PART X

ISOLATION OF VOLATILE PRODUCT
AND ITS CHEMICAL STUDIES
STEAM DISTILLATION OF VARIOUS PARTS OF PLANT AND
STUDY OF ISOLATED VOLATILE PRODUCT

The volatile product was isolated quantitatively in a similar way to isolation of volatile oils from plants by steam distillation method of B.P. (1958), using Clevenger apparatus for oils lighter than water.

Isolation of volatile product from flowers

To 200 gm. of the crushed flowers one litre flask added 500 ml. of distilled water and then collected the product in a graduated tube by steam distillation process.

1. Yield of the product from the sample of flowers collected from Rohtak district (Punjab) = 0.2 % v/w.

2. Yield of the product from the sample of flowers collected from Kamaal district (Punjab) = 0.45 % v/w.

The volatile product distilled within half an hour. It was collected and the process of steam distillation continued. It was found that a yellow crystalline product deposited along the sides of the condenser. After four hours of steam distillation process, the crystals from the condenser were removed with ethanol, in which they were insoluble. Filtered, dried in a vacuum desiccator, yield 0.042 % w/w, m.p. 117°C.

The yellow crystalline product was giving odour like sulphur and it was confirmed to be only sulphur by qualitative and quantitative elemental analysis.

It was observed that sample of flowers from which...
G.L.C. OF STEAM DISTILLED PRODUCT
(VOLATILE OIL)

APPARATUS ------------- PERKIN ELMER MODEL-154
TEMPERATURE ------------- 144°C
ENERGY SUPPLY ------------- 60% /N2 PRESSURE ------------- 4 LB
BRIDGE VOLTAGE ------------- 7 VOLTS
SENSITIVITY ------------- 4 ATTENUATION
AMOUNT INJECTED ------------- 0.04 ml. SOLUTION IN ACETONE
volatile product was not isolated within few days after the collection gave less amount of volatile product and more quantity of sulphur. If the flowers were kept more than a month and then steam distilled gave all sulphur but no volatile product.

Isolation of volatile product from seeds.

Fresh red barries were collected and the seeds were separated from the mucilaginous pulp by immersing in cold water and passing through sieve, which retained the seeds.

The seeds were crushed and volatile product was isolated as from flowers. yield 0.6 % w/w.

The volatile product separated as an oily layer. The product distilled within half an hour. The process was continued after the collection of the product and sulphur was separated as in case of flowers, yield 0.05 % w/w. The volatile product was found to be absent in the fruit pulp, stem bark and root bark.

Gas liquid chromatography of volatile product.

G.L.C. of the product was carried out using Perkin Elmer Model-154 to see whether product was a single chemical or composed of many volatile chemicals. The details of the G.L.C. is represented in the Fig. 1. The solution of the product in acetone was injected. There were only two peaks, the first peak was of acetone and the 2nd peak of volatile product. The presence of one the peak confirmed that the product was a single chemical.

Physical properties.

It is highly soluble in benzene, ether,
alcohol and acetone, but very slightly soluble in water. It has deep penetrating odour and is highly volatile, on cooling to 10°C, it crystallises to white needles. The product was always kept in refrigerator as it was highly unstable at room temperature and changed to black mass within a few days at room temperature.

Chemical investigations

Qualitative elemental analysis showed the presence of C, H, N, O and S.

(Quantitative elemental analysis)

<table>
<thead>
<tr>
<th>Element</th>
<th>% found</th>
<th>% calculated from C₆H₆N₂S₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>32.50</td>
<td>32.72</td>
</tr>
<tr>
<td>H</td>
<td>4.00</td>
<td>3.64</td>
</tr>
<tr>
<td>N</td>
<td>12.81</td>
<td>12.72</td>
</tr>
<tr>
<td>S</td>
<td>43.45</td>
<td>43.63</td>
</tr>
<tr>
<td>O</td>
<td>7.18</td>
<td>7.27</td>
</tr>
</tbody>
</table>

Molecular weight found by cryoscopic method = 220

Empirical formula and molecular formula were the same, C₆H₆N₂S₂O₂.

Function of sulphur

The sulphur in the plant products is usually in the form of sulphone, disulphide, thiol, thiokeone, thiocyanate and isothiocyanate etc.

1. The aqueous solution of the volatile product was neutral in reaction to litmus, which indicated the absence of sulphonic and sulphinic acids.

2. To a suspension of the product in water (1 ml.) added 0.5 ml. of warm dilute sodium hydroxide solution. Clear solution was formed, without indicating any decomposition, showing the probability of sulphur compounds containing phenolic
hydroxyl group or mercaptans.

3. To a suspension of small amount of product in water, added 1 ml. of mercuric chloride solution (2%), a precipitate was formed, indicating the probability of mercaptans, sulphides and disulphides. The precipitate was collected on a filter paper and dried, it did not melt up to 360°C. Mercuric derivatives of some mercaptans, sulphides and disulphides have definite melting point.

4. To a solution of product in dilute sodium hydroxide added freshly prepared sodium nitroprusside solution, an intense pink colour was formed which was stable only for about 75 seconds, indicating the presence of mercaptans.

5. A drop of the volatile product was mixed on a watch glass with a drop of iodine-azide solution (3 gm. of sodium azide in 100 ml. of 0.1 N iodine), there was evolution of bubbles of nitrogen, thus indicating the presence of either C=S (thioketone) or C-SH (mercaptan group).

To differentiate whether the test was because of thioketone or mercaptan group, the small amount of product (0.2 ml.) was warmed for a short time with alcoholic solution of iodine (5 ml.) and then few drops of this solution were mixed on a watch glass with a drop of sodium azide solution, there was evolution of bubbles of nitrogen, indicating the presence of C=S (thioketone group). The mercaptans on treatment with alcoholic iodine solution are converted into disulphide, which give negative test with sodium azide solution.

The above tests indicated the possibility of the
presence of mercaptan, thioketone and disulphide groups.

Preparation of derivative with 2:4 dinitro chlorobenzene

To an alcoholic solution of volatile product (0.5 g.m. in 10 ml. rectified spirit), added 2 ml. of 10 % sodium hydroxide and 5 ml. of alcoholic solution of 2:4 dinitrochlorobenzene (1 g.m. in 5 ml. of rectified spirit), and then refluxed the mixture on a water bath for 10 minutes, when a yellow crystalline product separated out. Filtered, dried in a vacuum desiccator, m.p. 194°C.

Quantitative elemental analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>% found</th>
<th>% calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>39.64</td>
<td>39.23</td>
</tr>
<tr>
<td>H</td>
<td>1.78</td>
<td>1.90</td>
</tr>
<tr>
<td>N</td>
<td>14.79</td>
<td>15.25</td>
</tr>
<tr>
<td>S</td>
<td>8.75</td>
<td>8.72</td>
</tr>
<tr>
<td>O</td>
<td>34.50</td>
<td>34.87</td>
</tr>
</tbody>
</table>

Empirical formula worked out to be C_{12}H_{7}N_{4}O_{8}S

M.Wt. = 358.

The molecular weight almost corresponds to the empirical formula, and thus the empirical formula is the molecular formula.

Function of nitrogen.

The nitrogen in most of the volatile sulphur containing compounds is in the form of primary, secondary tertiary amines; thiocyanates or nitriles.

1. Rimnis test\textsuperscript{4} for primary amines

No violet or red colour was formed when to the
suspension of the product added 1 ml. of pure acetone and a drop of freshly prepared aqueous solution of sodium nitroprusside, thus showing the absence of primary amines.

2. **Tests for secondary amines with sodium nitroprusside and acetaldehyde**

To a drop of volatile product on a spot plate added a drop of reagent solution (1% sodium nitroprusside solution to which 10% by volume of acetaldehyde was added. The reagent was prepared freshly) and then sufficient amount of 2% $\text{Na}_2\text{CO}_3$ solution to make the reaction mixture alkaline. No violet colour was formed, indicating the absence of secondary nitrogen.

3. **Test for secondary amine by copper dithiocarbamate formation**

To a drop of acidic solution of volatile product added a drop of Cu $\text{SO}_4$ solution (5%) and the mixture was made alkaline with $\text{NH}_4\text{OH}$, and to it added 2 drops of mixture of 1 volume of carbon disulphide and 3 volumes of benzene and shook thoroughly. No change in the benzene layer indicating the absence of secondary amine.

**Test for nitrile group** - $\text{C}=\text{N}$

Boiled the aqueous suspension of the volatile product with sodium hydroxide, the vapours evolved turned the paper previously dipped in nessler's solution yellow brown on prolonged reaction, indicating the evolution of ammonia vapours.

The presence of nitrile group was also confirmed by the presence of peak in I.R. spectrum at 2230 c.m.\(^{-1}\)

The aqueous solution of the product did not discharge the colour of potassium permanganate, and gave no precipitate with
INFRA RED SPECTRUM OF STEAM DISTILLED PRODUCT

WAVELENGTH (MICRONS)

TRANSMITTANCE (%)
Fig....2

ULTRA VIOLET SPECTRUM OF STEAM DISTILLED PRODUCT
2:4 dinitrophenylhydrazine and thus showing the absence of unsaturation and C=O grouping.

Absorption analysis.

(a) Ultraviolet: absorption spectrum of the solution of product in methanol was determined on a Hilger Unispeck spectrophotometer model H 700 using hydrogen discharge tube and 1 cm. Cuvettes. The curve is represented in Fig. No. 2.

The maximum absorption peak was obtained at 243 μm. No absorption peak was obtained in visible range. The presence of this peak is an indication of mercaptan group in the molecule.

STUDIES OF I.R. SPECTRUM OF VOLATILE PRODUCT

I.R. spectrum of the oil was taken in Nujol.

<table>
<thead>
<tr>
<th>Absorption peaks in cm⁻¹</th>
<th>Intensity</th>
<th>Expected functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3350</td>
<td>m</td>
<td>(-N-H)</td>
</tr>
<tr>
<td>2900</td>
<td>s</td>
<td>Nujol</td>
</tr>
<tr>
<td>2380</td>
<td>m</td>
<td>Indefinite</td>
</tr>
<tr>
<td>2230</td>
<td>s</td>
<td>Nitrile group (-C≡N)</td>
</tr>
<tr>
<td>2125</td>
<td>s</td>
<td>-do-</td>
</tr>
<tr>
<td>1550</td>
<td>m</td>
<td>-NH deformation</td>
</tr>
<tr>
<td>1465</td>
<td>s</td>
<td>-N=C = S</td>
</tr>
<tr>
<td>1410</td>
<td>s</td>
<td>-CH₂</td>
</tr>
<tr>
<td>1375</td>
<td>m</td>
<td>-CN vibrations of aliphatic amines</td>
</tr>
</tbody>
</table>

m. means medium        s. means strong.
I.R. Spectrum of the volatile product suggests the presence of nitrile group, \(-\text{N-C}=\text{S}\) group, and suggests the absence of sulphone group \(-\text{S-O}\) (1060-1040 cm\(^{-1}\)) and free OH group (3650-3590 cm\(^{-1}\)).

**SUMMARY AND DISCUSSION**

Volatile product has been isolated by steam distillation process from flowers (0.45%) and seeds (0.6%). The volatile oil isolated from flowers and seeds was found to be of same nature. Fruit pulp, root bark and stem bark gave no volatile oil by steam distillation process.

Volatile product had been confirmed to be a single chemical by G.L.C. method. It had formula corresponding to \(\text{C}_6\text{H}_6\text{N}_2\text{O}_3\text{S}_3\), and showed the presence of nitrile group, which had been confirmed by I.R. spectrum and chemical tests. I.R. spectrum also suggested the possibility of \(-\text{N-H}\) grouping. The presence of thioketone \(\text{C} = \text{S}\) group had been confirmed by chemical tests. Chemical tests also showed the possibility of mercaptan and disulphide groups. The presence of sulphone and OH groups were eliminated as the respective bands of these groups (1060-1040 cm\(^{-1}\)) and (3650 -3590 cm\(^{-1}\)) were absent in the I.R. spectrum of the volatile product. Chemical tests also showed the absence of unsaturation and carbonyl group in the molecule.

To confirm mercaptan group, the trial had been made to prepare derivative with 2:4 dinitrophenyl chloride. A yellow crystalline product was obtained, m.p. 194°C. Elemental analysis and molecular weight determination gave molecular
formula corresponding to \( C_{12}H_8O_6S \). This formula suggested the formation of compound of structure (I), and thus indicated that one of the \(-S-\) in the molecule was placed in such a way which could be easily abstracted from the molecule.

All the facts mentioned above lead to propose basic structure (II) of a molecule which should contain \(-O-\) in a cyclic form, as \(-O-\) does not seem to exist in any other functional form. The proposed thio-oxazolidon ring for the molecule, has also been found by Astwood, Greer and Ettlingar (1949) to exist in a sulphur containing volatile fraction isolated from *Brassica napobrassica*, which they identified as \( \ell \)-5-Vinyl-2-thio-oxazolidon (structure III). The possibility of disulphide and \(-CH_2-\) groups is indicated from chemical tests and I.R. spectrum. Nitrile group has also been confirmed in the molecule. Keeping all these facts the following (IV & V) structures may tentatively be proposed.

Structure No. VI and VII may explain the behaviour of the compound to give properties both of thioketone and mercaptan groups.
Further studies are still in progress to confirm the structure of the molecule by carrying out certain degradative studies and then finally through its synthesis.

---

**Note:** Antibacterial, antifungal and anthelmintic activity of the volatile product has been determined and are given on page 192, 199 and 217 respectively.
REFERENCES


5. Feigl, F., ibid, p. 260.

6. Feigl, F., ibid, p. 262.