EXPERIMENTAL

Isolation of Aegelenine from the Leaves
of Aegle marmelos Correia

Dried and milled leaves of Aegle marmelos Correia (3.0 kg) were extracted with ether (5 litres) in a soxhlet apparatus for fifty hours. The deep green ethereal extract was digested with 500 c.c. of hydrochloric acid (4N) in five instalments. The green acid solution was cooled in ice and basified with liquor ammonia (pH 7-8) when a flocculent mass separated. The precipitated base was shaken with ether (5 x 200 c.c.). The greenish ethereal solution having a deep violet fluorescence was washed with water, dried over anhydrous sodium sulphate and concentrated to a thick green pasty mass (3 gm.). The latter was then treated with 200 c.c. of aqueous potassium hydroxide solution (2 percent) and filtered. The aqueous alkaline filtrate was cooled in ice, washed with ether (200 c.c.) and acidified with concentrated hydrochloric acid (pH 2-3). The aqueous acid solution was basified with sodium carbonate (pH 7-8) and extracted with ether (5 x 100 c.c.) which was washed with water, dried over anhydrous sodium sulphate and concentrated. The pale yellow crystalline solid (0.1 gm.) left after removal of ether crystallised from acetone in prisms, m.p. 248-50° (with sublimation). Repeated crystallisations from methyl alcohol, and ethylacetate did not raise
the melting point of the base. Further purification of aegelenine was achieved by chromatographic resolution over Brockmann alumina. The base (0.1 gm.) was dissolved in ethyl acetate (30 c.c.) and adsorbed on a column of Brockmann alumina (20 cm. x 1.7 cm.) (Table VII) forming a deep green fluorescent zone in presence of ultra-violet light. The column was washed with benzene, then with a mixture of this solvent and ethyl acetate and finally with ethyl acetate when the alkaloid migrated out. The fractions were collected in portions of 30 c.c. each.

Table VII

<table>
<thead>
<tr>
<th>Number of Fractions</th>
<th>Eluents</th>
<th>Residue on evaporation of solvent and m.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>Benzene</td>
<td>Nil</td>
</tr>
<tr>
<td>6 - 10</td>
<td>Benzene:ethyl acetate (1:1)</td>
<td>Nil</td>
</tr>
<tr>
<td>11 - 25</td>
<td>Ethyl acetate</td>
<td>White residue (95 mg.) m.p. 248-50°</td>
</tr>
<tr>
<td>26 - 28</td>
<td>Ethyl acetate</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Fractions (11-25) were freed from solvent and crystallised from acetone, in needles m.p. 248-50°. The base retained one third molecule of acetone as solvent of crystallisation which escaped
when dried in vacuo over P₂O₅ at 136° for twelve hours. Found in a sample dried in vacuo over P₂O₅ for sixty-four hours at 136°: C, 70.18; H, 4.81; N, 11.76; H⁺, 0.40. Mol. wt., 232, 229.2 (Rast). Calcd. for C₁₄H₁₂O₂N₂: C, 70.0; H, 5.0%; N, 11.66; 1 H⁺, 0.41; Mol. wt. 240. Aegelenine showed no optical rotation \([\alpha]_{D}^{30°} = + 0^\circ\) (pyridine).

**Paper Chromatography of Aegelenine**

The homogeneity of aegelenine was proved by paper chromatography. Whatman filter paper No. 1 (11" x 11") impregnated with 2 per cent aqueous formamide solution was used. 0.02 c.c. of an absolute alcoholic solution of pure base (2. mg. in 2 c.c.) was concentrated in a small zone around a point on the paper, 2.5 cm. above the lower end of the paper. Unidimensional ascending method was adopted for the resolution. Different developing solvents, viz., (a) n-butanol saturated with N/10 hydrochloric acid, (b) n-butanol:formic acid:water (12:1:7), (c) water:acetic acid (9:1) were used. All the paper chromatograms showed a single blue violet fluorescent spot when exposed to ultra-violet light and a single brown spot when sprayed with Dragendorff's reagent (0.003 gm/100 c.c. of 20 per cent aqueous acetic acid). Rᵢ values of aegelenine are recorded in Table VIII.
### Table VIII

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temp.</th>
<th>Time required to reach the solvent front</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) n-Butanol saturated with N/10 hydrochloric acid</td>
<td>30°</td>
<td>8 hours</td>
<td>0.35</td>
</tr>
<tr>
<td>(b) n-Butanol:formic acid:water (12:1:7)</td>
<td>30°</td>
<td>7 hours</td>
<td>0.65</td>
</tr>
<tr>
<td>(c) Acetic acid:water (1:9)</td>
<td>30°</td>
<td>2 hours</td>
<td>0.74</td>
</tr>
</tbody>
</table>

**Aegelenine Chloroplatinate**

Aegelenine (0.1 gm.) was dissolved in 4N hydrochloric acid (10 c.c.). An aqueous solution of platinic chloride (5%) containing a few drops of hydrochloric acid was added dropwise to the acidic solution of the base till the precipitation was complete. It was then boiled till a clear yellow solution was obtained which was allowed to cool when aegelenine chloroplatinate crystallised in flakes decomposing above 235° (without melting). The orange yellow chloroplatinate hydrolysed to an amorphous solid when boiled with water containing a few drops of concentrated hydrochloric acid.
Found in a sample dried over P₂O₅ in vacuo at room temperature for sixty-four hours: Pt, 22.9, 23.01. Calcd. for C₂₆H₄₄N₄H₂PtCl₆: Pt, 21.9 per cent.

**Aegelenine Methiodide**

Aegelenine (25 mg.) was dissolved in methyl alcohol (10 c.c.) and the solution was cooled in ice. To the cold solution methyliodide (0.5 c.c.) was added. The yellow solution was kept overnight and concentrated to a bulk of 3-4 c.c. when aegelenine methiodide crystallised out in brown needles which upon repeated crystallisation from methanol melted at 263-66° (decomp.).

Found in a sample dried in vacuo over P₂O₅ at 136°: C, 47.52; H, 3.59; N, 7.40. Calcd. for C₁₄H₁₂O₂N₂.CH₃I: C, 47.12; H, 3.92; N, 7.32 per cent.

**Aegelenine Picrate**

A methanolic solution of picric acid (30 mg. in 10 c.c.) was added to a solution of aegelenine in methyl alcohol (25 mg. in 10 c.c.). The yellow solution was warmed for a few minutes on the water-bath and kept overnight when aegelenine picrate separated in thin elongated needles, m.p. 244-46° (decomp.).
Found in a sample dried in vacuo over P\textsubscript{2}O\textsubscript{5} at 136° C, H, 3.74; N, 15.01. Calcd. for C\textsubscript{20}H\textsubscript{15}O\textsubscript{9}H\textsubscript{5} : C, 51.17; N, 14.92 per cent.

**Aegelenine Hydrochloride**

Aegelenine (0.1 gm.) was dissolved in concentrated hydrochloric acid (20 c.c.) when the solution turned yellow. Upon concentration (5 c.c.) a yellow solid separated which crystallised from acetone in needles, m.p. 236-44°. Repeated crystallisation from acetone raised its melting point to 248-50°. It was found to be identical with aegelenine from melting point and mixed melting point determination.

**Potassium Salt of Aegelenine**

Aegelenine (0.2 gm.) was dissolved in a warm aqueous solution of potassium hydroxide (20 c.c., 10%). The solution on cooling deposited yellow needles, which crystallised from ethyl alcohol in flakes. It decomposed at 260° without melting.

Found in a sample dried over P\textsubscript{2}O\textsubscript{5} at 60° C, H, 3.71; N, 10.23; Calcd. for C\textsubscript{14}H\textsubscript{11}O\textsubscript{2}N\textsubscript{2}K : C, 60.43; H, 3.94; N, 10.07 per cent.

**Acetylation of Aegelenine**

(a) Aegelenine (0.2 gm.) was dissolved in freshly distilled acetic anhydride (5 c.c.) and dry pyridine (2 drops). The solution was then refluxed in an oil bath at 140° for twelve hours. Excess acetic anhydride was distilled off under reduced pressure. The syrupy residue was dissolved in acetic acid (3 c.c.) and diluted with water (10 c.c.). The solution was cooled in ice and basified with sodium carbonate (pH 7-8) and the precipitate extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulphate and
concentrated. The residue obtained crystallised in long, colourless needles from aqueous methyl alcohol m.p. 152-53° (yield, 0.105 gm.).

Found in a sample dried in vacuo over P₂O₅ at 80° for thirty-six hours: C, 68.83; H, 4.02; N, 9.45; -COCH₃, 15.82; Mol. wt., 278, 282 (Rast). Calcd. for C₁₄H₁₁N₂·(CO.CH₃): C, 68.08; H, 4.96; N, 9.93; -COCH₃, 15.24 per cent, Mol. wt. 282.

(b) Acetylation of Aegelenine (under mild conditions)

A solution of aegelenine (0.1 gm.) in freshly distilled acetic anhydride (2 c.c.) was treated with dry pyridine (2 drops) and allowed to stand at room temperature for four days. On working up the reaction product in a similar manner as described above, unreacted aegelenine was obtained, m.p. 248-50° which showed no depression in melting point when admixed with pure aegelenine.

Methylation of Aegelenine

Aegelenine (0.2 gm.) was dissolved in dry methyl alcohol (20 c.c.) and to the cooled solution (temp. < 3°) a fresh and dry ethereal solution (200 c.c.) of diazomethane (liberated from 3gm. of nitrosomethylurea) was added, a few c.c. at a time.
with stirring. The solution was kept at 0° for a few hours and then left at room temperature for twenty four hours. The solvent was removed carefully on a water-bath. The residue was taken up in benzene (1 c.c.) and subjected to chromatographic resolution over Brockmann alumina (32 cm. x 1.7 cm.) and eluted with petroleum ether (b.p. 40-60°) and benzene, fractions being collected in 10 c.c. portions (Table IX).

Table IX

<table>
<thead>
<tr>
<th>Number of Fractions</th>
<th>Eluent</th>
<th>Residue on evaporation of solvent and m.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether (b.p. 40-60°)</td>
<td>Small amount of yellow solid (8-10 mg.) m.p. 94-95.5°</td>
</tr>
<tr>
<td>2</td>
<td>Do</td>
<td>White solid (0.1 gm.) m.p. 98-100°</td>
</tr>
<tr>
<td>3</td>
<td>Do</td>
<td>Solid (30 mg.) m.p. 100-102°</td>
</tr>
<tr>
<td>4-5</td>
<td>Do</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Do</td>
<td>Very small amount of white solid, m.p. 102°</td>
</tr>
<tr>
<td>7-8</td>
<td>Do</td>
<td>Nil</td>
</tr>
<tr>
<td>9-10</td>
<td>Benzene</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Fractions (2-6) on repeated crystallisation from petroleum ether (b.p. 60-80°) yielded colourless flakes, m.p. 104° (Yield, 0.14 gm).

Found in a sample dried in vacuo over P₂O₅ at 50° for 12 hours: C, 71.03; H, 5.32; N, 11.30; -OCH₃, 12.28.
Calcd. for C₁₅H₁₄O₂N₂, C, 70.86; H, 5.51; N, 11.02; -OCH₃, 12.20 per cent.

Methiodide of Methoxy Aegelenine

A solution of methoxy aegelenine in dry acetone (25 mg. in 5 c.c.) was treated with methyl iodide (10 drops) and the solution kept at room temperature for half-an-hour when yellow needles separated from the solution which were crystallised from methyl alcohol m.p. 245° (decomp.).

Found in a sample dried over P₂O₅ in vacuo at 136°:
C, 48.61; H, 4.02; N, 7.3; Calcd. for C₁₅H₁₄O₂N₂-CH₃I:
C, 48.48; H, 4.28; N, 7.07 per cent.

Acetylation of Methoxy Aegelenine

Methoxy aegelenine (0.1 gm.) was dissolved in freshly distilled acetic anhydride (5 c.c.) containing dry pyridine (3 drops). The solution was refluxed in an oil-bath for twelve
hours. Excess acetic anhydride was distilled off under reduced pressure, the syrupy mass dissolved in acetic acid (5 c.c.) and then added to ice cold water (20 c.c.). The aqueous solution was basified with sodium carbonate (pH 7-8) and the precipitated base was extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue was crystallised from petroleum ether (b.p. 60-80°), m.p. 104° (Yield, 80 mg.). It was proved to be unchanged O-methyl aegelenine from melting point, mixed melting point (undepressed) and methoxy estimation.

Found in a sample dried over P₂O₅ in vacuo at 50° for 8 hours: -OCH₃, 12.32; Calcd. for C₁₅H₁₄O₂N₂: -OCH₃, 12.20 per cent.

Action of Carbonyl Reagent on Aegelenine

Action of 2:4-Dinitrophenylhydrazine

A solution of 2:4-dinitrophenylhydrazine in spectral alcohol (0.15 gm. in 10 c.c.) containing a few drops of concentrated sulphuric acid was added to a solution of aegelenine in aldehyde free ethanol (0.1 gm. in 6-7 c.c.) and the solution refluxed on a hot plate for four hours. The reddish yellow solution was cooled and kept in the frigidairé overnight. The
separated yellow solid, m.p. 220-35°, was thoroughly washed with water and then crystallised from ethyl acetate, m.p. 248-50° (Yield, 40 mg.). It showed no depression in melting point when admixed with a pure sample of aegelenine.

Found in a sample dried in vacuo over P₂O₅ at 136° for 24 hours: C, 70.35; H, 4.66; N, 11.72; Calcd. for C₁₄H₁₂O₂N₂: C, 70.0; H, 5.0; N, 11.66 per cent.

Reduction of Aegelenine

1. Reduction of Aegelenine with Lithium Aluminium Hydride

   (a) Reduction at room temperature

   A solution of aegelenine (0.2 gm.) in dry tetrahydrofuran (100 c.c.) was added dropwise to a well stirred and cooled slurry of lithium aluminium hydride (0.25 gm.) in dry tetrahydrofuran (30 c.c.). The mixture was stirred for sixteen hours at room temperature, then decomposed by addition of water and filtered. The residual grey mass was refluxed with tetrahydrofuran (50 c.c.) for four hours and filtered. The filtrates were combined, dried over anhydrous sodium sulphate and concentrated. The residue was crystallised from acetone, m.p. 248-50° (Yield, 0.16 gm.). It was found to be unchanged aegelenine from melting point and mixed melting point determination with pure aegelenine.
(b) Reduction at elevated temperature

A cold solution of aegelenine (0.3 gm.) in dry tetrahydrofuran (150 c.c.) was added slowly to a well stirred and cooled slurry of lithium aluminium hydride in the same solvent. The mixture was stirred on a steaming water-bath for seventy two hours, then cooled in freezing mixture. The mass was decomposed by ice-cold water. It was processed as in (a). The brown residue, m.p. 230-45°, was crystallised repeatedly from acetone, m.p. 248-50° (Yield, 0.12 gm.) and the mother liquor was kept (A). The melting point of this product was not depressed when admixed with pure aegelenine.

The deep brown mother liquor (A) was concentrated (5 c.c.) and chromatographed over Brockmann alumina (12 cm. x 1.7 cm.). Fractions were collected in 10 c.c. portions (Table X).

Table X

<table>
<thead>
<tr>
<th>Number of Fractions</th>
<th>Eluents</th>
<th>Residue on evaporation of solvent and m.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Benzene</td>
<td>Nil</td>
</tr>
<tr>
<td>4-10</td>
<td>Benzene:ethyl acetate (1:1)</td>
<td>Nil</td>
</tr>
<tr>
<td>11-16</td>
<td>Ethyl acetate</td>
<td>Nil</td>
</tr>
<tr>
<td>17-24</td>
<td>Ethyl acetate</td>
<td>Solid (50 mg.) m.p. 245-47°</td>
</tr>
<tr>
<td>25-28</td>
<td>Ethyl acetate</td>
<td>Nil</td>
</tr>
<tr>
<td>29-34</td>
<td>Alcohol</td>
<td>Brown gummy mass (Non-crystallisable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Yield, 80 mg.)</td>
</tr>
</tbody>
</table>
Fractions (17-24) crystallised from acetone, m.p. 248-50°. It was shown to be unchanged aegelenine from melting point and mixed melting point determination.

(c) A solution of aegelenine in dioxan (0.3 gm. in 80 c.c.) was slowly added to a well stirred slurry of lithium aluminium hydride (0.5 gm.) in dioxan (30 c.c.). The mixture was stirred at 120° for twenty four hours and was worked up as described above when unchanged aegelenine could only be obtained from the reaction products. It crystallised from acetone, m.p. 248-50° (Yield, 80 mg.) and did not depress the melting point of pure aegelenine.

2. Reduction with Sodium Borohydride

Aegelenine (0.2 gm.) was dissolved in dry methyl alcohol (70 c.c.) and cooled in ice. To this solution was added sodium borohydride (0.5 gm.) in small portions with stirring and kept overnight. It was then refluxed on a water-bath for two hours. The residue obtained by evaporation of the solvent was treated with water (20 c.c.) and the aqueous alkaline solution (pH 9-10) was extracted with ether (50 c.c.) (A). The aqueous portion was acidified with acetic acid (pH 6-7) when a base separated. It was extracted with ether (100 c.c.) (B). The acid solution was basified with dilute ammonium hydroxide (pH 8-9) and extracted with ether (50 c.c.) (C).
Ether solutions (A), (B), and (C) were washed with water, dried over anhydrous sodium sulphate and concentrated. The solutions (A) and (B) did not yield any residue while ether solution (C) yielded a solid, m.p. 245-48°C (Yield, 0.18 gm.) which crystallised from acetone, m.p. 248-50°C. Melting point, mixed melting point and analysis showed it to be identical with aegelenine.

Found in a sample dried over P₂O₅ in vacuo at 136°C for 24 hours: C, 70.41; H, 4.58; N, 11.52; Calcd. for C₁₄H₁₂O₂N₂: C, 70.0; H, 5.0; N, 11.66 per cent.

3. Reduction with Adam's Catalyst

Aegelenine (0.1 gm.) dissolved in aldehyde free ethanol (30 c.c.) was treated with hydrogen in presence of Adam's platinum dioxide catalyst (0.15 gm.) which was previously saturated with hydrogen in the same solvent (10 c.c.) and stirred for twelve hours. It showed no uptake of hydrogen. The alcoholic solution was filtered from the catalyst and the residue was washed with more alcohol (50 c.c.). The combined filtrates were concentrated (2-3 c.c.) when crystalline solid separated, m.p. 248-50°C (Yield, 95 mg.). Melting point and mixed melting determination with pure aegelenine proved it to be identical with aegelenine.
Hydrolysis of Aegelenine and Its Derivatives

(a) Hydrolysis of Aegelenine with Alkali

A solution of aegelenine (50 mg.) in a solution (10 c.c.) of potassium hydroxide (10 per cent) in diethyleneglycol was refluxed for one hour. The solution was cooled, poured in water (30 c.c.), acidified with hydrochloric acid (pH 3-4) and washed with ether (A). The acidic solution was basified with sodium carbonate (pH 7-8) and the precipitate extracted with ether (B). Ether solutions (A) and (B) were washed with water, dried over anhydrous sodium sulphate and distilled when no residue was left by (A). The solution (B), however, yielded a solid which crystallised from acetone, m.p. 248-50° (Yield, 45 mg.). It did not depress the melting point of pure aegelenine when admixed.

(b) With Alcoholic Alkali

Aegelenine (0.12 gm.) was refluxed with alcoholic potassium hydroxide (50 per cent, 50 c.c.) for fifteen hours at 120°. Alcohol was removed and the residue treated with water (50 c.c.). The yellow aqueous solution on working up as in (a) yielded unchanged aegelenine (0.1 gm.).

(c) With Amyl Alcoholic Alkali (50 per cent)

A solution of aegelenine (0.1 gm.) in n-amyl alcoholic
Caustic potash (10 c.c., 50 per cent) was refluxed for five hours at 150-60°. The reaction mixture was cooled and treated with water (40 c.c.). The aqueous solution was saturated with ammonium chloride and extracted with ether (A). The aqueous portion was next acidified with concentrated hydrochloric acid (pH 3-4) extracted with ether (B), then saturated with sodium acetate when the pH of the solution was raised to 5-6 and again extracted with ether (C). Finally, the aqueous solution was basified with sodium carbonate (pH 7-8) and the precipitated base was extracted with ether (D). The ether solutions were washed with water, dried over anhydrous sodium sulphate and distilled. The ether extracts (A), (B) and (C) yielded no residue while from the ether extract (D) a solid was obtained (Yield, 60 mg.) which crystallised from acetone, m.p. 248-50° and was found to be unchanged aegelenine.
CHART I
Hydrolysis of Aegelenine

Aegelenine
Dissolved in amyl/alcoholic caustic potash and refluxed
Hydrolysis product
Treated with water
Aqueous solution
Saturated with ammonium chloride, concentrated and extracted with ether

Aqueous layer
Acidified with concentrated hydrochloric acid and extracted with ether

Ether (A)
Concentrated
Oily residue
Sublimed under high vacuum
Solid, m.p. 79-80° (m-Hydroxydiphenylamine)

Aqueous portion (Discarded)
Washed with sodium-bicarbonate solution

Ether (B)
Concentrated
Residue
Sublimed under high vacuum
Solid, m.p. 214° (p-Hydroxybenzoic acid)

 Ether (C)
Concentrated
Residue
Sublimed under high vacuum
Solid, m.p. 214° (p-Hydroxybenzoic acid)
Aegelenine (0.1 gm.) was refluxed with amylalcoholic caustic potash (10 c.c., 100 per cent) at 170-75° for nineteen hours. The reaction product was treated with water (30 c.c.). The aqueous alkaline solution was treated with ammonium chloride (2 gm.) and concentrated to about 20 c.c. on the water-bath. The aqueous solution (pH 5-6) was extracted with ether (50 c.c.) (A). The aqueous layer was thoroughly cooled in ice and acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (50 c.c.) (B). The ethereal solution (B) was washed with a small portion of water and then with sodium-bicarbonate solution (10 per cent; 20 c.c.) to separate the acidic fraction from the phenolic product if any. The alkaline extract was acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (50 c.c.) (C). The ether solutions (A), (B) and (C) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated when only (A) and (C) yielded residue. The concentrates of (A) and (C) were sublimed in high vacuum.

Fraction (A) sublimed at 80-90°/0.01 mm. as a colourless syrupy mass which soon solidified. It crystallised from petroleum ether (b.p. 60-80°) in flakes, m.p. 79-80°, yield (20 mg.). It produced a deep violet colouration with concentrated sulphuric acid and sodium nitrite. It was characterised as m-hydroxy-diphenylamine, with which it showed no depression in melting point when admixed.
Found in a sample dried at 40° in vacuo over P₂O₅ for 10 hours: C, 76.93; H, 6.21; N, 7.82. Calcd. for C₁₂H₁₄O₂: C, 77.83; H, 5.94; N, 7.66 per cent.

Fraction (C) upon sublimation in high vacuum at 150-60°/0.01 mm. furnished a white solid (Yield, 20 mg.) which crystallised in needles from a mixture of benzene and acetone, m.p. 214°. Mixed melting point of this product with p-hydroxybenzoic acid remained unchanged.

Found in a sample dried over P₂O₅ in vacuo at 136° for 24 hours: C, 60.72; H, 4.18. Calcd. for C₇H₆O₃: C, 60.87; H, 4.34 per cent.

2. Hydrolysis of Aegelenine with Acid

(a) A solution of aegelenine (20 mg.) in concentrated hydrochloric acid (5 c.c.) was refluxed for five hours. The reaction product was cooled, extracted with ether (A) and then basified with sodium carbonate (pH 7-8). The precipitate was taken up in ether (B). (B) gave a small amount of a gummy mass which was subjected to high vacuum sublimation when a solid sublimed at 150-60°/0.01 mm., m.p. 247-50° (8-10 mg.). It was found to be unchanged aegelenine.

(b) Aegelenine (0.2 gm.) was dissolved in alcoholic hydrochloric acid (50 c.c., 50 per cent) and the solution was
refluxed in a sealed tube for sixty-five hours at 120-30°C. The reaction mixture was freed from alcohol and extracted with ether (A). The yellow coloured ether solution was washed with a small amount of water and then with sodium-bicarbonate solution (20 c.c., 5 per cent). The latter was cooled in ice and acidified with concentrated hydrochloric acid (pH 3-4) and digested with ether (B). Ether solutions (A) and (B) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated. Ether extract (A) left no residue. Ether solution (B) yielded a small amount of a deep brown gummy mass (5-8 mg.) which distilled at 110°C/0.01 mm. yielded a small amount of an oil (1-2 mg.). No further investigation on this product could be done due to paucity of materials. The original aqueous solution was basified with sodium carbonate (pH 7-8) and extracted with ether. The ether solution on working up did not yield any residue.

3. Hydrolysis of Acetylaegelenine

Acetylaegelenine (25 mg.) was warmed with aqueous potassium hydroxide (5 c.c., 5 per cent) for half-an-hour. The yellow aqueous solution on acidification and subsequent basification with sodium carbonate (pH 7-8) yielded aegelenine which crystallised from acetone, m.p. 248-50°C (20 mg.).
4. Hydrolysis of Methoxyaegelegenine

(a) Methoxyaegelegenine (50 mg.) was refluxed with alcoholic potassium hydroxide (5 c.c., 5 per cent) for seven hours on a water-bath. The reaction mixture was freed from alcohol and poured in water (20 c.c.). The flocculent precipitate was taken up in benzene. The benzene solution was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue crystallised from petroleum ether (b.p. 60-80°), m.p. 104° (Yield 45 mg.). It did not depress the melting point of pure methoxyaegelegenine.

(b) A solution of methoxyaegelegenine (50 mg.) in hydrochloric acid (10 c.c., 50 per cent) was refluxed for ten hours at 120°. The yellow acidic solution was worked up as above when unchanged methoxyaegelegenine was obtained (Yield, 40 mg.).
Fusion of Aegelenine with Potassium Hydroxide

Aegelenin*

Fused with KGE

Alkaline melt

Treated with water

Aqueous solution

Extracted with ether

Ether (A)

Ether (B)

Saturated with

VCl and extracted

with ether

Extracted with 2N HOI

Acidic extract Ether

Ether (C)

Concentrated

V residue

Concentrated

Solid, m.p. 214° (p-Hydroxybenzoic acid)

Solid, m.p. 79-80° (m-Hydroxydiphenylamine)

 Ether

Concentrated

Concentrated

Solid, m.p. 214° (p-Hydroxybenzoic acid)

Ether (C)

 Ether (O)

Concentrated

Ether (E)

Concentrated

Residue

Alkaline extract Ether

Ether (A)

Extracted with ether

Extracted with ether

Alkaline extract Ether

Ether (C)

Concentrated

Concentrated

Solid, m.p. 79-80° (m-Hydroxydiphenylamine)

Ether (C)

Sublimed

under high vacuum

Solid, m.p. 214° (p-Hydroxybenzoic acid)
Alkali Fusion of Aegelenine (CHART II)

Aegelenine (0.3 gm.) was fused with solid potassium hydroxide (1.5 gm.) in a nickel crucible at 270-80° for five minutes in a metal-bath. The fused mass was continuously stirred to prevent frothing. The molten mass was cooled and digested with water (30 c.c.) and extracted with ether (A) (50 c.c.). The ether extract (A) was washed with hydrochloric acid (2N, 20 c.c.) to separate the basic components from the neutral products if any. The acidic solution was then basified with sodium carbonate (pH 8-9) and extracted with ether (C) (3 x 20 c.c.). Ether solutions (A) and (C) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated. No residue, however, was obtained from either of the ether solutions (A) and (C). The original aqueous alkaline solution (B) was saturated with ammonium chloride (2 gm.) and concentrated to 20 c.c. It was cooled in ice and extracted with ether (E) (3 x 20 c.c.). The cold aqueous solution was acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (F) (4 x 20 c.c.). Ether solution (F) was then washed with an aqueous solution of sodium-bicarbonate (10 per cent, 4 x 5 c.c.) to separate the acidic material from the phenolic fraction if any. The alkaline extract was cooled thoroughly in ice, acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (g) (4 x 20 c.c.). Ether...
solutions (E), (F) and (G) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated when the ether extracts (E) and (G) yielded residue.

Identification of p-Hydroxybenzoic acid

The ether concentrate (G) sublimed at 150-60°/0.01 mm to furnish a white solid (10-12 mg.). It produced a red colouration with neutral ferric chloride solution, crystallised from water, m.p. 214° and identified as p-hydroxybenzoic acid from its colour reaction, melting point and mixed melting with an authentic sample of the acid.

Found in a sample dried over P₂O₅ in vacuo at 136° for 40 hours: C, 60.95; H, 4.40; Calcd. for C₇H₆O₂: C, 60.87; H, 4.34 per cent.

Identification of m-Hydroxydiphenylamine

The oily residue from fraction (E) sublimed as a colourless viscid mass (Yield, 5-6 mg.) at 80-90°/0.01 mm which soon solidified on cooling. It crystallised from petroleum ether in flakes, m.p. 79-80°, and developed a deep violet colouration with concentrated sulphuric acid and sodium nitrite. An alcoholic solution of the product developed a blue-violet colouration with aqueous ferric chloride solution.
It showed no depression in melting point when admixed with a synthetic sample of m-hydroxydiphenylamine.

Found in a sample dried over P$_2$O$_5$ in vacuo at 40°:

C, 77.42; H, 6.01; N, 7.73; Calcd. for C$_{12}$H$_{11}$ON: C, 77.83; H, 5.94; N, 7.56 per cent.

**Oxidative Experiments with Aegelenine**

**Oxidation of Aegelenine with Potassium Permanganate**

1. **Oxidation at elevated temperature**

To a boiling solution of aegelenine (0.3 gm.) in acetone (100 c.c.) was added a solution of potassium permanganate (0.5 gm.) in the same solvent (100 c.c.). The solution became pink and it was refluxed for half-an-hour. The colour was discharged by addition of methyl alcohol and the solution filtered. The precipitated manganese dioxide was boiled with acetone (100 c.c.) for half-an-hour and filtered. The filtrates were combined and concentrated. The residue was boiled with a saturated solution of sodium-bicarbonate (100 c.c.) and filtered. The alkaline filtrate was cooled and acidified with hydrochloric acid (2N) (pH 3-4) and extracted thoroughly with ether (200 c.c.). The ether solution was washed with water, dried over anhydrous sodium sulphate and concentrated. The yellow residue crystallised
in yellow coloured needles from methyl alcohol m.p. 265° (dec.) (Yield, 7-8 mg.). The compound did not depress the melting point of 7-hydroxy-1-phenyl-2-keto-1,2-dihydro-4-quinazolone, m.p. 265°.

Found in a dry sample: C, 66.42; H, 3.84; N, 10.90. Calcd. for C_{14}H_{10}O_{3}N_{2}: C, 66.13; H, 3.93; N, 11.02 per cent.

2. Oxidation at low temperature

A solution of potassium permanganate (0.2 gm.) in acetone (100 c.c.) was added gradually to a well stirred solution of aegelenine (0.1 gm.) in the same solvent (50 c.c.) cooled in ice. Stirring was continued for two hours and the reaction mass left at room temperature for another hour. The pink colour was discharged by adding methyl alcohol and filtered. The filtrate was concentrated and the residue (10-12 mg.) was sublimed at 90°/0.01 mm. The sublimate (1-2 mg.), m.p. 103° was found to be basic in nature. Paucity of material did not permit its identification.

Synthesis of 1-Phenyl-2-keto-1,2-dihydro-4-quinazolone

(a) Preparation of N-phenylantranilic acid

A mixture of O-chlorobenzoic acid (10 gm.), aniline (40 c.c.), anhydrous potassium carbonate (40 gm.) and precipitated copper powder (0.1 gm.) was refluxed at 180° for four hours.
The dark-brown reaction product was added to water (100 c.c.) and the aqueous solution was steam distilled to remove excess aniline present. The aqueous solution was treated with norite (5 gm.) and filtered. The yellow filtrate was cooled in ice and acidified with concentrated hydrochloric acid (pH 3-4). The solid separated crystallised from aqueous alcohol, m.p. 180°C. (Yield, 12 gm).

Found in a sample dried over P₂O₅ in vacuo at 100°C for 20 hours: C, 73.44; H, 5.30; N, 6.23; Calcd. for C₁₅H₁₁₂N₂: C, 73.24; H, 5.16; N, 6.56 per cent.

(b) Preparation of Methyl N-phenylanthranilate

A solution of N-phenylanthranilic acid (10 gm.) in ether (150 c.c.) cooled in freezing mixture was treated with an ethereal solution of diazomethane (100 c.c.) (regenerated from 30 gms of nitrosomethylurea). The yellow solution was left overnight at room temperature and subsequently concentrated. The residual thick brown oil distilled at 185-90°C/12 mm (Yield, 8 gm.).

(c) Condensation of Methyl N-phenylanthranilate with urethane

Dry urethane (0.89 gm.) and an ethereal solution of methyl N-phenylanthranilate (2.27 gm.) were added to molecular sodium (0.23 gm.) when an immediate reaction started with evolution of hydrogen. The mixture was heated on a water-bath for four hours. The yellow reaction product was taken up.
in alcohol (5 c.c.) and the alcoholic solution added to water (50 c.c.). The aqueous solution was cooled and washed with ether and acidified with concentrated hydrochloric acid (pH 3-4). The yellow solid that separated was filtered and crystallised from alcohol in colourless needles, m.p. 296° (dec.), (Yield, 0.3 gm.).

Found in a sample dried over P₂O₅ in vacuo for 48 hours at 136°: C, 70.44; H, 4.37; N, 11.89. Calcd. for C₁₄H₁₀O₂N₂: C, 70.56; H, 4.21; N, 11.76 per cent.

(d) The above reaction of urethane (0.89 gm.) and methyl N-phenylanthranilate (2.27 gm.) was carried out with two molar sodium (0.46 gm.). The mixture was heated on the water-bath for four hours. From the reaction product 2,4-diketo-N-phenylquinazolone was isolated (Yield, 0.27 gm.).

Synthesis of m-Hydroxydiphenylamine

A mixture of aniline (37.2 gm.), resorcinol (11 gm.), anhydrous calcium chloride (22 gm.) and aniline hydrochloride (1 gm.) was heated in a sealed tube at 280° for eight hours. The reaction product was dissolved in concentrated hydrochloric acid (200 c.c.). The deep-brown acid solution was diluted with water (500 c.c.) and the residue separated was filtered off. The aqueous solution was cooled in ice and saturated with sodium
acetate when m-hydroxydiphenylamine crystallised out in flakes.
It crystallised from petroleum ether (b.p. 60-80°), m.p. 78-80°
(Yield, 6.2 gm.).

Found in a dry sample, C, 77.53; H, 6.02; N, 7.83
Calcd. for C_{12}H_{11}ON: C, 77.83; H, 5.94; N, 7.56 per cent.
Fusion of 1-Phenyl-2-Keto-1:2-Dihydro-4-Quinazolone with Potassium Hydroxide

1-Phenyl-2-Keto-1:2-Dihydro-4-Quinazolone

Fused with KOH

Fusion product

Treated with water containing ammonium chloride

Aqueous solution

Extracted with ether

 Ether (A)

Acidified with concentrated hydrochloric acid and extracted with ether

Aqueous solution (B)

Extracted with 2N hydrochloric acid

Acid solution

Ether (A)

Basified with sodium carbonate and extracted with ether

Concentrated

Residue

Ether (F) Aqueous portion (Discarded) Solid, m.p. 54° (Salicylic acid)*

Ether (D) Bicarbonate extract

Concentrated

No residue

Acidified with concentrated hydrochloric acid and extracted with ether

Aqueous fraction (Discarded)

Concentrated

Residue

Sublimed under high vacuum

Solid, m.p. 152-55° (Salicylic acid)
N-phenylquinazolone (0.3 gm.) was fused with potassium hydroxide (1.5 gm.) at 280° for five minutes. The fused mass was cooled and digested with water (20 c.c.) containing ammonium chloride (1 gm.). The aqueous solution was extracted with ether (3 x 50 c.c.) when the basic and neutral fractions passed into ether (A). The aqueous solution (B) was cooled in ice, acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (3 x 20 c.c.) (C). The ether solution (A) was washed with 2N hydrochloric acid (20 c.c.) to separate the neutral components from the basic fraction if any. The acid extract was basified with sodium carbonate (pH, 8-9) and extracted with ether (F).

The ether solution (C) was washed with sodium-bicarbonate solution (5 per cent, 3 x 20 c.c.) to separate the acidic materials from the phenolic compounds. The alkaline washing was acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (3 x 20 c.c.) (F). The ether solutions (A), (D), (E) and (F) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated. Only the ether solutions (A) and (E) yielded residue.

**Identification of Diphenylamine**

The residue from the ether solution (A) sublimed under high vacuum at 70-80°/0.01 mm as a colourless oil which soon
solidified (Yield, 7-8 mg.). It crystallised from a mixture of benzene and petroleum ether, m.p. 54° and developed deep-blue colouration with concentrated sulphuric acid and sodium nitrite. It was identified as diphenylamine from melting point and mixed melting point determination.

Found in a sample dried in vacuo over P₂O₅ at 40°: N, 8.56. Calcd. for C₁₂H₁₁N: N, 8.3\% per cent.

**Identification of Salicylic acid**

The solid residue from the ether solution (R) was purified by sublimation under high vacuum at 150-200/0.01 mm. The sublimate (Yield, 5-6 mg.) crystallised from hot water in flakes, m.p. 153-54°. It produced a deep-violet colouration with ferric chloride. It showed no depression in melting point when admixed with an authentic sample of salicylic acid.

Found in a dry sample: C, 60.63; H, 4.11. Calcd. for C₇H₆O₃: C, 60.86; H, 4.34 per cent.
Acridone
Fused with KOH
Alkali melt
Treated with water containing NH₄Cl
Aqueous solution
Extracted with ether
Aqueous layer (B)
Acidified with Conc. HCl and extracted with ether
Aqueous solution (Discarded)
Ether (C)
Concentrated
No residue

Ether (A)
Extracted with 2N, HCl
Acidic extract
Ether
Concentrated
No residue

Aqueous solution (Discarded)
Concentrated

Ether (D)
Concentrated
No residue
Alkali Fusion of Acridone (CHART IV)

Acridone (0.3 gm.) was fused with solid potassium hydroxide (1.5 gm.) at 290-300°C for five minutes. The fusion product was digested with water containing ammonium chloride (2 gm.) and the cold aqueous alkaline solution was extracted with ether (5 x 20 c.c.) (A). The aqueous layer (B) was cooled and acidified with concentrated hydrochloric acid (pH 3-4) and extracted with ether (3 x 20 c.c.) (C). The ethereal solution (A) was washed with hydrochloric acid (2N, 30 c.c.) to separate the basic fragments from the neutral components. The acidic extract was cooled, basified with sodium carbonate (pH 8-9) and extracted with ether (D). The ether solutions (A), (C) and (D) were washed with small portions of water, dried over anhydrous sodium sulphate and concentrated when no residue was obtained from any of the solutions.

Alkali Fusion of Carbazole

Carbazole (0.5 gm.) was fused with potassium hydroxide (2.5 gm.) at 290°C for five minutes. The product on working up following the procedure described above did not afford any neutral, basic or acidic substance.
Synthesis of 1-Phenyl-7-hydroxy-2-keto-1,2-dihydro-4-quinazolone

(a) Preparation of m-Bromonitrobenzene

A mixture of dry nitrobenzene (90 gm.) and reduced iron powder (3 gm.) was taken in a round-bottomed flask fitted with a condenser and a dropping funnel and was heated in an oil-bath at 135-45°. Dry bromine (20 c.c.) was added to the reaction mixture in a period of one hour and heating (temperature 135-45°) was continued for another hour before adding a second portion of iron powder and bromine. Iron powder (6 gm.) and bromine (40 c.c.) were added in two more instalments in a similar manner, followed by the final addition of one gram iron powder. Heating was continued for sixty minutes. The deep brown reaction product was cooled and poured into water (500 c.c.) and extracted with benzene. The benzene extract was washed repeatedly with water, dried over anhydrous sodium sulphate and concentrated. The thick brown residue distilled at 136-80/18 mm. The yellow distillate (89 gm.) soon solidified.

Found in a pure sample: Br, 39.92; Calcd. for C₆H₄NO₂Br: Br, 39.60 percent.

(b) Reduction of m-Bromonitrobenzene

A solution of m-bromonitrobenzene (50 gm.) in aqueous ethyl alcohol (200 c.c., 50% w/w.) was taken in a three-necked flask (1 litre). Reduced iron powder (45 gm.) was added to the solution. Concentrated hydrochloric acid (10 c.c.) in aqueous
ethyl alcohol (25 c.c. 50% w/w.) was slowly added to the reaction mixture in a period of fortyfive minutes, while the latter was stirred and heated (temperature 60-70°) for three hours. It was filtered while hot and the residue was washed with hot ethyl alcohol (100 c.c.). The washing was added to the filtrate and the combined alcoholic solution was concentrated (200 c.c.) and diluted with water (500 c.c.). The cold aqueous solution was basified with sodium hydroxide solution (50%, aqueous) and extracted with benzene. The latter was washed with water, dried over anhydrous sodium sulphate and concentrated. The oily residue distilled at 138°/18 mm. (Yield, 39 gm.).

Found in a pure sample: Br, 46.28; Calcd. for C₆H₇N Br: Br, 46.51 percent.

(c) Preparation of m-Bromophenol

m-Bromoaniline (50 gm.) was added to a dilute solution of sulphuric acid (60 c.c. concentrated sulphuric acid in 90 c.c. water) when the amine sulphate precipitated out. It was diazotised with an aqueous solution of sodium nitrite (21 gm. in 50 c.c. water). The diazotised solution was slowly added to boiling dilute sulphuric acid (200 c.c. concentrated sulphuric acid in 150 c.c. water) in which a rapid stream of steam was passed when m-bromophenol distilled over with steam. The distillate was extracted with benzene. The benzene solution was dried over anhydrous sodium sulphate and concentrated. The concentrate distilled as a colourless liquid at 138°/12 mm. (Yield, 25 gm.).
Found in a pure sample: Br, 46.37; Calcd. for $C_6H_5OBr$: Br, 46.24 percent.

(d) Preparation of 2-Bromo-4-hydroxybenzaldehyde

A mixture of m-bromophenol (17 gm.), slaked lime (50 gm.), sodium carbonate (40 gm.) and water was stirred and heated at 60°. Chloroform (26 gm.) was added dropwise to the reaction mixture in a period of one hour. The reaction mixture was finally boiled for another hour. It was then cooled and acidified with dilute hydrochloric acid (pH 3-4) and subjected to steam-distillation to remove excess chloroform and unreacted bromophenol. The aqueous solution was filtered while hot and cooled when 2-bromo-4-hydroxybenzaldehyde crystallised out. It crystallised in colourless needles from aqueous alcohol, m.p. 156-7° (Yield, 4 gm.).

Found in a dry sample: Br, 40.17; Calcd. for $C_7H_5O_2Br$: Br, 39.80 percent.

(e) Preparation of 2-Bromo-4-hydroxybenzoic acid

To a suspension of 2-bromo-4-hydroxybenzaldehyde (1 gm.) in hot water (50 c.c.) at 60°C, was added an aqueous solution of potassium permanganate (1 gm. in 20 c.c.) over a period of thirty minutes with stirring. Heating (60-70°) and stirring was continued for one hour. The aqueous solution was filtered, and the residue was washed with hot aqueous potassium hydroxide
solution (2%, 100 c.c.). The filtrate and washings were concentrated (30 c.c.), cooled in ice and acidified with concentrated hydrochloric acid (pH 2-3). The precipitated acid was taken up in ether which was washed with a small amount of water, dried over anhydrous sodium sulphate and concentrated. The residue crystallised from a mixture of benzene and acetone in colourless needles, m.p. 207° (Yield, 0.7 gm.).

Found in a dry sample : C, 38.70; H, 2.38; Calcd. for C₇H₅O₃Br : C, 38.72; H, 2.30 percent.

(f) Acetylation of 2-Bromo-4-hydroxybenzoic acid

A solution of 2-bromo-4-hydroxybenzoic acid in freshly distilled acetic anhydride (10 c.c.) was refluxed with pyridine (5 drops) for four hours. The reaction product was added to water (50 c.c.), and kept overnight. The solid separated was taken up in ether which was worked up following usual procedure. The ether residue crystallised from aqueous ethyl alcohol in flakes, m.p. 142° (Yield, 4 gm.).

Found in a dry sample : C, 41.51; H, 2.92; Calcd. for C₉H₇O₄Br : C, 41.70; H, 2.70 percent.

(g) Preparation of 5-Acetoxy-2-carboxy-diphenylamine

A mixture of 2-bromo-4-acetoxylbenzoic acid (1.5 gm.) and anhydrous potassium carbonate (1.3 gm.) in amyl alcohol (6 c.c.)
was stirred at 136° for 6 minutes when a voluminous precipitate appeared. The reaction mixture was cooled and treated with aniline (3 c.c.) and copper powder (20 mg) and heated with stirring few at 145-50° (bath temperature) for thirty minutes. The cold reaction product was treated with water (40 c.c.) and steam-distilled to remove excess aniline and amyl alcohol. The aqueous solution was filtered, washed repeatedly with ether, cooled in ice and acidified with hydrochloric acid (pH 3-4) and extracted with ether (A). The ether extract (A) was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue crystallised from a mixture of acetone and benzene, m.p. 184° (dec.) (Yield, 0.3 gm.).

Found in a dry sample : C, 66.66; H, 4.91; N, 5.30;
Calcd. for : C\textsubscript{16}H\textsubscript{13}O\textsubscript{4}N : C, 66.42; H, 4.79; N, 5.16 percent.

(h) Preparation of Methyl ester of 5-acetoxy-3-carboxy-diphenylamine

An ether solution (50 c.c.) of 5-acetoxy-2-carboxy-diphenylamine (2 gm.) was cooled in freezing mixture and treated with an ethereal solution of diazomethane (20 c.c.) (regenerated from 5 gm. of nitrosomethylurea) and kept overnight. The ether solution was concentrated carefully on water-bath when an oily residue was left which was directly used for further reaction.
Condensation of Methyl ester of 5-acetoxy-2-carboxy-
diphenylamine with urethane

Dry urethane (0.89 gm.) and an ether solution of methyl
ester of 5-acetoxy-2-carboxy-diphenylamine were added to mole-

cular sodium (0.23 gm.) when an immediate reaction with warmed
on water-bath for four hours. It was then treated with alcohol
(5 c.c.) followed by water (50 c.c.) and heated (60-70°) for four
hours. The solution was then cooled, washed with ether and
acidified (pH 3-4) and extracted with chloroform. The latter was
washed with water, dried over anhydrous sodium sulphate and
concentrated. The residue crystallised in yellow needles from
methyl alcohol, m.p. 265° (dec.) (Yield, 0.11 gm.).

Found in a dry sample: C, 66.30; H, 3.71; N, 11.31;
Calcd. for C_{14}H_{10}O_{3}N_{2}: C, 66.13; H, 3.93; N, 11.02 percent.