.80 (d, J=6.8 Hz, 3H)</td>
</tr>
<tr>
<td>26,27-CH₃</td>
<td>0.88 (d, J=6.5 Hz, 6H)</td>
</tr>
<tr>
<td>21-CH₃</td>
<td>0.92 (d, J=6.5 Hz, 3H)</td>
</tr>
<tr>
<td>19-CH₃</td>
<td>1.02 (s, 3H)</td>
</tr>
<tr>
<td>3-ax-H</td>
<td>3.56 (m, 1H)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>5.36 (m, 1H)</td>
</tr>
<tr>
<td>-CH₂ and -CH protons of cyclic system and side chain</td>
<td>1.07-2.34</td>
</tr>
<tr>
<td>OH</td>
<td>4.25 (m, 1H)</td>
</tr>
</tbody>
</table>

s= singlet, d= doublet, m= multiplet, spectrum run in CDCl₃ at 90 MHz, using TMS as internal standard

**RA-6**

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot along with some minor
impurities. It was purified by crystallization into pure component RA-6 (225 mg) m.p. 299-300°C. It responded positively to Leibermann-Burchard test.

Its ir spectrum showed the characteristic bands at 3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210 cm\(^{-1}\). The \(^1\)H-nmr spectrum of RA-6 (Table-4) indicated the presence of seven tertiary methyl groups at δ 0.71 (3H), 0.82 (6H), 0.84 (3H), 0.89 (6H) and 1.10 (3H). The two unresolved multiplets at δ 4.39 and 5.20 are ascribed to one proton α-to hydroxylic group and one olefinic proton. Acetylation of RA-6 with acetic anhydride and pyridine afforded a monoacetate RA-6 (Ac). The ir spectrum of RA-6 (Ac) displayed bands at 1724, 1686 and 1250 cm\(^{-1}\) characteristic of the acetyl function. The \(^1\)H-nmr spectrum of the acetate RA-6(Ac) showed seven tertiary methyls as four independent singlets at δ 0.75 (3H), 0.85(6H), 0.94 (9H) and 1.12 (3H) and one singlet for an acetyl function at δ 2.02, an unresolved multiplet at δ 4.50 for one proton α- to the acetoxy and another multiplet at δ5.25 for the olefinic proton. These data further support to the β-amyrin skeleton of the compound with one acetyl function present. Formation of its methyl ester (m.p. 198 °C) with diazomethane and not with methanolic hydrochloric acid, coupled with difficulty of its hydrolysis suggested the attachment of carboxyl group at tertiary carbon atom (C-17). Its \(^1\)H-nmr spectrum showed one singlet of methyl ester at δ 3.58 and seven tertiary methyls as singlets at δ 0.70 (3H), 0.84 (6H), 0.86 (3H), 0.90(6H) and 1.10 (3H). Two unresolved multiplets centered at δ4.50 and δ5.30 can be attributed to one proton α- to methyl ether and one olefinic proton respectively.

On the basis of above spectral data and comparison of m.p., m.m.p. with an authentic sample confirmed the identity of RA-6 as oleanolic acid (VI).
**Table-4**

$^1$H-nmr data of RA-6, values on $\delta$- scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_3$</td>
<td>3</td>
<td>0.71 (s)</td>
</tr>
<tr>
<td>$2 \times \text{CH}_3$</td>
<td>6</td>
<td>0.82 (s)</td>
</tr>
<tr>
<td>$\text{CH}_3$</td>
<td>3</td>
<td>0.84 (s)</td>
</tr>
<tr>
<td>$2 \times \text{CH}_3$</td>
<td>6</td>
<td>0.89 (s)</td>
</tr>
<tr>
<td>$\text{CH}_3$</td>
<td>3</td>
<td>1.10 (s)</td>
</tr>
<tr>
<td>$\text{H-} \alpha \text{ to hydroxyl group}$</td>
<td>1</td>
<td>4.39 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.20 (m)</td>
</tr>
</tbody>
</table>

$s$= singlet, $m$= multiplet, spectrum run at 60 MHz in CDCl$_3$, using TMS as internal standard.

**RA-7**

RA-7 was eluted from the column with benzene-chloroform (8:2) mixture. It was crystallized from chloroform-ethanol as colourless needles (170 mg), m.p. 240°C. The elemental analysis agreed with molecular formula C$_{30}$H$_{48}$O. It gave positive Leibermann-Burchard test$^7$. The ir spectrum showed a strong carbonyl band at 1690-1700 cm$^{-1}$.

The $^1$H-nmr spectrum of RA-7 (Table-5) showed five singlets attributed to the protons of eight methyl groups at $\delta$ 0.83 (3H), 0.93 (6H), 0.97 (3H), 1.06 (9H) and 1.12 (3H). Methylene and methine protons appeared as multiplets at $\delta$ 1.32 and $\delta$ 1.50-1.90. The multiplets at $\delta$ 2.28-62.52 integrating for two protons were assigned to methylene protons at C-2 which is adjacent to carbonyl group at C-3. A double doublet at $\delta$ 5.56 integrating for one proton is attributed to olefinic proton.

These data compare well with Taraxerone$^{14}$, therewby support its structure (VII). The mass spectrum also supported the above structure, showed a molecular ion peak at m/z 424 and the base peak at m/z 300.
Table-5

$^1$H-nmr data of RA-7, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.83 (s)</td>
</tr>
<tr>
<td>2 x CH$_3$</td>
<td>6</td>
<td>0.93 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>0.97 (s)</td>
</tr>
<tr>
<td>3 x CH$_3$</td>
<td>9</td>
<td>1.06 (s)</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.12 (s)</td>
</tr>
<tr>
<td>-CH and -CH$_2$ protons</td>
<td>21</td>
<td>1.32 (m), 1.5-1.9 (m)</td>
</tr>
<tr>
<td>-CH$_2$ protons at C-2</td>
<td>2</td>
<td>2.28-2.52 (m)</td>
</tr>
<tr>
<td>Olefinic proton</td>
<td>1</td>
<td>5.56 (dd)</td>
</tr>
</tbody>
</table>

$s$=singlet, $dd$=double doublet, $m$=multiplet, spectrum run in CDCl$_3$ at 60 MHz, using TMS as internal standard.

RA-8

RA-8 was eluted from the column by benzene-chloroform (7:3) mixture. It was crystallized from methanol as reddish buff needles (120 mg), m.p. 155°C. It gave blue colour with alc. ferric chloride solution and brown spot under uv light. It was characterized as ethyl gallate by m.p., m.m.p., spectral studies and co-chromatography with an authentic sample.

The $^1$H-nmr spectrum of RA-8 (Table-6), showed a triplet at $\delta$ 1.31 integrating for three protons and a quartet at $\delta$ 4.24 integrating for two protons corresponded to methyl and methylene protons respectively.

On the basis of above results, RA-8 was characterized as ethyl gallate (VIII).
Table-6

$^1$H-nmr data of RA-8, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>3</td>
<td>1.36 (t, $J=7.0$ Hz )</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>2</td>
<td>4.24 (q, $J=7.0$ Hz )</td>
</tr>
<tr>
<td>H-2, 6</td>
<td>2</td>
<td>7.11 (s)</td>
</tr>
</tbody>
</table>

s= singlet, t=triplet, q=quartet, spectrum run in DMSO-$d_6$ at 100 MHz, using TMS as internal standard.

RA-9

RA-9 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg), m.p.160-162 °C. Homogeneity of the compound was established by TLC examination using different solvent systems indicating it to be a pure compound. It showed blue fluorescence under uv light. Elemental analysis and the molecular ion peak at m/z 248 agreed with the molecular formula as C$_{13}$H$_{12}$O$_5$.

The ir spectrum (Fig. 1) was helpful in identifying two functional groups present as COOH and OH groups. In the hydroxyl regions the absorption was evident between 3500 cm$^{-1}$ and 2100 cm$^{-1}$ with prominent peaks appearing at 3496, 3429, 3110 and 2817 cm$^{-1}$. The O-H stretching of the carboxyl group is reported to appear as a broad band with a series of minor peaks between 3000 and 2500 cm$^{-1}$ with the main peak around 3000 cm$^{-1}$ and a satellite peak near 2650 cm$^{-1}$ $^{15a}$. The low frequency region of the hydroxylic absorption can thus be attributed to the O-H stretching in the carboxyl. The peaks with high frequency region 3496 and 3429 cm$^{-1}$, therefore can be attributed to a hydroxyl function.
A corresponding peak at 1669 cm\(^{-1}\) in the carbonyl region can thus be attributed to the carbonyl group of the carboxyl, either aromatic or more likely of \(\alpha, \beta\)-unsaturated character. A band in the range 1440-1375 cm\(^{-1}\) has been said to arise out of C–O vibration of the carboxyl group\(^{15b}\). A band of fairly high intensity appearing at 1394 cm\(^{-1}\) in this instance can be reasonably assumed to confirm the presence of a carboxyl function in RA-9. Another band appearing between 1320-1210 cm\(^{-1}\)\(^{15c}\) and found to be the most intense appearing between 1600-700 cm\(^{-1}\) has also been attributed to a carboxyl function. The band appearing at 1276 cm\(^{-1}\) in the ir spectrum of RA-9, can thus be assumed to arise only due to the presence of a carboxyl function.

Another band appearing at 910 cm\(^{-1}\) can now be attributed with O–H deformation vibration of the COOH group, which has been reported to appear at 935 ± 15 cm\(^{-1}\)\(^ {15d}\).

Another feature which stands out in the ir spectrum is a band appearing at 1606 cm\(^{-1}\) equal in intensity of the carbonyl of the carboxylic group appearing at 1669 cm\(^{-1}\). This band based on its frequency and intensity can be reasonably attributed to a conjugated \(-\text{C} = \text{C} -\) system or to an aromatic system.

RA-9, can therefore be tentatively concluded to possess either the system IX or X, preferably X as it has already concluded to carry a carboxyl function.

\[\text{C} = \text{C} \quad \text{C} = \text{C} \quad \text{O} \]

\[\text{C} = \text{C} \quad \text{C} = \text{C} \quad \text{OH} \quad \text{O} \quad \text{C} = \text{C} \quad \text{C} = \text{C} \quad \text{OH} \]

IX (X)

Additional information can be had from the \(^1\text{H-nmr}\) and the \(^{13}\text{C-nmr}\) spectra of RA-9. The \(^1\text{H-nmr}\) spectra (Fig.2, Table-7), shows in all, the presence of 12 protons. The spectrum was determined in a mixture of CDCl\(_3\) and DMSO-d\(_6\) and hence the peak appearing at 82.39 was excluded from consideration. The peak appearing at the highest field at 82.71 from its integral is found to represent six protons and from its sharp nature can be concluded to represent two methyl groups situated in identical environments. Two sets of signals integrating for two protons each appeared in the region of 86.8 and 87.4. These signals when integrated with the postulated partial structure (X) for RA-9, can lead to the conclusion that one proton each from these sets can represent hydrogen each attached to \(\alpha, \beta\)-carbons represented in (X), leading to the partial structure (XI).
RA-9 has already been found to have the molecular formula C\textsubscript{13}H\textsubscript{12}O\textsubscript{5}, out of which XI accounts for three carbons, three hydrogens and two oxygens. Additionally two methyl groups and a hydroxyl group have been concluded to be present in RA-9, on the basis of its \textsuperscript{1}H-nmr and ir spectra. This information taken together with XI, now accounts for five carbons, ten hydrogens and three oxygens (XI, C\textsubscript{3}H\textsubscript{3}O\textsubscript{2} + 2 \times CH\textsubscript{3} + 1 \times OH = C\textsubscript{5}H\textsubscript{10}O\textsubscript{3}). The partial structure for RA-9 can therefore now be expanded to (XII).

The signals for two methyl groups in RA-9 appear in the region of aromatic methyls. Assuming thus RA-9 to be aromatic in character, six carbons are accounted for bearing two carbons, two hydrogens and two oxygens yet to be accounted for. The two sets of relatively deshielded signals representing two hydrogens each, appearing in the region of δ6.8 and 7.4 came in handy in further expanding the partial structure (XII). One hydrogen each from these two signals at δ6.81 (J=9.0 Hz) and δ7.43 (J=9.0 Hz) have been assigned to hydrogens attached to the α,β-unsaturated carbons of the acid moiety (XI) coupling constant of which indicate the hydrogens to be cis-oriented.

Chemical shifts of one proton each from the two sets, δ7.41 (J= 3.0Hz) and δ 6.83 (J=3.0 Hz) correspond to the well documented values of the α'- and β'- protons of a furan ring in benzofurans at δ 7.3 and δ6.6 respectively\textsuperscript{16}.

Thus RA-9 is concluded to have a benzofuran moiety, two carbons, two hydrogens and one oxygen are further accounted for, thus enabling the partial structure for RA-9 to be expanded as (XIII).
It can be noted that all the thirteen carbons are accounted for in (XIII) and hence appendages have to be attached to the eight carbons of the benzofuran nucleus. However, the furan ring, as evidenced by the presence of both the \( \alpha \) and \( \beta \)-hydrogens in the \( ^1H \)-nmr spectrum is unsubstituted. Hence the groups to be accounted further in formulation of the structure for RA-9 have to be appended to four aryl carbons, leading to the partial structure (XIV). However, one more oxygen is to be accounted for.

Considering that there is no further locations it can be attached to, or any other atom which it can carry, it has to be present as an ether function. This further limits the location of this oxygen as present between an aryl carbons and the acid unit. The other alternative is automatically excluded in the absence of an aromatic methoxyl signal in the \( ^1H \)-nmr spectrum. RA-9 can therefore be represented by (XV) or any of its regiomers.

The \( ^1H \)-nmr spectrum of RA-9, still requires further assignments. The signal appearing at \( \delta 6.06 \) and broadish in nature can be assigned to the phenolic proton
considering that DMSO-d$_6$ is used as a co-solvent. The most deshielded of the signals which appears at $\delta 9.89$ should arise only out of the carboxylic proton.

The final structure of RA-9 has now to be one of the possible regioisomers (XVI→XXVII).

(XVI) \[ \text{O-CH=CH-CO}_2H \]

(XVII) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XVIII) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XIX) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XX) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XXI) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XXII) \[ \text{O-CH}=\text{CH-CO}_2H \]

(XXIII) \[ \text{O-CH}=\text{CH-CO}_2H \]
Of the above structures, the one that can be assigned to RA-9 should have some features present to enable the methyl signals in both the $^1$H-nmr and $^{13}$C-nmr to coincide and have identical chemical shifts. Three oxygens are directly attached to the phenyl ring and these are the one which can cause shielding as well as deshielding around the ring and more particularly on the methyl groups. Analyzing all the possible structures (XVI→XXVII), the structures XVII, XVIII, XXIV and XXV emerge as the possible structure for RA-9.
Of the above (XVIII) appears to be the structure which can correspond to RA-9, not only with respect to situations of oxygen, but also with respect to the side chain which is capable of having similar effect on both the methyl groups. RA-9 can therefore be assigned the structure (XVIII).

The signals in the $^{13}$C-nmr spectrum (Fig.3) can be assigned as below by employing additive factors $^{17}$.

The mass of RA-9 is compatible with the proposed structure (XVIII) as has been detailed in the scheme (I).

On the basis of above discussion, RA-9 has been characterized as (2E)-3(4-hydroxy-5,7-dimethylbenzo[3,4-b]furan-6-yloxy-prop-2-enoic acid named as alatic acid (XVIII) which is being reported for the first time.
**Table-7**

$^1$H-nmr data of RA-9, values on $\delta$-scale

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x CH$_3$</td>
<td>6</td>
<td>2.71 (s)</td>
</tr>
<tr>
<td>$\alpha$- H of the acid moiety</td>
<td>1</td>
<td>6.81 (d, $J=9.0$ Hz)</td>
</tr>
<tr>
<td>$\beta$- H of the acid moiety</td>
<td>1</td>
<td>7.43 (d, $J=9.0$ Hz)</td>
</tr>
<tr>
<td>4-OH</td>
<td>1</td>
<td>6.06 (br s)</td>
</tr>
<tr>
<td>OH of acid moiety</td>
<td>1</td>
<td>9.89 (br s)</td>
</tr>
<tr>
<td>H-2 ($\alpha$-H of furan ring)</td>
<td>1</td>
<td>7.41 (d, $J=3.0$ Hz)</td>
</tr>
<tr>
<td>H-3 ($\beta$-H of furan ring)</td>
<td>1</td>
<td>6.83 (d, $J=3.0$ Hz)</td>
</tr>
</tbody>
</table>

$s$=singlet, br $s$= broad singlet, $d$=doublet, spectrum run at 300 MHz using TMS as internal standard.
EXPERIMENTAL
RA-1
Elution of the column with petroleum ether-benzene (9:1) mixture and crystallized from chloroform-petrol as white needles (100 mg), m.p. 138°C.
Analysed for C\textsubscript{10}H\textsubscript{10}O\textsubscript{4}
Calcd.: C, 61.85; H, 5.15%
Found: C, 61.79; H, 5.12%

UV data \(\lambda_{\text{max}}\) (CHCl\textsubscript{3}) nm
265, 340.

IR \(\nu_{\text{max}}\) cm\textsuperscript{-1}
1720, 1500, 1435.

\(\text{^1H-nmr} (100 \text{ MHz, CDCl}_3)\) values on \(\delta\)-scale
3.97 (6H, s, 2x OCH\textsubscript{3}), 8.13 (4H, s, Ar-H).

Mass m/z
194 [M\textsuperscript{+}], 163[M\textsuperscript{+}-OCH\textsubscript{3}] (100%), 179 [M\textsuperscript{+}-CH\textsubscript{3}], 164 [M\textsuperscript{+}-2 x CH\textsubscript{3}], 135 [M\textsuperscript{+}-COOCH\textsubscript{3}], 76 [M\textsuperscript{+}-2 x COOCH\textsubscript{3}].

RA-2
Elution of the column with petroleum ether-benzene (7:3) mixture and crystallized from chloroform-methanol as white needles (190 mg) m.p. 198°C, R\textsubscript{f}= 0.63 (benzene-chloroform, 8:2). It gave positive Leibermann-Burchard test.

IR \(\nu_{\text{max}}\) cm\textsuperscript{-1}
3360 (OH), 2960, 2880, 1650, 1465 (C=C), 1040 and 980.

\(\text{^1H-nmr} (100 \text{ MHz, CDCl}_3)\) values on \(\delta\)-scale
0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H,s), 1.0 (3H, s), 1.14 (3H, s), 1.83, 2.01 (22H, -CH\textsubscript{2} and -CH protons of cyclic system), 3.01 (1H, dd, J=9.0 Hz and 7.0 Hz), 4.88 (1H, br s, \alpha\text{- to OH} ), 5.21 (1H, m, olefinic proton).

Mass m/z
426 [M\textsuperscript{+}].
Acetylation of RA-2

Crystalline RA-2 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand overnight at room temperature and then heated on a water bath for four hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was separated, washed well with water and dried. On crystallization from chloroform-methanol, it gave colourless needles (25 mg), m.p. 241-42°C, $[\alpha]_{D}^{23} +68.9^\circ$.

$^1$H-nmr(100 MHz, CDCl$_3$) values on $\delta$-scale

0.84 (3H, s, H-28), 0.89 (12H, s, CH$_3$-23, 24, 29, 30), 0.96 (6H, s, CH$_3$-25, 26), 1.14 (3H, s, CH$_3$-27), 2.08 (3H, s, OAc), 4.54 (1H, dd, J= 6.0 Hz, H-3α), 5.20 (1H, t, J= 3.5 Hz, H-12).

RA-3

Elution of the column with petroleum ether-benzene (6:4) and crystallized from chloroform-methanol as white needle-shaped crystals (150 mg), m.p. 262-64°C.

Analysed for C$_{30}$H$_{50}$O

Calcd.: C, 84.50; H, 11.30 %

Found: C, 84.47; H, 11.10 %

$^1$H-nmr (200 MHz, CDCl$_3$) values on $\delta$-scale

0.72 (3H, s, CH$_3$), 0.87 (3H, s, CH$_3$), 0.89 (3H, s, CH$_3$), 0.92 (3H, s, CH$_3$), 0.95 (6H, s, 2 x CH$_3$), 1.05 (3H, s, CH$_3$), 1.18 (3H, s, CH$_3$), 1.25, 1.34, 1.45, 1.52, 1.58 (23 H, m, - CH$_2$ and CH- protons), 2.26-2.41 [3H, m, (C$_2$-2H and C$_4$-1H)].
Mass m/z

426 [M⁺] (92.0%), 411 (40.0%), 341 (32.0%), 303 (70.0%), 273 (100.0%).

RA-4

Elution of the column with petroleum ether-benzene (1:1) and purified by repeated crystallization from methanol-chloroform afforded RA-4 as crystalline solid (170 mg) m.p. 214-15°C, [α]^D_{D}+23.64° (CHCl₃). It gave positive Leibermann-Burchard, Noller’s test and yellow with tetranitromethane.

IR KBr ν cm⁻¹

3360, 1030 (OH), 1645 (C=C), 1385 (geminal dimethyl), 885 (terminal methylene).

Analysed for C₃₀H₅₀O
Calcd.: C, 84.95; H, 11.50 %
Found: C, 84.93; H, 11.45 %

Acetylation of RA-4

Crystalline RA-4 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand over night at room temperature. After usual work-up the solid obtained was crystallized from chloroform-methanol as colourless flakes (25 mg), m.p. 218-20°C.

IR KBr ν cm⁻¹

875 (terminal methylene), 1245 (acetate), 1640 (C=C), 1730 (C=O).

¹H-nmr (100 MHz, CDCl₃) values on δ-scale

0.82, 0.87, 0.94, 1.04 (21H, s, 7 x CH₃), 1.27, 1.41, 1.46, 1.47 (m, –CH and –CH₂ protons), 2.03 (OCOCH₃), 4.28-4.77 (1H, m, α- to acetate), 4.59-4.67 (2H, d, J=8.0 Hz, olefinic protons).

Analysed for C₃₂H₅₄O₂
Calcd.: C, 82.05; H, 11.11 %
Found: C, 82.14; H, 11.17%
RA-5

Elution of the column with benzene only and crystallized from chloroform-methanol as white needle-shaped crystals (150mg) m.p. 136-37°C.

\[
\text{KBr IR } \nu_{\text{max}} \text{ cm}^{-1}
\]

3340 (OH), 1055, 1655, 1460, 840 (C=C), 1375 (C-Me2).

\[
\text{\textsuperscript{1}H-nmr (90 MHz, CDCl}_3\text{) values on } \delta \text{--scale}
\]

0.70 (3H, s, 18-Me), 0.80 (3H, d, J=6.8 Hz, 29-Me), 0.88 (6H, d, J=6.5 Hz, 26,27-Me), 0.92 (3H, d, J=6.5 Hz, 21-Me), 1.02 (3H,s, 19-Me), 3.56 (1H, m, 3-ax-H), 5.36 (1H, m, olefinic proton), 1.07-2.34 ( -CH\textsubscript{2} and -CH protons of cyclic system and side chain), 4.25 (1H, m, OH).

\[
\text{Mass m/z}
\]

414 [M\textsuperscript{+}].

\textbf{Acetylation of RA-5}

Crystalline RA-5 (30 mg) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml) and allowed to stand over night at room temperature and then heated on water bath for two hours. After cooling, the mixture was poured on crushed ice and left over night. The solid was collected, washed well with water and dried. On several crystallization from chloroform-methanol, it gave colourless flakes (20 mg), m.p.114-15°C, [\alpha]_{D}^{17} = -48.5° (CDCl\textsubscript{3}).

Analysed for C\textsubscript{31}H\textsubscript{52}O.

Calcd.: C, 81.57; H,11.40%

Found : C, 81.52; H,11.37%

\[
\text{KBr IR } \nu_{\text{max}} \text{ cm}^{-1}
\]

2930, 2850, 1730, 1660, 1460, 1380, 1260
**Benzoate formation**

The compound RA-5 (40 mg) was treated with benzoyl chloride (1 ml) and pyridine (0.5 ml) and the mixture was left over night at room temperature and then heated for six hours on a water bath. The reaction mixture was cooled and ice was added. The solid thus separated was filtered, washed with aqueous solution of potassium hydroxide (KOH) 2% and then with water. It was crystallized from methanol (25 mg), m.p. 145-46°C, [α]D17 - 7.52°.

**RA-6**

Elution of the column with benzene-chloroform (9:1) afforded a fraction which on TLC examination showed the presence of one major spot along with some minor impurities. It was purified by crystallization into pure component RA-6 (225 mg), m.p. 299-300°C. It responded positively to Leibermann-Burchard test.

![nujol](image)

**IR ν cm⁻¹**

3200 (OH), 2750-2610 (COOH), 1655 (C=O), 1210

**¹H-nmr (60 MHz, CDCl₃) values on δ-scale**

0.71 (3H, s, CH₃), 0.82 (6H, s, 2 x CH₃), 0.84 (3H, s, CH₃), 0.89 (6H, s, 2 x CH₃), 1.10 (3H, s, CH₃), 4.39 (1H, m, α- to hydroxyl group), 5.20 (1H, m, olefinic proton).

**Acetylation of RA-6**

Crystalline RA-6 (100 mg) was treated with acetic anhydride (1 ml) and pyridine (2 ml) and allowed to stand over night at room temperature. After usual work-up the solid obtained was crystallized several times from methanol to give RA-6 (Ac) (80 mg), m.p. 258-60°C.

![nujol](image)

**IR ν max cm⁻¹**

1724, 1686 and 1250
$^1$H-nmr (60 MHz, CDCl$_3$) values on $\delta$-scale

0.75 (3H, s, CH$_3$), 0.85 (6H, s, 2 x CH$_3$), 0.94 (9H, s, 3 x CH$_3$), 1.12 (3H, s, CH$_3$), 2.02 (3H, s, OCOCH$_3$) 4.50 (1H, m, H-$\alpha$ to acetoxyl), 5.25 (1H, m, olefinic proton).

Methylation of RA-6 (Ac)

The acetate RA-6 (Ac) (40 mg) was dissolved in ether and treated with an excess of ethereal solution of diazomethane. After leaving the contents at room temperature over night, the ether was evaporated and the product crystallized from methanol-chloroform as colourless shining plates (30 mg), m.p. 220-21 °C.

$^1$H-nmr (60 MHz, CDCl$_3$) values on $\delta$-scale

0.70 (3H, s, CH$_3$), 0.84 (6H, s, 2 x CH$_3$), 0.86 (3H, s, CH$_3$), 0.90 (6H, s, 2 x CH$_3$), 1.10 (3H, s, CH$_3$), 3.58 (3H, s, methyl ester), 4.50 (1H, m, H-$\alpha$ to methyl ether), 5.30 (1H, m, olefinic proton).

RA-7

RA-7 was eluted from the column with benzene-chloroform (8:2) mixture. It was crystallized from chloroform-ethanol as colourless needles (170 mg), m.p. 240°C. It gave positive Leibermann-Burchard test.

IR $\nu_{\text{max}}$ cm$^{-1}$

1690-1700 (C=O), 1655, 1055, 840 (C=C)

$^1$H-nmr (60 MHz, CDCl$_3$), values on $\delta$-scale

0.83 (3H, s, CH$_3$), 0.93 (6H, s, 2 x CH$_3$), 0.97 (3H, s, CH$_3$), 1.06 (9H, s, 3 x CH$_3$), 1.12 (3H, s, CH$_3$), 1.32, 1.50-1.90 (21 H, m, CH and CH$_2$ protons), 2.28-2.52 (2H, m, -CH$_2$ protons at C-2), 5.56 (1H, dd, olefinic proton).
**Mass m/z**

424 [M⁺] (37.0%), 409 (18.3), 300 (100), 285 (45.4), 257 (9), 232 (7.3), 218 (16.5), 205 (62.6), 204 (95), 189 (10), 149 (10), 133 (25), 121 (12), 119 (10), 109 (15), 107 (10), 95 (17), 93 (10), 91 (8), 67 (15), 57 (15), 43 (12).

**RA-8**

RA-8 was eluted from the column by benzene-chloroform (7:3) mixture. It was crystallized from methanol as reddish buff needles (120 mg), m.p. 155°C. It gave blue colour with alc. ferric chloride solution and brown spot under uv light.

Analysed for C₉H₁₀O₅

Calcd.: C, 54.9; H, 5.05 %

Found: C, 54.7; H, 5.00 %

^1H-nmr (100 MHz, DMSO-d₆), values on δ-scale

1.31 (3H, t, J=7.0 Hz, CH₃), 4.24 (2H, q, J=7.0 Hz, CH₂), 7.11 (2H, s, H-2, 6).

**Mass m/z**

195 [M⁺] (45.0%), 183 (8.0%), 170 (20.0%), 153 (100.0%), 125 (20.0%), 107 (4.0%), 79 (9.0%), 51 (6.0%).

**RA-9**

RA-9 was eluted from the column by benzene-chloroform (1:1) mixture. It was crystallized from chloroform-methanol as white crystals (190 mg), m.p.160-162 °C.

Analysed for C₁₃H₁₂O₅

Calcd.: C, 62.90; H, 4.83 %

Found: C, 62.84; H, 4.79 %

IR KBr cm⁻¹

3496 (OH), 3429, 1669 (C=O), 1606, 1440-1375 (C-O vib. of COOH), 1394, 1320-1210, 1276 (COOH), 910 (OH def. of COOH).
$^1$H-nmr (300 MHz, CDCl$_3$ + DMSO-d$_6$), values on $\delta$-scale

2.71 (6H, s, 2 x CH$_3$), 6.81 (1H, d, J=9.0 Hz, $\alpha$-H of acid moiety), 7.43 (1H, d, J=9.0 Hz, $\beta$-H of acid moiety), 6.06 (1H, br s, 5-OH), 9.89 (1H, br s, COOH), 6.83 (1H, d, J= 3.0 Hz, H-2 of furan ring), 7.41 (1H, d, J=3.0 Hz, H-3 of furan ring).

$^{13}$C-nmr (300 MHz, CDCl$_3$), values on $\delta$-scale

161.57 (C$_{3}$-$\beta$), 161.02 (COOH), 155.07 ($\beta$-C), 152.87 (C-6), 152.85 (C-4), 125.40 (C-2), 113.02 (C-5), 112.45 (C-7), 110.7 (C$_{4}$-$\beta$), 102.97 (C-3), 162.97 ($\alpha$-C), 18.45 (2 x CH$_3$).

**Mass m/z (rel. int.)**

REFERENCES
    a) 186, b) 194, c) 195, d) 197