CHAPTER 1
THE CHEMICAL INVESTIGATION OF
RHYNCHOSIA SUAVEOLENS
1.1 INTRODUCTION

*Rhynchosia suaveolens* DC (Leguminoseae) is distributed in plains of Ceylon and both sides of Western Peninsula.²

The plant is an undershrub 1-3 ft. high with many stiff erecto-patent branches, clothed with fine short grey pubescence sometimes lengthened out and half turning at the ends. Stipules are minute lanceolate, leaflets are almost membranous, pale green, minutely downy, especially beneath, sometimes stipellate, the end on roundish with a long cusp, distinctly stalked, 1-3 in. long. Peduncles are slender erectopatent, downy, seldom above 1 in.; Pedicels are shorter than the calyx. Calyx is ¼ in., downy; teeth are linearsetaceous. Ped oblong, 1/4-3/4 in. long, turgid, 2-seeded.

The genus is comprised of about 80 species.

The seeds of the plant *R. minima* DC, are said to be bitter and poisonous. Extract of the seeds shows sp. agglutinating activity with certain types of human blood cells. Leaves are reported to be used as abortifacient.

Only four or five species have been so far worked up phytochemically and in most of the cases they yielded flavonoids and their O- and C-glycosides. The compounds isolated from five species of this genus have been summarised in Table 1. *R. pyramidalis* was claimed to possess androgenic and aphrodisiac action which could not be confirmed in laboratory animals.¹¹

1.2 PREVIOUS WORK

No work on *Rhynchosia suaveolens* has been reported in the literature.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant</th>
<th>Compound(s) isolated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>R. beddomei</em></td>
<td>Rhynchosin (5-deoxyflavonol), 3',4'-di-O-methyluteolin-7-O-β-D-glucopyranoside, D-inositol, naringenin, rutin, vitexin, isovitexin, orientin, isoorientin, vicenin-2 and lucenin-2.</td>
<td>2, 3</td>
</tr>
<tr>
<td>2.</td>
<td><em>R. cyanosperma</em></td>
<td>Tirumalin</td>
<td>(+)-(2R,3R)-8-C-pemyltaxifolin-7,4'-dimethyl ether</td>
</tr>
<tr>
<td>3.</td>
<td><em>R. minima</em></td>
<td>Proanthocyanidins, hydroquinone diacetate, protocatechuic acid, gallic acid, sitosterol, 6-C-glucosylapigenin, 6-C-glucosyl-8-C-arabinosylapigenin, di-C-hexosylapigenin, C-pentosyl-C-hexosylapigenin, 6-C-hexosyl-8-C-pentosylapigenin, 6,8-di-C-glucosylapigenin and 6-C-glucosyl-8-C-xylosylapigenin.</td>
<td>6, 7, 8</td>
</tr>
<tr>
<td>4.</td>
<td><em>R. phaseoloides</em></td>
<td>Ethyl ester of gallic acid and some indole derivatives.</td>
<td>9</td>
</tr>
<tr>
<td>5.</td>
<td><em>R. pyramidalis</em></td>
<td>An unidentified alkaloid (picrate m.p.132°).</td>
<td>10</td>
</tr>
</tbody>
</table>
Rhynchosia suaveolens
(5 Kg.)

↓

Alcoholic Extract
(375.0 g)

↓

200.0 g EtOH extract

Hexane

↓

Hexane-soluble fraction
(70.5 g)

Hexane-insoluble fraction

C₆H₆

↓

C₆H₆-soluble fraction
(56.0 g)

C₆H₆-insoluble fraction

Me₂CO

↓

Me₂CO-soluble fraction
(9.8g)

Me₂CO-insoluble fraction

MeOH

↓

MeOH-soluble fraction
(63.0 g)

Scheme 1
1.3 PRESENT WORK

In pursuance of a research programme aimed at the development of drugs from natural sources in this Institute, a 50% aqueous EtOH extract of the whole plant of *R. suaveolens* was found to exhibit antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. With a view to isolate the active principle(s) responsible for biological activity a detailed chemical investigation of *R. suaveolens* has been undertaken according to the Scheme 1.

Subsequent studies led to the location of this activity in benzene-soluble fraction (Scheme 1) from which two new biphenyl derivatives designated as substances A and B have been isolated and characterised (Table 2).

**Table 2: Substances isolated from the plant *R. suaveolens***

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Substance</th>
<th>Mol. formula</th>
<th>m.p. °C</th>
<th>Identified as</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Substance A</td>
<td>C_{18}H_{20}O_{2}</td>
<td>51-52</td>
<td>4-(3-Methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol</td>
<td>0.103684</td>
</tr>
<tr>
<td>2.</td>
<td>Substance B</td>
<td>C_{19}H_{20}O_{4}</td>
<td>138</td>
<td>2-Carboxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol</td>
<td>0.01377</td>
</tr>
</tbody>
</table>

1.3.1 Substance A, m.p. 51-52° was optically inactive. The IR spectrum showed the absorption at 1600 and 1490 cm\(^{-1}\) due to aromatic ring, 755 and 700 cm\(^{-1}\) for a monosubstituted phenyl ring and 835 cm\(^{-1}\) for -CH=C- in the molecule. The compound was indicated to be phenolic in nature showing the absorption at 3350 cm\(^{-1}\) for OH group.\(^{12}\)
\[
\begin{array}{cccc}
\text{R} & \text{R'} & \text{R'\text{'} } \\
1 & \text{H} & \text{H} & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
2 & \text{H} & \text{OCOCH}_3 & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
4 & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 & \text{H} & \text{H} \\
5 & \text{COOH} & \text{H} & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
6 & \text{COOH} & \text{OCOCH}_3 & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
7 & \text{COOCH}_3 & \text{H} & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
8 & \text{COOCH}_3 & \text{OCOCH}_3 & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 \\
9 & \text{CH}_2-\text{CH}=\text{C} (\text{CH}_3)_2 & \text{H} & \text{COOH} \\
\end{array}
\]

Fig. 1
The UV spectrum of substance A showed an absorption at 262 nm (log ε 4.4) suggesting a biphenyl nucleus with auxochromic group\(^1\).

The mass spectrum of the substance A showed the molecular ion peak at m/z 268 corresponding to the molecular formula \( \text{C}_{18}\text{H}_{20}\text{O}_2 \) which on subsequent fragmentation gave major fragment ion peaks at m/z 253, 212, 199, 165, 77. The molecular formula of substance A suggested the presence of nine double bond equivalents. Eight of these were accounted for by the presence of two aromatic rings, one may be present as an extra nuclear equivalent.

The \(^1\)H NMR spectrum showed the presence of seven aromatic protons identifying a biphenyl system comprising of one monosubstituted phenyl ring which resonated at \( \delta \) 7.4 as a multiplet and another two aromatic proton singlet at \( \delta \) 6.64. A three proton singlet was accounted for a methoxy group which appeared at \( \delta \) 3.80. The spectrum exhibited the presence of an isopentenyl side chain at one of the two aromatic rings evidenced by two vinylic methyl singlets at \( \delta \) 1.70 and 1.78. Two benzylic protons were located as a doublet centred at \( \delta \) 3.38 (J=6 Hz), while one olefinic proton as a triplet at \( \delta \) 5.23 (J=6 Hz). The signal for one hydroxy group was masked by the vinylic proton.

Substance A formed a mono-acetate 2 (Fig.1), m.p. 61°, as revealed by its molecular ion peak at m/z 310 corresponding to the molecular formula \( \text{C}_{20}\text{H}_{22}\text{O}_3 \). Its IR spectrum showed the absorption band at 1757 cm\(^{-1}\) for a phenolic ester. The \(^1\)H NMR spectrum of 2 exhibited a three proton singlet at \( \delta \) 2.26 due to methyl of the acetoxy group and a five proton multiplet for a monosubstituted phenyl group at \( \delta \) 7.35. The aromatic two proton singlet got split
into two broad singlets as a result of experiencing deshielding of expected magnitude which resulted into the magnetic non-equivalence of these two aromatic protons suggesting the presence of a phenolic hydroxy group ortho and para to these protons. This is in accordance with the fact that when a substituted phenol is acetylated the para proton gets deshielded to a greater extent (0.3 ppm) than the ortho (0.17 ppm)\textsuperscript{14}, hence, the protons at $\delta$ 6.83 and 6.9 were thus located ortho and para to the hydroxy group respectively.

These data suggested the substance A to be a biphenyl in which one ring is unsubstituted whereas the other carries an isopentenyl, a methoxy and a hydroxy substituents. The hydroxy group does not have any substituent at its ortho and para positions. Since biphenyls with substituents ortho to the biphenyl-link show marked reductions in their extinction coefficients, a structure with no substitution on the respective ortho positions was favoured. The optical inactivity of substance A further supported that the ortho positions of the biphenyl chromophore were free which allowed free rotation of the rotamers. Thus a tentative structure at this stage can be assigned to substance A as

![Chemical structure]

The relative positions of these two substituents ($S^1$ and $S^2$) were determined as follows.
A detailed study of the $^1$H NMR spectrum of acetylated substance A revealed the benzylic CH$_2$ to be deshielded ($\Delta$, 3.38-3.23 = 0.15). This suggested the orthogonality of the isopentenyl side chain and the hydroxy group. This observation was confirmed by treating the substance A with mixture of HCl-AcOH at 120-25$^\circ$ which afforded 3. Its mass spectrum showed the molecular ion peak at m/z 268 corresponding to the molecular formula C$_{18}$H$_{20}$O$_2$. The IR spectrum of 3 showed the absorption at 1120 cm$^{-1}$ due to asymmetrical stretching of the ether linkage of chroman ring suggesting it to be an isomer of substance A formed as a result of acid catalysed cyclisation. The $^1$H NMR spectrum of 3 exhibited two triplets. The one at $\delta$ 2.63 (J=7 Hz) was due to the benzylic CH$_2$ and the other at $\delta$ 1.72 (J=7 Hz) was accounted for CH$_2$ flanked by benzylic methylene and the quaternary carbon of the chroman ring. A six proton singlet of the two methyl groups of the chroman ring appeared at $\delta$ 1.27 followed by two meta-coupled doublets in the aromatic region at $\delta$ 6.64 and 6.53 (J=2 Hz). This confirmed the proximity of the hydroxyl and the isopentenyl side chain. On the basis of the above evidence the structure 4 for substance A was ruled out. The final structure was, however, assigned on the basis of the study of benzene induced shift, NOE and $^{13}$C NMR spectra of substance A and its derivatives. The $^1$H NMR spectrum of substance A recorded in C$_6$D$_6$ created solvent induced magnetic non-equivalence of the two aromatic proton singlets which split to two meta-coupled doublets ($\delta$ 6.54 and 6.48, J=2 Hz) and thus suffered shielding of unequal magnitude. Hence, a different environment around the methoxyl could be inferred. Further, the methoxy signal is shifted
upfield indicating that the methoxy group has an unsubstituted ortho position.

In the NOE difference spectrum of 3, irradiation at the frequency of methoxy group enhanced the intensity of only one (δ 6.53) of the two aromatic doublets (δ 6.64, 6.53) suggesting the former to be adjacent to the methoxy group.

The proton noise decoupled (pnd) $^{13}$C NMR spectrum of substance A showed sixteen signals for the presence of eighteen carbon atoms (Fig.2). The three most upfield signals at δ 17.77, 22.23 and 25.73 were due to β-methyl, benzylic methylene and α-methyl groups respectively of the isopentenyl side chain. The single frequency off reasonance decoupled (sford) spectrum showed the presence of six quaternary carbons as revealed by the pnd spectrum where the low peak intensity showed the poor relaxation. The most deshielded singlet was found at δ 158.37 which was due to C-5 followed by singlets of C-3, C-1, C-1', C-3'' and C-4 at δ 155.39, 141.17, 140.57, 133.76 and 114.79 respectively. A quartet for methoxy carbon appeared at δ 55.85. The C-2 and C-6 which were ortho to hydroxy and methoxy groups appeared as two doublets at δ 107.85 and 102.59 respectively and were found to be in agreement with the calculated chemical shift values of these two carbons taking into account the substitution pattern of the biphenyl$^{17}$. The olefinic carbon, C-2'' exhibited its signal as a doublet at δ 122.17. The other proton-bearing five carbons (C-2', C-3', C-4', C-5', and C-6') of the monosubstituted phenyl ring exhibited the expected chemical shift values. Being magnetically equivalent C-2' and C-6' appeared as a doublet at δ 126.94 and C-3' and C-5' at δ 128.64 between which appeared the resonance signal of C-5' at δ 127.25.
POSSIBLE BIOGENETIC PATHWAY OF SUBSTANCE A AND B

Scheme 2
Finally on the basis of the above evidences combined with biogenetic considerations (Scheme 2), the substance A was identified as 4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol (1).

1.3.2 Substance B: m.p. 138° was optically inactive. The IR spectrum of substance B exhibited the absorption signals for the presence of chelated hydroxyl and carboxylic acid functionalities at 3375 and 1653 cm\(^{-1}\) respectively together with the bands characteristic of a monosubstituted phenyl ring at 755 and 700 cm\(^{-1}\) and trisubstituted double bond at 835 cm\(^{-1}\).

The UV spectrum of the substance B showed an absorption at 268 nm (log \(\varepsilon\) 4.1) suggesting a biphenyl with auxochromic group. The marked reduction in the extinction coefficient of substance B suggested that the position ortho to the biphenyl-link was occupied by a substituent creating moderate steric crowding.

The mass spectrum of substance B showed the molecular ion peak at m/z 312 corresponding to the molecular formula C\(_{19}\)H\(_{20}\)O\(_4\). The molecular formula led to the calculation of the double bond equivalence (DBE) to be ten. Nine of these were accounted for the biphenyl system and a carboxylic function with one extranuclear double bond equivalent.

Further insight into its structure was gained by the study of its \(^1\)H NMR spectrum which exhibited the signal for a chelated hydroxyl at \(\delta\) 11.6\(^{18}\). A doublet integrating for two protons appeared at \(\delta\) 3.37 (J=6 Hz) and was accompanied by one proton triplet at 5.22 (J=6 Hz) and two singlets at \(\delta\) 1.73 and 1.66 integrating for two methyl groups. This indicated the presence of an isopentenyl side chain. Another singlet for a methoxy group appeared at \(\delta\) 3.79. A five proton multiplet due to a monosubstituted phenyl
ring and a singlet for a lone aromatic proton were located at δ 7.23 and 6.22 respectively.

Substance B gave an acetate 6 m.p. 140°. The mass spectrum exhibited the molecular ion peak at m/z 354 corresponding to the molecular formula C_{20}H_{22}O_{5}. In the \(^{1}\)H NMR spectrum of 6, the lone aromatic proton has suffered a deshielding of 0.4 ppm suggesting its placement \textit{para} to the hydroxy group. This was also confirmed by observing a positive Gibb's test by substance B.

Substance B on treatment with diazomethane, gave a methyl ester 7; the molecular ion [M\(^+\)] at m/z 326 suggesting the molecular formula as C_{20}H_{22}O_{4}. A singlet at δ 3.37 due to a carbmethoxy group appeared in the \(^{1}\)H NMR spectrum of 7 which suggested that only carboxyl group got methylated in preference to the hydroxy group. This preferential methylation is in contradiction to an earlier report where the hydroxy group was methylated in preference to the carboxyl group in a similar environment in the case of a phytoalexin isolated from \textit{Cajanus cajan}\(^{19}\). This observation got further support when 7 was acetylated to give 8, m.p. 111.5° which showed the molecular ion peak at m/z 368 corresponding to the molecular formula C_{22}H_{24}O_{5}. The \(^{1}\)H NMR spectrum of 8 exhibited singlets for methoxy (δ 3.86), carbmethoxy (δ 3.46) and acetoxy (δ 2.28) groups. The derivative 8 was also obtained by reacting 6 with diazomethane.

\(^{1}\)H NMR spectra of 6 and 8 when compared with that of 5, a shielding of benzylic CH\(_2\) in the former two cases was observed which suggested the close vicinity of the hydroxyl and the isopen-tenyl side chain in substance B. The magnitude of shielding in 6 was greater (0.18 ppm) than in 8 (0.11 ppm).
The vicinal disposition of the hydroxyl and carboxyl as indicated in IR and $^1$H NMR spectra of substance B got further support from EI mass spectral studies which also furnished informations about the orthogonality of monosubstituted phenyl ring and carboxyl group.

The mass spectra of substance B and its derivatives 6, 7 and 8 showed structurally diagnostic features. Substance B (5) and its methyl ester derivative 7 exhibited the expulsion of elements of H$_2$O and MeOH respectively from their respective molecular ions as a result of a rearrangement called ortho-effect which refers to the hydrogen transfer via six-membered transition state at vicinally substituted aromatic compounds with the result that the fragments (H$_2$O and MeOH) come into existence (Scheme 3). The ortho-effect has been observed primarily only for radical cations but Bowie$^{20}$ demonstrated that the mass spectra of doubly charged molecular cations of salicylic and anthranilic acids eliminate H$_2$O with high intensity, while this effect does not operate for the meta/para isomers from which OH is exclusively eliminated.

Due to the presence of bulky groups at positions ortho to the carboxyl in substance B and its derivatives 6, 7 and 8, the ortho effect operates in two ways. The molecular ions of compounds 6 and 8 lose a ketene$^{21-24}$ to give fragment ions at m/z 312 and 326 which correspond to the molecular ion peaks of substance B and 7 respectively. These on subsequent expulsion of H$_2$O and MeOH via route a give the common fragment ion I at m/z 294 and via route b another common fragment ion II at m/z 294. The fragment I loses CH$_3$ and subsequently CO to give fragment ions at m/z 279 and 251.
MASS FRAGMENTATION PATTERN OF 5, 6, 7 and 8

Scheme 3
Compound 6 and 8 may alternatively adopt two other pathways. In one, 6 and 8 lose H₂O and MeOH by abstracting the proton from C-1' of the monosubstituted phenyl ring to give fragment ion IV at m/z 336 which on subsequent loss of an acyl radical yield III at m/z 293. In other route, 6 and 8 lose an acyl radical first to give fragment ions V and VI at m/z 311 and 325 respectively. V and VI subsequently lose H₂O and MeOH respectively to give III. Compounds 6 and 8 follow route b predominately while 5 and 7 prefer the route a.

It was inferred from the above evidence that the ortho effect is operative not only via route a but also via route b which in turn suggests that hydroxyl and monosubstituted phenyl ring in substance B can be placed at positions ortho to the carboxyl group.

During a benzene induced shift experiment the methoxy group in substance B suffered a strong upfield shift (0.6 ppm) which suggested the location of the carboxyl function either ortho or para to this group. At this stage an alternative structure 9 for substance B could be proposed. The absence of any change in the chemical shift of the methoxy in substance A and B confirmed that carboxy group cannot be placed ortho to methoxy in the latter, thus discarding structure 9. The final proof in favour of 5 was achieved by its decarboxylation. A brief exposure to 170-75° resulted in the quantitative conversion of substance B into A.

The above evidences together with the support from the biogenesis (Scheme 2) confirmed the structure of substance B to be 2-carboxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol (5).
1.3.3 Biogenesis

The biosynthesis of most of the naturally occurring biphenyls is believed to involve aldol condensation of an intermediate β-triketo acid derivative (Scheme 2)\(^{28}\). In accordance with the polyacetate hypothesis\(^{29}\) the triketo-intermediate is derived by condensation of a shikimate-derived benzoic acid derivative with three malonate units. Alternatively, claisen condensation of the acyclic intermediate could produce benzophenones. It was reported that the following phenyl dipyranone served as a model for the postulated triketoprecursor and can be converted into biphenyls and benzophenones.

![Phenyl dipyranone](image)

In an attempted biogenetic-type synthesis of biphenyls, Douglas and Money\(^{30}\) transformed phenyl dipyranone into phenolic compounds of predictable structure by using the appropriate basic conditions. Therefore, a biogenetic argument supports the structures of substance A and B as the metabolites can be considered to be derived from a phenacylpolyacetate (Scheme 2). An aldol condensation will lead to the natural biphenyls 1 and 5.
1.3.4 EXPERIMENTAL

All melting points are uncorrected. The UV spectra were recorded on Hitachi-320 automatic recording spectrometer while IR spectra were taken on Perkin-Elmer Infracord-157 instrument. The $^1$H NMR and $^{13}$C NMR spectra were recorded on Perkin-Elmer R-32 (90 MHz), Varian EM-360L (60 MHz), CFT-20 (80 MHz for $^1$H NMR and 20 MHz for $^{13}$C NMR) spectrometers with TMS as an internal standard. The EI and FD mass spectra were taken on Jeol JMS-D300 and Jeol JMS-OSIG2 instruments respectively. Silica gel G was used for thin layer chromatography (TLC). The spots were visualised either by keeping the TLC-plate in iodine atmosphere or spraying with 1% Cerric sulphate solution in 2N-H$_2$SO$_4$. The column chromatography were performed over silica gel (60-120 mesh, BDH).

Isolation of Constituents: The air dried finely powdered whole plant Rhynchosia suaveolens DC (Leguminoseae) (5 kg) was percolated with 95% EtOH (4x20 litre). After removal of the solvent in vacuo at 60°C, the percolate gave a residue (375 g). 200 g of this residue was fractionated successively with hexane, benzene, acetone and methanol to afford 70.5 g, 56.0 g, 9.8 g, and 63.0 g of concentrates respectively.

The Benzene Soluble Fraction:

The green coloured benzene soluble fraction showed two major constituents on TLC along with some minor components. The major spots were designated as A and B according to decreasing order of their $R_f$ values. The C$_6$H$_6$-soluble residue (16 g) was chromatographed over silica gel (600 g), eluted with hexane, containing increasing amounts of benzene, chloroform and then methanol. One
hundred and thirty fractions of 500 ml each were collected and were mixed on the basis of components observed by TLC examination. The results are summarised in Table 3.

Table 3: Column chromatography of Benzene-Soluble fraction (16g).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Eluant</th>
<th>No. of Fractions collected</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane</td>
<td>1-8</td>
<td>-</td>
<td>No product</td>
</tr>
<tr>
<td>2.</td>
<td>Hexane:C₆H₆(75:25)</td>
<td>9-24</td>
<td>0.244</td>
<td>Greenish yellow oils</td>
</tr>
<tr>
<td>3.</td>
<td>Hexane:C₆H₆(50:50)</td>
<td>25-64</td>
<td>4.0</td>
<td>Yellowish red viscous liquid (contain substance A)</td>
</tr>
<tr>
<td>4.</td>
<td>C₆H₆</td>
<td>65-86</td>
<td>1.305</td>
<td>Contain Substance B</td>
</tr>
<tr>
<td>5.</td>
<td>C₆H₆:CHCl₃(75:25)</td>
<td>87-99</td>
<td>0.14</td>
<td>Green pigments</td>
</tr>
<tr>
<td>6.</td>
<td>C₆H₆:CHCl₃(50:50)</td>
<td>100-110</td>
<td>0.13</td>
<td>Green pigments</td>
</tr>
<tr>
<td>7.</td>
<td>CHCl₃</td>
<td>111-120</td>
<td>0.34</td>
<td>Green pigments</td>
</tr>
<tr>
<td>8.</td>
<td>CHCl₃:MeOH(95:5)</td>
<td>121-130</td>
<td>6.0</td>
<td>Brown green pigments</td>
</tr>
</tbody>
</table>

Fractions 25–33 were mixed together, which on concentration gave a yellowish red oily residue (2.4 g). This was chromatographed over silica gel column (100 g), eluted with hexane, containing increasing amounts of benzene. Forty six fractions of 250 ml each were collected and mixed according to TLC pattern. The results of column chromatography are summarised in Table 4.
Table 4: Column Chromatography of the residue (2.4 g)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Eluant</th>
<th>No. of fraction collected</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane</td>
<td>1-4</td>
<td>-</td>
<td>No product</td>
</tr>
<tr>
<td>2.</td>
<td>Hexane:C\textsubscript{6}H\textsubscript{6}(90:10)</td>
<td>5-8</td>
<td>-</td>
<td>No product</td>
</tr>
<tr>
<td>3.</td>
<td>Hexane:C\textsubscript{6}H\textsubscript{6}(80:20)</td>
<td>9-16</td>
<td>-</td>
<td>No product</td>
</tr>
<tr>
<td>4.</td>
<td>Hexane:C\textsubscript{6}H\textsubscript{6}(70:30)</td>
<td>17-32</td>
<td>0.89</td>
<td>Yellowish red oil (contain substance A)</td>
</tr>
<tr>
<td>5.</td>
<td>Hexane:C\textsubscript{6}H\textsubscript{6}(60:40)</td>
<td>33-42</td>
<td>1.4</td>
<td>Yellowish red oil (contain substance A)</td>
</tr>
<tr>
<td>6.</td>
<td>C\textsubscript{6}H\textsubscript{6}</td>
<td>43-46</td>
<td>0.05</td>
<td>Red oil</td>
</tr>
</tbody>
</table>

Fractions 17-35 were mixed together to yield 0.98 g residue which was crystallised with hexane to afford 0.79 g of substance A.

Fractions 68-74 (Table 3) were mixed together and concentrated to give 0.25 g of green residue which on crystallisation with hexane-CH\textsubscript{2}Cl\textsubscript{2} yielded 0.065 g of substance B. On addition of hexane the mother liquor afforded an additional amount (0.04 g) of substance B.

**Substance A, 4-(3-Methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol, (1):**

0.79 g of this compound eluted with a mixture of C\textsubscript{6}H\textsubscript{6}-hexane (3:7) had m.p. 51-52° (hexane); IR $\nu_{\text{KBr}} \text{ cm}^{-1}$: 3500-3000 (OH), 2900 (C-H stretching), 1600, 1588, 1566, 1490, 1445, 1401, 1340, 1220, 1160, 1082, 960, 880, 835, 755, 728, 700; UV $\lambda_{\text{MeOH}}^{\text{max}} \text{ nm (log } \epsilon) : 262 (4.4), 205, 13^C \text{ NMR (CDCl}_3) : 6158.37 (s, C-5), 155.39 (s, C-3), 141.17 (s, C-1), 140.57 (s, C-1), 128.64 (d, C-3' and C-5'), 127.25
Id, C-4'), 126.94 (d, C-2', and C-6'), 114.79 (d, C-4), 107.85 (d, C-2), 102.59 (d, C-6), 55.85 (q, ArOMe) isopentenyl side chain 133.76 (d, =C <), 122.17 (d, HC=), 25.73 (q, Me), 22.33 (t, CH₂), 17.77 (q, Me); ¹H NMR (CDCl₃): δ 7.4 (m, 5H), 6.64 (d, 2H), 5.23 (t, J=6 Hz, 1H), 3.80 (d, Ar-OMe), 3.38 (d, J=6 Hz, 2H), 1.78 and 1.70 (2s, 3H each); ¹H NMR (C₆D₆): δ 7.3 (m, 5H), 6.54 (d, J=2 Hz 1H), 6.48 (d, J=2 Hz, 1H), 5.37 (t, J=6 Hz, 1H), 3.32 (d, Ar-OMe), 3.54 (d, J=6 Hz, 2H), 1.66 and 1.53 (2s, 3H each); MS m/z: 268 [M⁺], 253, 212, 199, 165, 77.

Acetylation of Substance A:

A solution of substance A (40 mg) in a mixture of acetic anhydride (1 ml) and pyridine (2.5 ml) was left overnight at room temperature. The reaction mixture was worked up by removing the solvents in vacuo. The reaction mixture was taken in CHCl₃ (15 ml) and washed with water (2x10 ml). The organic layer was dried over anhydrous Na₂SO₄ and evaporated to yield 35 mg of a residue which was crystallised from MeOH-H₂O to give white silny flakes of 3-Acetoxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl], 2 (30 mg), m.p. 61°: IR νKBr cm⁻¹: 2900, 1757, 1573, 1456, 1412, 1375, 1340, 1213, 1159, 1088, 1023, 925, 900, 860, 775, 760, 708; ¹H NMR (CDCl₃): δ 7.35 (m, 5H), 6.9 (d, 1H), 6.83 (d, 1H), 5.1 (t, J=6 Hz, 1H), 3.83 (d, Ar-OMe), 3.23 (d, J=6 Hz, 2H), 2.26 (d, 3H), 1.71 and 1.64 (2s, 3H each); MS m/z: 310 [M⁺], 267, 253, 213, 200, 165, 115, 77.

Cyclisation of Substance A to 3:

A mixture of substance A (100 mg) glacial acetic acid (4.3 ml) and concentrated HCl (0.1 ml) was refluxed at 120-25° for
2 hr. The reaction mixture was diluted with water and extracted with CHCl₃ (3x5 ml). The organic layer was dried over anhydrous Na₂SO₄ and evaporated to give 2,2-dimethyl-5-methoxy-7-phenyl chroman, 3, as a viscous liquid (95 mg). IR νKBr cm⁻¹: 2950, 1600, 1582, 1500, 1466, 1420, 1168, 1240, 1172, 1120, 920, 867, 783, 720; ¹H NMR (CDCl₃): δ 7.6-7.16 (m, 5H), 6.64 (d, J=2 Hz, 1H), 6.53 (d, J=2 Hz, 1H), 3.79 (s, Ar-OMe), 2.63 (t, J=7 Hz, Ar-CH₂), 1.72 (t, J=7 Hz), 2H), 1.27 (s, 6H); MS m/z: 268 [M⁺], 253, 213, 165, 115, 77.

Substance B, 2-Carboxy-4-[3-methyl-but-2-enyl]-5-methoxy-[1,1'-biphenyl]-3-ol, 5:

105 mg was obtained from the C₆H₆-eluate, m.p. 138° (hexane-CH₂Cl₂); UV λmax nm (log ε): 305, 268, (4.15), 231; IRνKBr max cm⁻¹: 3375, 3000-2800, 1653, 1625, 1600, 1560, 1440, 1370, 1336, 1276, 1227, 1170, 1130, 1098, 980, 911, 840, 818, 780, 712; ¹H NMR (CDCl₃): δ 7.23 (m, 5H), 6.22 (s, 1H), 5.22 (t, J=6 Hz, 1H), 3.79 (s, Ar-OMe), 3.37 (d, J=6 Hz, 2H), 1.73 and 1.66 (2x, 3H each); ¹H NMR (C₆D₆): δ 7.07 (s, 5H), 6.07 (s, 1H), 5.57 (t, J=6 Hz, 1H), 3.70 (d, 2H), 3.13 (s, Ar-OMe), 1.47 (s, 6H); MS m/z: 312 (64.4) [M⁺], 294 (39.7), 293 (24.9), 279 (100), 251 (77.9), 239 (74.2), 213, 165, 139,115, 77.

Acetylation of Substance B:

A solution of substance B (40 mg), in a mixture of acetic anhydride (1 ml) and pyridine (2.5 ml) was kept overnight at room temperature. The reaction mixture was worked up as in case of 2 to yield 2-Carboxy-3-acetoxy-4-[3-methyl-but-2-enyl]-5-methoxy-[1,1'-biphenyl] 6 (30 mg), m.p. 140° (MeOH-H₂O); IR νKBr cm⁻¹:
2900, 1768, 1602, 1560, 1420-60, 1370, 1325, 1290, 1230, 1200, 1165, 1080, 1015, 943, 922, 862, 800, 780, 720, 702; $^1$H NMR (CDCl$_3$): $\delta$ 7.28 (s, 5H), 6.62 (s, 1H), 5.07 (t, J=6 Hz, 1H), 3.77 (s, Ar-OMe), 3.19 (d, J=6 Hz, 2H), 2.2 (t, 3H), 1.67 and 1.6 (2x, 3H each); FDMS m/z: 354 [M$^+$], 336 (14.8), 311 (26.7), 294 (33.9), 293 (64.0), 279 (67.9), 251 (48.8), 239, 223, 165, 115.

**Methylation of Substance B:**

Substance B (25 mg) was dissolved in dry ether (5 ml) and a dried solution of CH$_2$N$_2$ in ether (10 ml, obtained from 100 mg of N-nitrosomethylurea) was added into it and left overnight at room temperature. The solvent was evaporated in vacuo to give 2-carbomethoxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol 7 (20 mg) as an amorphous residue. $^1$H NMR (CDCl$_3$): $\delta$ 7.26 (m, 5H), 6.25 (s, 1H), 5.23 (t, J=6 Hz, 1H), 3.8 (s, Ar-OMe), 3.37 (s, -OCOMe), 3.37 (t, J=6 Hz, 2H), 1.76 and 1.65 (2x, 3H each); MS m/z: 326 (64.3), [M$^+$], 294 (43.4), 293 (33.8), 279 (100), 251 (70.4), 239, 223, 165, 115.

**Acetylation of compound 7:**

A solution of 7 (20 mg), acetic anhydride (0.5 ml) and pyridine (1.5 ml) was kept overnight at room temperature. Usual work up followed by purification through crystallisation yielded 2-carbomethoxy-3-acetoxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl] 8 (15 mg) m.p. 111.5° (MeOH-H$_2$O); $^1$H NMR (CDCl$_3$): $\delta$ 7.34 (s, 5H), 6.74 (s, 1H), 5.13 (t, J=6 Hz, 1H), 3.86 (s, Ar-OMe), 3.46 (s, 1H), 5.13 (t, J=6 Hz, 1H), 3.86 (s, Ar-OMe), 3.46 (s, -OCOMe), 3.26 (d, J=6 Hz, 2H), 2.28 (s, 3H) and 1.74 and 1.68 (2x, 3H each); FDMS m/z: 368 [M$^+$], 336 (15.7), [M-MeOH$^+$]. 325 (42.3,
294 (31.5), 293 (100), 279 (41.4), 251 (32), 239 (27.2).

Decarboxylation of Substance B:

Substance B (20 mg) when heated at 170-75° for 5 minutes afforded on work up a residue (17 mg) identical in all respect with substance A.
REFERENCES


