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THE "CHEMICAL INVESTIGATION
OF
KARAFF SEEDS"
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INTRODUCTION.
**Introduction.**

There is no exaggeration in the statement that plants have sustained and are sustaining human life on this planet. True, we have been much better served by the synthetic chemist in a variety of ways in this world of ever changing economic reactions. The crude drugs have given place to pure compounds, which have the advantage of fixed and definite action and no complications; still crude drugs are used firstly because, our search has not yet been successful in fixing up the active constituents of many of these drugs; secondly there is the economic question; and finally these drugs provide mixtures of medicinal substances with well established and desirable therapeutic action. The chapter opened by the synthetic chemist with the dye-stuffs has now passed on to a stage, where it is very doubtful if we can fruitfully go any further. The hope that Photosynthesis when realised will solve all our difficulties of world shortages, is a statement to be taken with great care and caution. We are yet far from the solution of the problem, though a number of facts have emerged from these studies which are of some consequence.

The recent developments in the field of enzyme chemistry and traceable isotopes have led us to the exclusive importance of chlorophyll amongst the plant pigments, still the next stage, for a clear elucidation of the building up of the plant wealth, which is so fast dwindling, needs solution of a number of problems. Before this stage could be arrived at we want a number of exact
statistical data about plants themselves. The composition of any plant is the first important point which requires attention. True, nature is regular, but this regularity more often than not is shown in a range, the ends in which though not opposite, are far apart.

Therefore, even if we succeed in clearly explaining a specific case, by phosphorolytic reactions, in the plant metabolism, still at every stage it will need modification. Before this explanation could be attempted, thorough knowledge about the constituents of a plant cannot be over emphasised.

It is amazing how man through ages has selected plants for his food, which are today well-known for their dieto-and medico-therapy, and it appeared of some interest to investigate karaff seeds which have recently developed a considerable importance as a vegetable.
THEORETICAL.
The Pigments from the Plants.

The red, the blue and the violet of the plant world is a phenomenon of common occurrence, and is attributed to the pigment anthocyanin, though occasionally other pigments may be responsible for this manifestation of colour, as Molisch found in the case of the leaves of aloes (Carotin). It is also common knowledge that the nature of the cell sap, acidic, alkaline or neutral is responsible for the red, the blue and the violet of the plants. Thus the same anthocyanin is red (red rose) in one case and blue (Corn flower) in the other case. The varying shades of the flowers may be due to a slight modification in the molecule or to other factors, such as the concentration of the anthocyanin, the presence of yellow pigments (flavones, flavonols, lipochromes), combination with tannins or with other substances (co-pigments) which materially alter the shades of the anthocyanins.

The yellow colour in the vegetative organs and the petals of many plants is due to various pigments such as lipochromes (Polyenes-lycopene and Carotin) and different hydroxy flavones and their derivatives (hydroxy flavonols), and xanthones and occasionally to yellow hydroxy ketones which are genetically related to flavones. The group of yellow pigments, flavones and xanthones, on account of their close resemblance to anthocyanins, has been given the generic name anthoxanthin, by Willstatter and Ehrlich (1913), a term at first suggested by Marquart (1835).
The anthoxanthins occur naturally combined with sugars, some times uncombined, and are found occasionally associated with tannins. In combination they are feeble in colour, uncombined they are deeper in shade. The concentration of these substances in the plant in some cases may not be large enough to give rise to colour, as their occurrence in many a white petals is revealed only on exposure of the petals to ammonia vapours. They are abundantly found in the vegetable kingdom and have been used as dyeing materials from remote times.

From the extensive studies conducted by Kostanecki, Herzig, and A.G. Perkin in the case of anthoxanthins; by Willstatter, Robinson, Karrer and Freudenberg in the case of anthocyanins and by E. Fischer and Freudenberg (synthetic tannins), Pelouze, Schiff, Nierenstein, Trimble, and Perkin, in the case of tannins, during the last sixty years, a number of relations have been established between them, both in the test tube, and in the Plant world. Thus Shibata and Nagai (Bot. Mag. Tokio, 1916, 30, 149) found that the young plant contains red anthocyanin which gives place to a colourless flavone in the mature stage; at leaf fall the anthocyanin may reappear.

Sheildale (Proc. Camb. Phil. Soc. 1909, 15, 137; Jour. Gen. 1911, 1, 10) considers anthoxanthins (flavones and flavonols) as the precursors of anthocyanins. He further mentions that two factors— a flavonol and an oxidising enzyme — are necessary requisite for the production of an anthocyanin.

In the case of cultivation of Antirrhinum (Bio. Chem. J. Camb. 1913, vii, 441-4) two varieties among others arose as
sports, vix an ivory and a white, both incapable of producing an anthocyanin. When a white (certain ancestry) is crossed with an ivory, a plant having magenta flowers of the type is produced. The magenta flowers contained apigenin in the inner tissues of the corolla and the anthocyanin in the epidermis. Combes (Compt. rend. 1913, 152, 1002, 1454; 1914, 159, 272) claims to have obtained both the flavone and anthocyanin (Ampelopsis hederacea) together, and the conversion of the flavone into anthocyanin by reduction, and anthocyanin into flavone by oxidation. Jonesco (Comp. rend. 1921, 175, 830, 1006) considers the red obtained by Combes in his experiments to be due to acid used. Hall and Everest (Proc. Royal. Soc. B. 1921, 92, 150) from the studies on flower buds well marked for the anthocyanin content of their mature petals, observed that before the anthocyanin appeared, the petals were yellow or colourless, and developed colour with ammonia vapours. Alcoholic extract of the red rose and a mauve violet collected before the appearance of the anthocyanins treated with Magnesium, gave pale red and therefore they concluded that the young buds contain flavonols which would have given rise to anthocyanin by reduction at a later stage.

Thus there is some evidence (of a qualitative nature) of the simultaneous occurrence of an anthocyanin and anthoxanthin in the same plant and two schools of thought about the conversion of anthoxanthin and anthocyanin - the one favouring the oxidation and the other reduction. It is quite possible as Haas and Hill (An introduction to the Chemistry of Plant...
products, 1923, 353) point out that "the anthocyanin in a
given plant material might actually contain a greater number
of hydroxyl groups than the flavonol accompanying it, so that
while the conversion of the flavonol into the corresponding
anthocyanin would result from reduction, the introduction of
an increased number of hydroxyl group nevertheless involve
oxidation, so that both schools of thought would be justi­
fied".

Purely chemical evidence as detailed later supports
the reduction school of thought.

The parent substance of the anthocyanin class is the
nucleus known as Benzopyrylium chloride (1) discovered by
Decker and Fallenberg (Ann., 1908, I. 364) and on account of
the basic oxygen atom formulated by them, on the basis of
oxonium theory as under

\[
\begin{align*}
\text{(I)}
\end{align*}
\]

This (1) on attachment of a phenyl residue in position (2)
will give rise to 2-phenyl benzopyrylium chloride (flavylium
chloride), (II)

\[
\begin{align*}
\text{(II)}
\end{align*}
\]
which on the exchange of hydrogen atoms in the positions 3, 5, and 7 with hydroxyl groups (OH) will yield 3, 5, 7-trihydroxy flavylium chloride (III) the simplest intact structural unit of the anthocyanin.

![Chemical structure of flavylium chloride (III)](image)

On account of the claims of carboneum theory and also of the doubt about the location of the positive charge (carbon atom 2 or 4), these salts are usually formulated in the neutral state.

From the examination of the various anthocyanins it has been observed that the change from member to member in the group is obtained by the replacement of hydrogen atoms by hydroxyl groups in the benzene nucleus and thus there are four type groups (IV), (V), (VI) and very rare (VII) in which the (OH) in position 3 is missing.

![Chemical structures of anthocyanins IV, V, VI, VII](image)
The structure IV, V, VI have been synthesised (Robinson and Collaborators) by methods which leave no doubt about their validity. Taking the first type Pelargonidin (IV)

which represents the red form in acid solution, the replacement of the -Cl by -(OH) on treatment with an alkali gives rise to an anhydride (VIII)

which is the neutral form and gives violet shades. The blue would be obtained by the replacement of the hydrogen atom or atoms by a metal in alkaline solutions.

The degradation (by alkali fusion) of the anthocyanin yields Flavoglucinol (1,3,5. trihydroxy benzene)(IX) invariably as one of the products of fission and the fact is obvious from the above constitution.
The study of these compounds by Wilstätter, Robinson, Karrer and collaborators have established that the sugar residue attaches itself in position 3 or in positions 3 and 5 giving rise to -

(1) 3-mono glucosides or 3-galactosides (2) 3-rhamnoses or 3-other pentosesides (3) the 3-biosides, (4) 3,5-diglucosides and (5) the acylated anthocyanine.

The members of class (4) are widely distributed and well known. Some Anthocyanins yield on hydrolysis a sugar or sugars and a third compound invariably an organic acid. This is the group of acylated anthocyanins (5) above. The acid so found, thus far, are p-hydroxy benzoic acid, malonic acid, p-hydroxy cinnamic acid and p-coumaric acid (Karrer).

γ-pyrone (X), the simplest aromatic derivative of which is Benzopyrone (XI) commonly called Chromone, on attachment of a phenyl group in position 2, will yield 2-phenyl benzopyrone (XII) or flavone. Condensation of a benzene nucleus to Benzopyrone structure would give xanthone (XIII)

\[
\begin{align*}
\text{(X)} & \quad \text{(XI)} & \quad \text{(XII)} & \quad \text{(XIII)} \\
\end{align*}
\]

another mother substance for yellow plant pigments, but as yet very few members of this class are known.

Replacement of the hydrogen atom in position 3 by a hydroxyl group will yield a hydroxy flavone (XIV) called flavonal.
Variety of yellow pigments in the plant world from flavone and flavonol structures in the first instance is obtained by the introduction of hydroxyl groups in the benzene nucleus or in the benzo radical of the parent substance or in both, and then by a replacement of hydrogen of the hydroxyl group, or groups, by a sugar or sugar molecules, or by other groups (rarely), and lastly, by substitution in the hydroxyl group of the attached sugar residues.

The chemistry of this group of compounds which closely resembles anthocyanins owes much to the pioneer researches of Von Kostanecki for his large number of useful syntheses, which have made these compounds easily and readily available; to Herzig, whose patient study of Quercitrin (1884) from quercitron bark and Fisetin from young fustic, which marks a new era for the natural yellow colouring matters; and to A.G. Perkin for his exhaustive studies of these compounds from natural sources and their tinctorial properties.

These compounds are mostly yellow, high melting solids soluble in water, alcohol, dilute mineral acids and alkalies. They are precipitated as yellow, orange or red lead salts from their solutions by lead acetate. The solubility of these compounds in acids is due to the basic oxygen atom in 1-pyrone nucleus forming an additive compound, the oxygen atom becoming tetravalent.

These compounds are unstable and hydrolyse in water and do
not occur in plants, while in the case of anthocyanins such compounds are stable and occur naturally in the plants and provide the red of the plant kingdom.

Some of the representative members of the flavone and flavonol series are:

1. **Flavone**
   
   ![Flavone Structure](image)
   
   Chrysin.

2. **Flavanol**
   
   ![Flavanol Structure](image)
   
   Galangin.
The constitution of these compounds have been in the first instance determined (as usual) by degradation (alkali fusion) and then by synthesis.

It was in 1891, that Herzig submitted fully acetylated fisetin (tetra ether) to gentle hydrolysis (alkali fusion) and obtained fisetol diethyl ether and protocatechuic acid-diethyl ether. Fisetol diethyl ether was found to possess the following constitution (XV); and this led to a tetra-hydroxy Phenylpheno-γ-pyrone structure (XVI) for fisetin and then by analogy Quercetin was given the constitution of a hydroxy fisetin (XVII). Kostanecki (in 1893) from similar considerations represented chrysin as a dihydroxy phenylpheno-γ-pyrone (XVIII)

\[
\begin{align*}
\text{Fisetin (XVI)} & \quad \text{Quercetin (XVII)} \\
\text{Chrysin (XVIII)} &
\end{align*}
\]

In 1898, Smilewiec, Kostanecki and Tambor announced the synthesis of the first flavone-chrysin. This they obtained by the condensation of Ethyl benzoate (XIX) and Phloroaceto-phenone tri methyl ether (XX) in presence of sodium and the demethylation of condensation product through hydroiodic acid to chrysin (XXI).
This synthesis of chrysain was soon followed by other syntheses of chrysain by Kostanecki and co-workers, of flavone (1899), apigenin (1900), and luteolin, a little latter. Then flavonols were synthesised and soon (1904-1907), fisetin, quercetin, kaempferol and morin were synthetically produced. A large number of methods for the preparation of hydroxy flavones and flavonols have been devised by Kostanceki, Perkin and Robinson. They may be summed up as under:

Condensation of (a) alkylated O-hydroxy acetophenone (XXII) with esters of aromatic acids (XXIII) or alternatively (b) esters of salicylic acid (XXIV) with acetophenone (XXV). The condensing agent used is metallic sodium.
This (XXVI) is simple flavone. The various hydroxy derivatives of flavone may be obtained by condensing appropriate esters and acetophenone. Another useful method of obtaining the compound is through the chalkone (XXVII) and the flavonone (XXVIII) (a reduced flavone at position 2 and 3) is from 0-hydroxy acetophenone and Benzaldehyde.
Thus, this synthesis provides a ready method of obtaining flavanones and flavonols, two derivatives of flavones, in themselves parent substances for a series of yellow pigments, though at present in the case of flavanones, few natural colouring matters are known to belong to this group such as Maringenin and Hesperidin.

By condensing derivatives of O-hydroxy acetophenone and of benzaldehyde a variety of flavanones, flavones and flavonols could be obtained.

In fact the success in the synthesis of these compounds is very great. Appropriate derivatives of acetophenones, esters of the acids (aromatic), derivatives of benzaldehyde and acid anhydrides could be condensed. A direct synthesis of Quercetin by Allen and Robinson (J.C.S.1924,125,2192;1926,2334) may be mentioned.(XXIX—XXXII).

\[
\text{K-veratrurate} \\
\text{w-Methoxy phloroacetophenone (XXIX)} + \text{Veratric anhydride (XXX)}
\]
(XXXI)3,3',4' trimethyl ether. Quercetin (XXXII)

Relation between anthoxanthine, anthocyanin and tannine (catechin).

Conversion of a flavonol (quercetin(XXXII) into anthocyanin. Cyanidin was early realised by Willstatter and Mallison (Swizungsber, Kgl. Preuss. Akad. d. wiss, 1914, 769).

\[
\text{(XXXII) } \xrightarrow{2H_{\text{HCl}}} \text{(XXXIII)}
\]

this (XXXIII) then loses water and passes on to cyanidin Chloride (XXXIV)

Next stage in the development arrived with the announcement of Freudenberg and Collaborators (Ann, 1925, 444, 135) that cyanidin chloride (XXXIV) could be catalytically hydrogenated to yield dl-epicatechin(XXXV).
to which Robinson and Appel (J.C.S. 1935, 426) added the reverse reaction i.e. the conversion of the catechin to anthocyanidin chlorides. d-Catechin tetra methyl ether (XXXVI) when brominated in dioxane (technical) solution gives bromocyanidin-tetra methyl ether bromide which on demethylation (accompanied by debromination) with hydroiodic acid and phenol (Ber. 1928, 61, 2505) in presence of phosphorous yields cyanidin chloride (XXXVIII) on the replacement of Iodine by Chlorine through precipitated Silver chloride in alcoholic solution.

Freudenberg drew attention to this close relationship much earlier than the above mentioned conversions were realised in the laboratories. He suggested diphenyl propane as the common parent substance for them. The support to this
idea also comes from the occurrence of chalkone, hesperetin and phloretin in the plant world which carry the same skeleton. One of the ways in which diphenyl propane (XXXIX) could be written is as shown below:

\[
\begin{align*}
\text{H}_2 & \quad \text{CH}_2 \quad \text{CH}_2 \\
7 & \quad 8 & \quad 9 \\
6 & \quad 5 & \quad 4' \\
2' & \quad 3' & \quad 6' & \quad 5' \\
\end{align*}
\]

(XXXIX)

From this, it is obvious how by introduction of hydroxyl groups in position 3', 5' and 3,5,7 and 9 and slight rearrangement, it should be possible to pass on from compound to compound.

Robinson (British Assoc. Reports 1921) from the close relationship between the sugars and the appearance of red pigment in the plant already established by the work of Ewart (Journ. Linner. Soc. Bot. 1895-7, 31, 445; Ann.Bot 1897, 11, 461) on Elodea Canadensis and other aquatic plants; Overton (Nature 1899, 59, 256; Jahrb Wiss Bot. 1899, 33) on Hydrochoris and other alpine plants and Wulff (Botanische Beobachtungen Aus Spitburgen Lund 1902) on arctic plants, suggested that C\textsubscript{15} nucleus of flavones, flavonols and anthocyanins may be derived from the condensation of two molecules of glucose to glycerose in aldol condensations.

Goodyear and Howarth (J.Chem.Soc. 1927, 3141) drew attention to the Pyran ring structure of the sugar (which is a part of the molecule of all these substances) and also to
the amylene oxide structure of the sugar shown below. This gives a new significance to the function of sugar in the plants. Also it is very interesting that of all the plant products carbohydrates are the only ones agreed upon to have been obtained through photosynthesis.

![Sugar Structures](image_url)
There are a number of points which indicate a close relation between carbohydrates and fats both in animals and plants. The fattening of animal kept on carbohydrate diet, the transformation of carbohydrates at low temperatures into fats in the plants, the development of fats in the immature seeds detached from the parent plant, all emphasise this close relationship.

The fats and oils are found in all parts of the plant, leaves, stems and roots, but by far the largest quantities are obtained from the fruits and seeds and therefore these attracted early attention. As early as 1861 (Compt. rend. 1861; 53, 3801) De Luca found that olives could make fat even after separation from the trees, while Pfeffer (Jahrb Wiss 1872, 8, 580) found that psaony seeds, detached from the plant at the immature stage when they contained no fat, on keeping developed fat to a certain amount.

Rousille (Compt. rend. 1872, 86, 610), could not detect any change in the amount of the fat of the olive leaves during the ripening of the fruit and Funaro (Landw Versuchsst., 1880, 25, 52) showed that the ether soluble fat from the olive leaves was materially different from fruit flesh olive oil.

Uhlmann (Dissertation, Zurich 1902) examined rather more closely the development of fat in various species of fruits from the earliest stage when there was no fat and only starch present, to the later stages of ripening and found that as oil gradually made appearance, the starch
granules diminished and seemed to dissolve in the 'oil plasma';
the development of oil in the later stages was rapid, and
finally the oil occupied the greater part of the cell under
observation. Uhlmann also noted, in most cases, the formation
of sugar.

Du Sablon (Compt. rend. 1896, 123, 1084; Rev. gen. bot.
1897, 9, 313) investigated the relative proportions of
starch, cane sugar and glucose present in almonds and walnut
at various stages of ripening and some of his results are
summarised below.

<table>
<thead>
<tr>
<th>Almonds</th>
<th>Date of gathering</th>
<th>% Fat</th>
<th>% Glucose</th>
<th>% Sucrose</th>
<th>% Starch &amp; Dextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 June.</td>
<td>2</td>
<td>6</td>
<td>6.7</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>4 July.</td>
<td>10</td>
<td>4.2</td>
<td>4.9</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>1 August.</td>
<td>37</td>
<td>0</td>
<td>2.8</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>1 September.</td>
<td>44</td>
<td>0</td>
<td>2.6</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>4 October.</td>
<td>46</td>
<td>0</td>
<td>2.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Walnut</th>
<th>Date of gathering</th>
<th>% Fat</th>
<th>% Glucose</th>
<th>% Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 July.</td>
<td>3</td>
<td>7.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 August.</td>
<td>16</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>15 August.</td>
<td>42</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>1 September.</td>
<td>59</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>4 October.</td>
<td>62</td>
<td>0</td>
<td>1.6</td>
</tr>
</tbody>
</table>
From these tables it will appear that the general decrease in carbohydrate value is marked by accompanying increase in fat content.

Valee (Compt. rend. 1903, 136:114) who re-investigated oil from almonds confirmed the work of Du Sablon.

Ivanow and Colleagues (Bei. Bot. Centr. 1912, 28:159) extended these studies to a number of seeds (Rape, hemp, poppy and flax seeds) and found identical results. They also obtained evidence, for considerable amount of free fatty acids in the oil, in the early stages of the development of the seeds, characterised by low Iodine values from which they concluded that these are saturated higher members of the fatty series. In the case of Linseed (only) they found that the characteristic iodine value attained maximum (175) in the final stages of ripening only and their scheme of the synthesis of fat from carbohydrate in the case of seed of flax is given below.

<table>
<thead>
<tr>
<th>Glycerol</th>
<th>Carbohydrate</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated fatty acids</td>
<td>unsaturated fatty acids</td>
</tr>
</tbody>
</table>

These observations, about the early development of free fatty acids and the appreciation of the iodine value, after the oil formation has ceased (linseed oil) drew further attention of Ivanow and collaborators as well as of other investigators.

Eyre and Fischer (J. Agr. Sci. 1915, 7:120) who repeated Ivanow's work on flax plant under far more exactly controlled conditions, confirmed the general conclusions of
Ivanow's work, and showed clearly that the oil accumulates rapidly during the early stages of seed development, after which further increase is comparatively slight. The table below shows some of their results.

**Seed of Flax.**

<table>
<thead>
<tr>
<th>Days after flowering</th>
<th>Percentage of Fat in dry seeds</th>
<th>Iodine Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.5</td>
<td>114</td>
</tr>
<tr>
<td>14</td>
<td>15.1</td>
<td>119</td>
</tr>
<tr>
<td>17</td>
<td>31.1</td>
<td>127</td>
</tr>
<tr>
<td>23</td>
<td>37</td>
<td>143</td>
</tr>
<tr>
<td>28</td>
<td>37</td>
<td>170</td>
</tr>
<tr>
<td>35</td>
<td>39</td>
<td>180</td>
</tr>
<tr>
<td>51</td>
<td>36.3</td>
<td>190</td>
</tr>
</tbody>
</table>

Byre who continued the work on the rate of formation and character of the oil in the seed of flax - Linum usitatissimum and L. caribrosum gave more detailed account later (Biochem. J. 1931, 25, 1902), the conclusions from which are reproduced below.

"The acidic constituents are formed first. Whether glycerol is formed at the same time only combining with the fatty acid at a later stage, or whether the formation of glycerol itself is delayed, is not clear. The observations reveal a remarkably rapid oil formation over a period of some fifteen days, during which a maximum of about 30% of oil, calculated on the dry weight of the seeds, is reached.

Changes in the nature of the oil continue after oil
formation has ceased; its unsaturated character, as measured by its iodine absorption, continues to increase. It is pointed out that this is apparently entirely separate change, independant of oil formation.

Syré suggests a reducing system for the formation of fatty acids from carbohydrates, which changes to oxidising system leading to the formation of unsaturated fatty acids.

Barker (J. Soc. Chem. Ind. 1932, 511, 218) has confirmed the main features of this work.

Ivanow and Klokow (Allgem. oel.u. Fett. Ztg, 1933, 20, 149) later working on Moscow linseed found that Linolenic acid content of oil rises with maturing of the seeds, whilst Oleic acid and more so Linoleic acid content of the oil diminishes. They also found acetaldehyde, propionic, hexanoic acids in the unripe sunflower seed and mustard seed.

Studies on similar lines have been conducted on cotton seed (Caskey and Gallup, J. Agr. Res. 1931, 42, 671; Lonzinger and Raskina, Maslob. Shir. Delo 1931, 2, 57), Nigir seed (Sahasrabuddhe, Indian J. Agric. Sci., 1932, 3, 57); and all these investigators confirm the main features of the work of Ivanow and Syré and their collaborators.

There is one case (Bauer, Fettchem. Umschm, 1934, 41, 1) of sunflower seeds (grown from the seeds of one flower head) where the Iodine value remained constant throughout, but even in this case the thio-cyanogen values varied, indicating a change in the character of the unsaturation of the ripening oil.
This is one set of evidence showing the synthesis of fats from carbohydrates in plants. There is another line of argument to emphasise the relationship and is based on the evaluation of respiratory quotient, which is, a volume ratio between carbon dioxide produced and oxygen consumed by the plants. In the normal stage of things in the plant this ratio is 1 : 1, but as Boer (Sec. Trav. Bot. Neirl 1928, 25, 117) has shown it depends upon the substrate and its concentration (Puriewitch, Jahrb. J. Wiss. Bot. 1900, 35, 73) and therefore when carbohydrates (high oxygen content compounds) are being transformed to fats (low oxygen content compounds) during the process of ripening of seeds and fruits, this ratio 1 : 1 will be materially altered.

This ratio in the case of plants has been studied amongst others by Godlewski on excised poppy and castor bean seeds (Jahrb. Wiss. Bot. 1882, 13, 49) and Gerber on castor oil seed and olive oil (Compt. rend. 1897, 125, 658-732; Jour. de Bot. 1901, 15, 121) and recently in the case of castor oil bean by Burr and Miller (Bot. Gazette. 1933, 22, 773). All these authors find values for respiratory quotient well over 1 : 1 ratio and therefore lend indirect support to the thesis that in plants, fats are synthesised from carbohydrates.

The above gradually developed evidence that oils and fats in the plant are mainly obtained from carbohydrates led to efforts to elucidate this synthetic mechanism in the plant. The photosynthetic origin of fats in the plants primarily based on the occurrence of fat like bodies in Vaucheria lost its charms, when these bodies did not respond
to (Mayer. Be. dent. bot. Gesell, 1917, 35, 586; 1918, 36, 5, 235, 674) characteristic biochemical tests for fats. The idea of origin of fats in the plants from proteins, came mostly from the evidence related to animals. There seems to be a close relationship between them in the plants also, but this relationship is not clear. Stark (J. Amer. Soc. Agron. 1924, 16, 636) working on soyabean has brought out the evidence that oil and protein content of soyabean are correlated and that the cultural condition which affects one, also affects the other but in the opposite direction.

**Mechanism of the Synthesis of Fat from Carbohydrates.**

There are three very significant observations in connexion with the occurrence of fats and oils in the plants.

(i) The abundance of C₁₈ acids (Stearic, Oleic, Linoleic, and Linolenic acids)

(ii) The occurrence of even number of carbon atoms in the molecule.

(iii) The straight chain carbon skeleton.

The occurrence of C₁₈ acids in such abundance in nature gave birth to the idea that these may be obtained from hexoses by the condensation of three molecules of C₆ sugars and was advanced by Emil Fischer (Be. 1890, 23, 2114; Untersuchungen Uber Kohlenhydrate Und Fermente, 1909).

The C₁₂ acids may be obtained by the condensation of two hexose molecules, and C₁₆ acids by the condensation of two molecules of pentoses and one of hexose, but there are no indications so far, that pentosans which are present in
about equal quantities in all naturally occurring carbohydrates, are transformed into fatty acids. If this partnership of pentoses is possible, then there is nothing to exclude the condensation of one molecule of hexose and one of pentose to give eleven carbon atom acids, and of the condensation of three molecules to give $C_{15}$ acids; thus giving rise to odd number of carbon atoms, so far not met with in nature.

Armstrong and Allan in a theoretical paper (J. Soc. Chem. Ind. 1924, 43, 216T) also subscribed to this hexose condensation origin of the fatty acids. They suggested the decomposition of a hexose into $C_3$ units (that is $C_6$--- $C_3$) and then the condensation of these units to give rise to higher acids in which oleic acid might be one of the end products. This suggestion of the splitting of the carbohydrate is quite in conformity with our views about carbohydrate fermentation, and as Hilditch (The chemical constitution of natural fats N.Y. 1943, 236) points out "attractive on account of its leaning towards $C_3$, $C_6$ and $C_9$ groups which is clearly observable in the natural unsaturated fatty acids as under.

<table>
<thead>
<tr>
<th>Type Grouping</th>
<th>Acids in which this grouping occurs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_9$</td>
<td>CH$_3$(CH$_2$)$_7$CH = or =</td>
</tr>
<tr>
<td></td>
<td>CH(CH$_2$)$_7$COOH</td>
</tr>
<tr>
<td></td>
<td>Oleic, Linoleic, Linolenic,</td>
</tr>
<tr>
<td></td>
<td>oleostearic, hexadeconic, tetra-</td>
</tr>
<tr>
<td></td>
<td>deconic, ricinoleic, erucic, and</td>
</tr>
<tr>
<td></td>
<td>also cetyl alcohol.</td>
</tr>
<tr>
<td>$C_3$</td>
<td>CH$_2$.CH$_2$.CH =</td>
</tr>
<tr>
<td></td>
<td>Linoleic and Linolenic.</td>
</tr>
<tr>
<td>$C_6$</td>
<td>CH$_3$(CH$_2$)$_4$.CH = or</td>
</tr>
<tr>
<td></td>
<td>CH$_3$(CH$_2$)$_4$.COOH</td>
</tr>
<tr>
<td></td>
<td>Linoleic and Petroselenic.</td>
</tr>
<tr>
<td>$C_{12}$</td>
<td>CH$_3$(CH$<em>2$)$</em>{10}$.CH =</td>
</tr>
<tr>
<td></td>
<td>Petroselenic.</td>
</tr>
</tbody>
</table>
The observations of Emde (Helv. Chem. acta. 1931, 14, 881) and Reichel (Angew Chem. 1938, 51, 190) that fructose is more readily converted to fatty acids than other carbohydrates is of some importance in this connexion.

Haehn and Kintoff (Be. 1923, 56, 437; Chemie der Zelle e Gewebe, 1925, 12, 115) have observed consistent formation of carbon dioxide during the synthesis of fatty acids from carbohydrates and this fact would need the decomposition of the carbohydrate molecule, and militate against the suggestion of the formation of fatty acids by the condensation of the carbohydrates, but it is to be noted that carbon dioxide even in this formation of fatty acids will also be invariably evolved in every case on account of the degradation of the higher acid to lower acid. The mode of this degradation is through B-oxidation, which requires the breakdown of the chain in pairs of two carbon atoms and is a process definitely established in the case of animal life.

Assuming that the higher acid is $\text{C}_{18}$, this degradation will give $\text{C}_{16}$, another very common acid in the plants. Armstrong and Allan (loc.cit.) were willing to accept B-oxidation device for the formation of abundant palmitic acid provided it was accompanied with hexa- or tetra-decenoic acid. Hilditch (loc.cit.) points out a serious difficulty in accepting this B-oxidation mechanism. This process will yield from Oleic acid $7:8$ hexa-decenoic acid and $5:6$ tetra-decenoic acid; these acids are known to have unsaturation at $9:10$, that is at the same place as of Oleic acid. In this connexion Smedley-Maclean's (The Metabolism of fat, 'London', 1943) remarks are significant that palmito-oleic acid ($\text{C}_{16}\text{H}_{26}\text{O}_2$—which is
much more widely distributed than was previously realised) contains its unsaturation at 9:10 that is, the double bond is not centrally placed and therefore Thiele's theory is not applicable. Also Grande (Skand. Arch. Physiol. 1934, 69, 189) has described the occurrence of dehydrogenases in many plant seeds capable of using stearic and palmitic acids as hydrogen donors, and recently the formation of oleic from stearic acid by such an enzyme has been demonstrated, and the position of its double bond established as identical with that of the natural 9:10 oleic acid (Lang and Adickes, Z. physiol. Chem. 1939, 262, 240, 249, 1940, 262, 123), and therefore after this convincing demonstration of formation of oleic from stearic acid through dehydrogenases, the position of the double bond between 9:10 carbon atoms cannot be regarded as throwing any light on the manner of synthesis from carbohydrate.

There is an alternative scheme described below for the synthesis of the fatty acids from carbohydrate in the plants. The main idea of which scheme is the building up of plant fatty acids from substances carrying two carbon atoms in the molecule.

It was suggested by Nencki (J. Prakt. Chem. 1878, 17, 105) that in the formation of butyric acid - a four carbon compound from lactic acid a three carbon compound - acetaldehyde was an intermediate stage. Two molecules of acetaldehyde condensed to give aldol which by a molecular rearrangement gave rise to butyric acid.
Magnus Levy (Engelmann's Archive, 1902, 365) and subsequently Leathee (Problems of Animal Metabolism, 'London', 1906) developed the idea of building up more and more complex aldols by the serial addition of two atoms of carbon.

The formation of an eight carbon atom fatty acid may be explained as under,

\[ 4 \text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{CH(OH)}\text{CH}_2\text{CHOH. CH}_2\text{CH(OH)}\text{CH}_2\text{CHO} \]

this aldehyde by the loss of four molecules of water will give an unsaturated aldehyde, which on reduction and oxidation will give an eight carbon fatty acid \( \text{CH}_3(\text{CH}_2)_6\text{COOH} \).

The objection that the condensation may give rise to branched chain compounds was soon shown unfounded by Harper (J. Chem. Soc. 1907, 91, 1831) by condensing two molecules of aldol to a straight chain carbon compound, and by Smedley (J. Chem. Soc. 1911, 99, 1627) by condensing two molecules of Crotonaldehyde to a straight chain eight carbon compound.

Smedley and Lubrzynska (Biochem. J. 1913, 7, 364) suggested pyruvic acid to be the building unit in the formation of fatty acids from carbohydrates as shown below:

\[ \text{CH}_3\text{COCOOH} + \text{CH}_3\text{COOCOOH} \rightarrow \text{CH}_3\text{CH(CH(OH))CH}_2\text{COOH} \]

\[ \rightarrow \text{CH}_3\text{CCH}_{\text{CHCH}}\text{CH}_2\text{COOH} \]

From this, higher fatty acids, may be built up on similar lines.
It is interesting in this connexion to note that Kostyschev (Zeit. Physiol. Chem. 1912, 72, 130, 359) considered the formation of pyruvic acid and acetaldehyde as intermediate stages in the formation of alcohol from glucose.

\[
\begin{align*}
(1) \quad & C_6H_{12}O_6 \rightarrow 2CH_3CO\cdot COOH + (4H) \\
(\text{ii}) & 2CH_3CO\cdot COOH \rightarrow 2CO_2 + 2CH_3CHO \\
(\text{iii}) & 2CH_3CHO + (4H) \rightarrow 2CH_2CH_2CH
\end{align*}
\]

The recent work of Kuhn and others (J.C.S., 1938, 506) on aliphatic polyenes from aldol like condensation of acetaldehyde and croton-aldehyde in presence of 'piperidine acetate' as a catalyst, has yielded a series of higher vinylene homologues of croton-aldehyde. All the aldehydes condense with malonic acid, yielding compound with one more double bond in the molecule giving mono-carboxylic acid (directly) in the case of lower aldehydes and dicarboxylic acid from higher polyenes (in presence of piperidine only). These dicarboxylic acids are converted to monocarboxylic acid by boiling with acetic acid and acetic anhydride instead of thermal decomposition. The colour in the polyene makes appearance with four conjugated double bonds in the molecule.

The hexa-decaphenatal obtained as deep red needles gave with malonic acid hexadecaphenatal malonic acid.

\[
CH_3(CH\cdot CH)_{7}\cdot CH\cdot C(COOH)\_2
\]

which absorbed eight hydrogen atoms on catalytic hydrogenation and on distillation yielded colourless oil which on solidification was identified as identical in all respects with the natural stearic acid.

This is for the first time a direct synthesis of stearic acid from a two carbon compound and is of great
importance in connexion with the biological synthesis of fatty acids in nature. Kuhn points out that "though we do not know the 'intermediate' occurring in nature, this synthesis may be founded on a catalytic condensation of eight molecules of acetaldehyde in a straight chain. Not only acetaldehyde but its derivatives, and predecessors like pyruvic acid may undergo an aldol condensation. During metabolism, reduction certainly occurs at an earlier stage than in our synthesis, since highly unsaturated fatty acids, which could be detected by their colour, have not as yet been observed".

Some efforts have been made through enzymes to establish the relation between fat and carbohydrate (in vitro) and investigate and elucidate the formation of fatty acids from carbohydrates and other substances and it is interesting to examine them now.

Maclean and Hoffert (Biochem. J. 1923, 17, 720; 1924, 18, 1274; 1922, 16, 370) have investigated the formation of fat in Saccharomyces and have shown that the fat is formed when yeast is suspended in an oxygenated sugar solution; that this sugar is replaceable by the salts of acetic, lactic and pyruvic acids. They have also shown that when phosphate is added to above sugar solution, the formation of fat is greatly increased and the production of fat is more regular in a medium containing fructose than in one containing glucose. From these results the authors emphasise that the formation of a fructose phosphate is a stage in the formation of fat from carbohydrate. In this connexion the observations of Cori and colleagues are interesting "that aerobic phosphorylation of glucose leads in cell free tissue extracts to the
Endomyces vernalis— an organism in which fat content could be raised to a high level, was used as a source of protein and fat food during 1914 war in Germany. Lindner (Woch.brauerei. 1919, 36, 188) has shown that it could assimilate alcohol and that alcohol could serve as a source of fat.

Haehn and Kintoff have shown that the fungus Endomyces vernalis (Ber.deut.Chem.Ges.1923,56,439) is capable of building fat when grown on a medium containing acetic anhydride and even ethyl alcohol as the only source of carbon, and latter (Chemieder zelle u Gewebe, 1925, 12,115) they obtained quantitative results with glucose and determined the percentage of free fatty acids (Oleic 23% to 30%) and their mean molecular weight (235-245) and Iodine value (64'6-105'2).

They grew the organism in media containing 1 to 4% of ethyl alcohol, aldol, glycerol, pyruvic acid, lactic acid and acetaldehyde and found their power to form fat was in the above order.

Stephenson and Whetham (Proc.Roy.Soc.'London' B,1922, 93,262;B,1923,25,200) found that Timothy grass baccillius can synthesise fat from sugar and when the supply of the carbohydrate is exhausted the fat rapidly disappears.

Recently Heichel and Schmidt have repeated and extended the investigations of Haehn and Kintoff on Endomyces vernalis and reported 25% conversion of glucose, fructose or cane sugar to fatty matter and that fructose was an excep-
tionally good source of fat. They also reported that while the higher (8 to 10 carbon atom) saturated aldehyde (octyl or decyl aldehyde) were converted to the corresponding higher saturated acids, the unsaturated aldehyde the hexenal, \( \text{CH}_3(\text{CH}_2)_2\text{CHO} \) and the octatrienal were transformed to higher fatty acids.

The scheme they suggested for the formation of the higher fatty acids from acetaldehyde and other aldehydes is given below:

\[
2\text{CH}_3\text{CHO} \rightarrow \text{CH}_3\text{CH}:\text{CH}:\text{CHO} + \text{CH}_3\text{CHO} = \text{CH}_3\text{CH}:\text{CH}:\text{CH}:\text{CHO} \]

Hexenal

(a) \(3\text{CH}_3\text{CH}:\text{CH}:\text{CH}:\text{CHO} = \text{CH}_3(\text{CH}:\text{CH})_8\text{COOH} \)

Octadecaoctaenoic acid.

(b) \(3\text{CH}_3\text{CH}:\text{CH}:\text{CH}:\text{CHO} \xrightarrow{\text{H}_2} 3\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}:\text{CH}:\text{CHO} \)

\( \xrightarrow{\text{H}_2} \text{CH}_3(\text{CH}_2\text{CH})_3(\text{CH}:\text{CH})_5\text{COOH} \)

Acid abc \( \xrightarrow{\text{H}_2} \) Oleic acid \( \xrightarrow{\text{H}_2} \) Stearic acid.

Further though they have not been able to reproduce Haehn and Kintoff's work with pure glycerol, they have shown that mixtures of glycerol with pyruvic acid, acetaldehyde, croton-aldehyde, hexenal and octatrienal yielded the mixtures of fatty acids and fat. They also state that the components of the product are Linoleic, Oleic and Palmitic acid but do not give any analytical data to support it.

This line of work is important and interesting and as Hilditch (loc.cit) points out should be pursued.

This, then, is the position of our knowledge about the elaboration of fats from carbohydrates in the plant. This knowledge has got gaps yet to be filled in is obvious;
but gradually a picture is emerging and our idea are getting clearer. The hexose condensation hypothesis for the manufacture of plant fatty acids still invites attention and stimulates interest. The natural occurrence of fatty acids with multiples of six carbon atoms is the mainstay of the advocates of this hypothesis. The presence of the dehydrogenases in the seeds of many a plant, capable of converting (in vitro) stearic acid into 9:10 oleic acid, has reduced the importance of unsaturation obtained in the plant fats. The non-existence of the hydroxylated long chain carbon compounds in the plant and the carbon to carbon attachment of carbohydrates, much against the present known mode of their reaction, are two objections against hexose condensation hypotheses of the elaboration of fats.

The initial stages of B-oxidation process as suggested by Hilditch (loc.cit), though not in this connection, may explain the non-existence of highly hydroxylated compounds as shown below:

\[
\begin{align*}
\text{CHOH} + \text{H}_2\text{O} & \rightarrow \text{CH}_2\text{HOOH} \\
\text{CHOH} \rightarrow \text{CH} & + \text{HOOH}
\end{align*}
\]

The carbon to carbon attachment in carbohydrate is difficult to visualise though heterotrophic bacteria (Wood and Werkman, Enzymology Vol. II 1942, 135) capable of fixing carbon dioxide by carbon to carbon bond are now known. A middle course i.e. the resolution of hexose into C₃ units advocated by Armstrong and Allan (loc.cit) appears to be
appreciated by Hilditch because of the prominence which
certain groupings obtained in the naturally occurring fatty
acids, receives on this suggestion.

The elaboration of fats from the decomposition products
of carbohydrate obtained in alcoholic fermentation of sugars
(acetaldehyde Menck, Magnus-Levy and Leathes; pyruvic
acid- Smedley-Maclean and Lubrzynska; crotonaldehyde-Kuhn
and collaborators; ethyl alcohol, acetaldehyde etc. through
Endomyces vernalis - Haehn and Kintoff, Reichel and Schmidt)
has been shown possible experimentally.

The abundant C_{18} acids along with other saturated
and unsaturated acids and cetyl alcohol has been obtained
and therefore, the problem in the main seems to have been
solved. The question now remains why and how different
acids are obtained in different species of plants. Apparently
this difference will finally depend upon the contents of
the seeds which during and after germination elaborate
different carbohydrates finally responsible towards the
close of a cycle of a plant, for different acids in the seeds.
Therefore, along with other studies for obtaining a clear
picture of this mechanism, a thorough examination of the
total contents of various seeds is obvious.
Celery.

Celery is second in importance of the salad crops, in value and popularity being exceeded only by "Lettuce", at one time a luxury, but now a common article in the diet and available throughout the year. It is a plant of marshy places and according to Sturtevant (E. L. Sturtevant-notes on edible plants N.Y. State Sta. Rept., part II, 1919) "its habitat extends from Sweden to Algeria, Egypt, Abyssinia and in Asia even to the Caucasus, Baluchistan and the mountains of India. It has been found growing wild in Terra del Fuego, in California and in New Zealand. The wild plant was probably used for medicinal purposes for hundreds of years before it was used as a food. There is no evidence that it was grown by ancients as a food plant, but if it was planted at all, it was for medicinal purposes. The first mention of its cultivation as a food plant was in 1625 in France. The first cultivated celery differed little from the wild plant".

Celery- "Apium graveolens" is a biennial plant, although grown as an annual crop. Under some conditions the plant behaves as an annual, developing flowers and seeds during the first year. Celery belongs to the family "Umbelliferae". The flowers are small, white and are borne in compound umbels among the leaves of the flower stalk, which grows to the height of 2 to 3 feet. Celery thrives best when the weather is relatively cool and rainfall moderate and well distributed during the growing season. It needs a long growing season. Distinct varieties are not so numerous nor so clearly separable from each other as is the case with
most of the vegetables.

According to Thompson there are not more than twenty American varieties that are distinct enough to justify separate naming. (Thompson: Vegetable crops, 232, Ithaca, 1939).

Both heredity and environment are involved in quality. It is well known that some varieties are of better quality than others and it is known also that the conditions under which the crop is grown affect quality. Celery to be classed as of good quality, must be crisp and tender and have a pleasant, sweet, nut like flavour. Some varieties possess all of these qualities, but those most commonly grown fall far short of meeting the requirements. Flavour seems to be influenced more by variety than by environmental factors.

In India the popular name is Karaffs and the Hindi name is Ajmanda and according to Watt (Watt: The Commercial Products of India, 72, 1903) is grown as a garden crop chiefly for the use of Europeans in the vicinity of the towns. Karaffs is abundantly mentioned and utilised in the Unani (Greek) system of medicine. They mention that each country has got its own variety but chiefly they classify it under the following five heads:

(i) Garden.
(ii) Hilly.
(iii) Rocky.
(iv) Herbaceous,

and (v) Aquatic.

and they utilise mostly the garden variety. They mention its uses as a deobstruent and resolvent, as a tonic, as a pectoral, as a diuretic and carminative adjunct to purgatives
(Dymock, Pharmacographia Indica, 1883). The officinal root is considered alterative and diuretic and given in anasarca and colic. The seeds are also used as stimulant and cordial. Its use is also mentioned for liver and spleen diseases.

Bengal and the Panjab cultivate it for its seeds and roots respectively. Locally seed is used as a spice while branch, stem and leaf stalk are used by Europeans. Roots are used in a number of preparations such as stew, soup, etc. (Nandkarni, Indian Materia Medica). In the wild state it is poisonous to a certain degree. (cf. Forstel.Pr.Soc.1786,67; Paulus.Aegineta'Adams,Communt' iii, 106; Pharmacog.md.ii, 122-24, Queensland.Agri.Jour.1903,xiii,237,U.S.Dept.Agri, Fowness Bull, 1902, No. 148).
Chemical work on Celery.

So far as it could be ascertained the only mention of a glucoside in celery (English equivalent of Indian Karaffs) is in 1843 (Ann. Chim. Phys. 1843, 'iii', 9, 250), as reported by Chopra (Indigenous Drugs of India, 1933, 463). There appears no subsequent work on this point, though profuse mention of a glucoside, apiin, from celery seed is made in the literature.

Apiin is the name given to a gelatinous substance, obtained, both by Rump (Buchner Report, f. Pharm. 1836, 6, 6) and Braconnot (Ann. Chim. Phys. 1843, 'iii', 9, 250) from the stem, leaves, and seeds of parsley.

Planta and Wallace (Ann. 1850, 24, 262) obtained apiin as a colourless mass (M.P. 180°C) and assigned to it the formula \( C_{24}H_{28}O_{13} \); the brown product obtained from this (apiin) by acid (dilute) hydrolysis was represented by the formula, \( C_{24}H_{20}O_{9} \).

Subsequently Lindenborn (Inaugural Dissertation, Wurzburg 1867) obtained apiin for the first time in a crystalline form (M.P. 228°C; C, 53.57%, H, 5.35%) and found that on acid hydrolysis apiin yielded glucose and apigenin (M.P. 343°C; Found C, 66.13%, H, 3.9%) and gave to apiin the formula \( C_{12}H_{14}O_7 \). He suggested that the acid hydrolysis of apiin, probably proceeded as under:

\[
\begin{align*}
C_{12}H_{14}O_7 \cdot H_2O & \rightarrow C_6H_4O_2 \cdot C_6H_12O_6 \\
\text{Later Von Gerichten (Ber. 1876, 9, 1124; Annalen 318, 124)}
\end{align*}
\]

investigated apiin (Merck's) and showed that on alkaline fusion it gave phloroglucinol and an acid not closely examined;
but from which, under more energetic conditions of reaction, proto-catechuic acid and some oxalic, formic and p-hydroxy-benzoic acids were obtained. As a result of this investigation he considered apigenin to be represented by \( C_{15}H_{10}O_5 \) and apiin by \( C_{27}H_{32}O_{16} \), yielding glucose and apigenin on acid hydrolysis as below.

\[
C_{27}H_{32}O_{16} + H_2O \rightarrow C_{15}H_{10}O_5 + 2C_6H_{12}O_6
\]

Subsequently von Gerichten (1901, loc.cit.) found that regulated acid hydrolysis of apiin (0.5% sulphuric acid and half an hour's refluxing) yields apirose (identified by conversion into an osazone M.P. 145-55°C) and glucoapigenin (M.P. 215-220°C; \( C_5H_{32}O_6 \), 38.48, 59.60%, H, 4.73, 5.00%) and he suggested the formula \( C_{26}H_{28}O_{14} \) for apiin. Glucoapigenin on prolonged acid hydrolysis decomposed into glucose (identified through its osazone) and apigenin. The acid hydrolysis proceeded as shown below.

The action of Nitric acid on apiin yielded picric and oxalic acids only.
Perkin (Proceedings, 1897, J.C.S., 1897, 805; Perkin and Everest. The Natural Organic Colouring Matters, 1918, 144) who examined aphin (Merck's), mentions a method for obtaining it in a colourless mass of fine, silky needles, from a boiling, saturated, water solution. His values for carbon and hydrogen contents of aphin agreed with those of Lindemborn and Von Gerichten (loc.cit.).

Perkin (loc.cit.) digested apigenin in a boiling solution of caustic potash and obtained from it two products Phloroglucinol (M.P. 210°C) and p-hydroxy acetophenone (M.P. 107°C). This latter compound further degraded to yield p-hydroxy benzoic acid (M.P. 209-10°C), but no protocatechuic acid, as observed by Von Gerichten, was formed.

Perkin (loc.cit.) methylated and ethylated apigenin and obtained the di-ethers only. These di-ethers on acetylation yielded mono-acetyl di-ethers, thus establishing the presence of three hydroxyl groups in apigenin, and also, that one of them is in the ortho position to carbonyl group. He also degraded through alkaline fusion these di-ethers and from a similarity and correspondence of the reactions of apigenin with those of Chrysain, C_{15}H_{10}O_{4}, which had already been given the formula of a di-hydroxy flavone, by Kostanecki (Ber; 1893, 26, 2901), Perkin assigned to apigenin the formula of a hydroxy chrysain to explain the various reactions.

Perkin (loc.cit.) mentions in connexion with the hydrolysis of aphin that this "in comparison to most
glucosides is but slowly decomposed by acids "and therefore he raised the time of digestion with dilute hydrochloric acid from ten hours (Von Gerichten loc. cit.) to twenty hours for the completion of reaction. Even then he obtained apigenin in only 40% yields of the apiin employed, " an amount considerably less than that indicated by theory". The loss in the yield he attributes to the brown product simultaneously produced with apigenin in the above reaction. In support for the long time necessary to hydrolyse apiin to apigenin he records carbon and hydrogen values for samples of apigenin analysed at various stages of the operation.

In connexion with protocatechuic acid which Von Gerichten (loc. cit.), obtained, and which Perkin (loc. cit.) could not reproduce; Von Gerichten subsequently (Ber. 1900, 33, 2334) found was due to his apiin (Mercks preparation) being contaminated with a glucoside of luteolin monomethylether, which he found was present in the leaves and stems of Parsley.

Later apigenin was synthesised by Czajkowski, Kostanecki and Tambor (Ber. 1900, 33, 1896) and the above formula assigned by Perkin was supported.

Power and Browning (J.C.S. 1914, 105, 1833) working on the flowers of Anthemis nobilis obtained a compound M.P. 178-80°C, which from a consideration of the composition of its acetyl-derivative and the hydrolytic products was found by them to be a glucoside of apigenin. This glucoside carried three molecules of water, two of which could be removed by prolonged heating at 125-130°C or drying over calcium chloride, but the third molecule of water could not be taken
away without rupturing the molecule. Therefore, they formulated glucose apigenin as \( \text{C}_{21}\text{H}_{20}\text{O}_{10}\cdot\text{H}_2\text{O} \). (Found C, 55.8\%; H, 5.0\%; 5.0\%; \text{C}_{21}\text{H}_{20}\text{O}_{10}\cdot\text{H}_2\text{O} \) requires C, 56.0\%; H, 4.8\%.

These authors also repeated Von Gerichten's (loc. cit.) regulated hydrolysis of apilin (commercial) and obtained only a product M.P. 192-195\(^\circ\)C and not the product M.P. 215-220\(^\circ\)C and therefore, they considered that Von Gerichten's product M.P. 215-220\(^\circ\)C was probably mixed up with the higher melting compound apigenin (M.P. 343\(^\circ\)C). Therefore, these authors claimed that the glucoside of apigenin M.P. 178-180\(^\circ\)C, which they have obtained is a new glucoside. Tasitro Nakaoki (J. Pharm. Soc. Japan 60, 502-6; Abstracts in English' 190-91) working on the yellow white flowers of the double variety of Zinnia, obtained in 12 yields, yellow crystals, which on recrystallisation from methyl alcohol-acetone mixture, gave M.P. 218-20\(^\circ\)C. From the results of the hydrolysis and the analytical values of this compound he assigned to it the formula \( \text{C}_{21}\text{H}_{20}\text{O}_{10}\cdot\text{H}_2\text{O} \) (a glucoside of apigenin). The anhydrous compound decomposed at 227-236\(^\circ\)C. The substance recrystallised from pyridine also decomposed at 226-227\(^\circ\)C.

Nagai (J. Agr. Chem. Soc. Japan 1341, 17, 483, Bull. Agr. Chem. Soc. Japan 17, 50 (in English) mentions the occurrence of 7-3-4'-trihydroxyflavone-7-glucoside in the leaves and stems of Euphorbia thymifolia, but no other details about this work are available.

Compared to the glucosides in the celery, the essential oils in the plant attracted much attention of the investigators in most countries.
Thus it has been the subject of a number of papers in Germany from 1892 onwards (The Volatile oils -Gildemeister and Hoffmann 1922, 318); in England it has been examined by Swenholt (Midland Drug and Pharm. Rev. 1910, 44, 220); in France by G. Igolam (Parfumes France 1937, 15, 219, 28; Parfume de France 1936, 14, 12); and in America by Cavanger (Bull. Natl. Ferniaury, Comm. 1839, 7, 231-233, 293-294).

Some of these investigators have also paid attention to the oil from the roots and leaves of this plant. The wild South African variety (Pencedanua Galbanum) was examined by Juritx (Chem. News, 1923, 126, 67)

The alcohols in the celery oil have been investigated by Huxicks and Stoll (Helv. Chim. Acta, 1923, 6, 846), but no mention appears of any work on karaffs seeds (Indian garden celery).

In the main the conclusions (Gildemeister and Hoffmann loc. cit.) are that the essential oil in the seed is about 2'5 to 3'0% and that it is made up of the following constituents.

1. Hydrocarbons 70%. These consist of d,Limonine (60%) and d,Selinene (about 10%)
2. Alcohols 2'5 to 3'0%.
3. Sedanolide 2'5 to 3'0%.
4. Sedanonic acid anhydride 0'5% (pure), and Residua 10'0%

The amount of the oil is least in the leaves (0.1%) and most (2.5 to 3.0%) in the seeds.
Freshly cut herb does not give as good a yield of the oil as the one which has been kept a little time.

The fixed oil from celery was investigated by Clemens Grimm (Bot. S. Inst. Hamburg, Pharm. Zent. 1911, 52, 661) who obtained it from the seeds through ether extraction in 16.7% yields and recorded its physical constants, and more fully by Christian and Hilditch (Biochem. J. 1929, 22, 327) who mentioned the occurrence of palmitic acid 3%, petroselenic acid 51%, oleic acid 26% and linoleic acid 20% in the seed oil.

They found unsaponifiable matter 2.2%.

During recent time celery has attracted the attention of investigators mostly in America and a number of papers on sugars, pectin contents, insulin, vitamins and other nitrogenous bodies in the celery roots, leaves and seeds have appeared. Some of these papers are purely agricultural studies while others are biological assays of the various constituents of roots, leaves and seeds of celery.

So, the present position is, that apart from the oils (essential and fixed) of the celery seeds, we know that it contains apiin. This apiin (from parsley seeds) is reported to have been obtained as a colourless mass M.P. 178-180°C, and in crystalline form M.P. 228°C. This crystalline structure was observed under microscope. Von Gerichten and Perkin, who investigated apiin and its compounds do not mention its melting point, but quote Lindenborn's M.P. 228°C. Hydrolysis of apiin gives considerably low yields of apigenin compared to that required by theory and mixed up with a brown product.
Two glucoseapigenins are recorded in literature. One by Von Gerichten M.P. 215-220°C and supported by Tasitro, who obtained a glucoseapigenin from Zinnia M.P. 215-220°C. This work of Von Gerichten on glucoseapigenin on repetition by Power and Browning did not repeat the product M.P. 215-220°C, but yielded a product M.P. 191-195°C. The second glucoseapigenin recorded is from Anthemis nobilis, by Power and Browning M.P. 173-180°C. It is noticeable that all these glucoseapigenins melt over a range of 3° to 5°C. Further, apin as well as all these glucoseapigenins are reported to crystallise out with varying molecules of water. Some of this water can be dehydrated over calcium chloride or removed by prolonged heating at 110 to 125°C, but one molecule of water can not be so removed from them without rupturing the molecule.

From the above account it will appear that no systematic and quantitative work (apart from oils) has been done on celery seeds. Our knowledge of a definite nature of the other constituents of the celery seeds is indeed very little. No work of any nature seems to have been done on Karaffs seeds (Indian celery). These seeds, though at one time imported, are now considerably grown in the country and therefore, it appeared of some importance and interest to investigate the seeds.

Work on Karaffs Seeds (Indian Celery).

Karaffs is abundantly grown round about Aligarh and therefore is easily available and the maintenance of the uniformity of the material analysed possible. The local Karaffs seeds considerably differ from the Bengal and the Panjab varieties in colour and taste.
The moisture and the ash content of the seeds were determined and found 2.5% and 12% respectively.

The inorganic elements found in the ash were calcium, magnesium, iron and aluminium.

The extractable matter from the seeds by the following organic solvents was found as noted below:-

- Ether 14.3%
- Petroleum ether 11.5%
- Carbon tetrachloride 14.9%
- Alcohol (95%) 13.0%

The percentage yield of the extract was maximum in the case of alcohol and minimum in the case of Petroleum ether. Experience showed that alcohol was a better solvent for the solid constituents of the seeds than water.

Essential Oils from the Seeds:

The seeds (500 g) were taken in a specially constructed round bottom, short neck, copper flask, tinned inside and steam(superheated, 120-125°C) distilled. The oil was collected from the distillate by ether extraction, purified and weighed (11.25 g). The yield obtained was 2.25% (Gildemeister and Hoffmann, loc.cit., 2.5 to 3.0%; Swenholt loc.cit. 2.44%).

The oil (13.53 g; $D_{28}^4 0.8426$) after the usual purification was fractionally distilled under reduced pressure (2 mm) and three fractions of the oil and a residue obtained.
The residue in the flask was acidic to litmus and was not further worked up.

Fraction 1, when redistilled at atmospheric pressure (74.65 cms) came over 174-175°C. Its specific gravity was found to be $d_{18}^{0} = 0.8495$. It yielded a dihydrochloride M.P. 48°C and two tetra-bromides M.P. 103°C and M.P. 105°C. These were very distinct in their crystalline (needles and plates) form. Its molecular weight as found by the boiling point method was 134°9 (Limonene, B.P. 174-175°C, dihydrochloride M.P. 49-50°C, tetrabromides M.P. 104-105°C and molecular weight 136).

The second fraction redistilled at atmospheric pressure (74.65 cms) came over a range of 6°C from 263°C to 269°C and was apparently a mixture. However, it yielded a dihydrochloride M.P. 72°C in good yields. Its molecular weight by the boiling point method was found to be 208. On combustion it gave C, 87.77%; and H, 11.53% (Selinene requires M.W. 204; C, 83.23%; H, 11.77%; and yields a dihydrochloride M.P. 72-74°C).

The third fraction being very small could not be distilled at atmospheric pressure and attempts to obtain derivatives from it did not succeed.
It was therefore, concluded that karaffs seeds contain 2.25% essential oil. The distillable fraction from this oil is 79.0%. Limonene (presumably both d,l,forms) occurs in the oil in 45.4% yields (Gildemeister and Hoffmann, loc.cit. 60.0% d-limonene).

Also that selinene occurs in the oil (fraction 2, 22%) in some quantity.

The residue is acidic to litmus and is 21.0% of the essential oils.

Fatty oils.

The fixed oil mixed up with colouring matter and essential oil was extracted with petroleum ether (60-80°C) from the seeds in 11.5% (average) yields. The extract was non-acidic in character and green in colour. This was first clarified by thoroughly agitating it with a thick paste of zinc chloride (obtained by leaving zinc chloride in an open dish overnight). The Zinc chloride was then washed off with water (distilled) and the oil mixed up with a 1% caustic soda solution (freshly prepared), and thoroughly shaken in a separating funnel. On separation of the oil from caustic soda solution, it was well washed free of the alkali. The oil was finally clarified by alcohol (obtained by distilling rectified spirit over lime) in which the oil did not seem to dissolve appreciably. The oil on separation from alcohol was light yellow in colour and was freed of alcohol by first warming on a water bath and thereafter exhausting it in a vacuum desiccator for a couple of days. Yield 8.6 to 9.0%. 
In this manner a total quantity of 350 gms. of the oil was collected. This was colourless transparent oil and was neutral to litmus. It gave refractive index 1.4738, Iodine value 95.4, Saponification value 238.3 and Specific gravity $d_1^{18}0.9275$ (Grimme loc. cit. green brown oil in 16.7% yields, congealing point 12°C, Refractive index $n_{d}^{35}1.4785$, Iodine value 94.8 and Specific gravity $d_{15}^{15}0.9236$).

The oil (300 g) was then saponified with alcoholic potash (potassium hydroxide 180 g, alcohol 1.51, 95%) by refluxing over a period of six hours. Most of the alcohol was then recovered and the soaps dissolved in water (excess). Non-saponifiable matter 2.5% (Hilditch loc. cit. 2.9%; Grimme loc. cit. 0.79%) was then removed by continuous extraction with ether (Hilditch's method). The aqueous layer on acidification with sulphuric acid (40%) liberated the acids, which were taken up in ether and washed free of the mineral acids. On recovery of ether, mixed fatty acids were obtained, in 83.3% yields. (Grimme loc. cit. 93.0%).

The mixed acids (250 g) were then separated into solid and liquid acids by lead salt-ether method and gave the solid acids (130.5 g) and liquid acids (119.5 g).

The solid acid fraction on keeping deposited a crystalline acid, which on recrystallisation gave M.P. 61°C, showing it to be palmitic acid. This was confirmed by a determination of the combustion values of the acid.

Found C, 74.76%, H, 12.02%. Palmitic acid $C_{16}H_{32}O_2$, requires C, 75.00%, H, 12.57%.
The solid acids (5g) were converted into soaps and treated with alkaline (N/2 caustic soda) potassium permanganate at 4°C. In the usual manner this gave rise to a colourless crystalline solid product which on recrystallisation gave M.P. 121-122°C.

The liquid acids (5g) when similarly treated gave rise to a product which could finally be separated (by fractional recrystallisation) into two products M.P. 170°C, and M.P. 123°C. These acids according to Hilditch (loc.cit) showed the presence of petroselinic, oleic, and linoleic acids.

Each group of the acids was then separately converted into its methylesters, but further work on these could not be taken up.

Examination of the unsaponifiable matter.

The unsaponifiable matter (75g) was dissolved in methyl alcohol and cooled in a freezing mixture, when a white flocculent precipitate was found. This was filtered while still at low temperature and on several recrystallisation from absolute alcohol had M.P. 69-70°C. (Found Carbon 84.9%, H, 14.4%).

The filtrate from the above was reduced in bulk and on treatment with digitonine in the usual manner gave a digitonide (3.80g). The digitonide (0.45g) was taken in acetic anhydride (25 c.c. with a trace of zinc chloride anhydrous) and refluxed for a couple of hours when it was transferred to another vessel. On cooling crystals gradually separated, but this was left overnight for complete separation of the crystalline product. The product was repeatedly
recrystallised from absolute alcohol and finally gave an acetyl derivative M.P. 123-124°C.

**Resinous part.**

The seeds (200g) after the removal of oils and colouring matter through petroleum ether were exhausted with either ether or carbon tetra-chloride. In each case the extract (4.2%, average) was brown resinous product and acidic to litmus and therefore it was treated with Caustic soda (N/2) and separated into acid resinous product and neutral resinous product. Attempts to obtain from either part a well-defined solid have not so far materialised (11 months).

**Alcoholic Extract.**

Thoroughly defatted seeds from the above operations were exhausted with successive portions (fresh) of alcohol, till the alcoholic extract was colourless. Usually with seeds (500g), alcohol (2.5 litres) in two portions, refluxing over a total period of sixteen hours, was enough for this purpose. The combined alcoholic extracts were then partially reduced in bulk at the water pump and on standing a couple of days, gave a gelatinous mass. This was filtered at the pump and the mass well pressed on the buchner funnel and kept aside marked (A). Yield 14.7g i.e. 2.94%.

The filtrate on further partial concentration and keeping overnight deposited a crop of colourless crystals. These were filtered and labelled (B). The yield was 4.68g (0.92%). The filtrate at this stage was deep red in colour and was further concentrated in a dessicator at the water pump. A test sample from this filtrate readily reduced Fehling’s solution and Ammonical Silver nitrate solution.
It gave positive molisch's test and an osazone M.P. 205-206°C, identical with a genuine sample of glucosazone (mixed melt, 204-205°C). This established the presence of glucose. Attempts were made to separate glucose (completely) by further concentration of the filtrate and by keeping over weeks in a dessicator under vacuum but only partial deposition of the sugar occurred. The concentrate which was practically a syrup at this stage was totally insoluble in petroleum ether, ether and benzene but soluble in chloroform with difficulty. The total concentrate was therefore, treated with phenylhydrazine in acetic acid and glucose separated. The filtrate was extracted free of the phenylhydrazine (through ether and benzene) and then concentrated under vacuum, but so far no definite solid product has separated. When tested for phosphorus, nitrogen and sulphur, it gave positive tests for these elements. Nitrogen appears to be in some quantity. A little of this concentrate when heated in a crucible, burnt with a smoky flame and left an ash, which shows the presence of calcium and magnesium.

Examination of the gelatinous mass (A).

A little of this gelatinous mass when melted on a nickel spatula, left a residue and therefore a quantity (1g) was taken in a crucible and ignited. The ash on analysis gave the presence of both aluminium and iron.

The entire lot of the gelatinous mass (13.0g) was then dissolved in alcohol (warm-excess) and filtered. The solution was then treated with lead acetate (alcoholic) drop by drop with constant stirring. The lead-salt precipitate obtained with the first few c.c.s. of the lead acetate solution
was rather ill-defined and sticky and therefore filtered off. Further addition (dropwise) of the lead acetate solution produced bright yellow precipitate with a strong suggestion of red in it. This dropwise addition of lead acetate was continued till a portion of the solution tested with ferrous sulphate (freshly prepared solution) developed a pink or red colour. The precipitate was well shaken, allowed to settle down, and then separated on a buchner funnel. The precipitate on the funnel was well washed with alcohol and the washings added to the filtrate. The precipitate was suspended in alcohol and the lead salt decomposed with hydrogen sulphide. Hydrogen sulphide was carefully added to avoid large excess. This solution was filtered off and the filtrate which was orange coloured, warmed to boiling on a water bath to expel hydrogen sulphide and kept aside. This on standing deposited clusters of shortneedles slightly yellowish in colour, which were separated from the solution and recrystallised from alcohol gave M.P. 245-246°C. This melting point even on four or five recrystallisations with ethyl and methyl alcohols could not be improved. The mother liquors gave a further crop of crystals on concentration. Total yield obtained was 3.65g.

The filtrate from the above lead salt on further addition of lead acetate (dropwise) and constant stirring of the solution gave a clear yellow precipitate which was separated and suspended in alcohol and decomposed with hydrogen sulphide. The lead sulphide was filtered, and the solution which was of light yellow (straw) colour, warmed.
to boiling, to expel hydrogen sulphide, and kept overnight, deposited straw colour dull short needles, M.P. 211-212°C. This melting point on recrystallisations could not be raised.

It has been observed that the substance M.P. 211-212°C is not completely precipitated by lead acetate solution, and a further quantity of this substance is obtained from the gelatinous mass, which is produced on the addition of water to the filtrate after the separation of the yellow lead salt.

Therefore, in subsequent work the filtrate at this stage was taken in a distilling flask and reduced in bulk by warming on a water bath under reduced pressure (water pump). The moment a solid made its appearance, the whole was poured into a large beaker and allowed to cool to 40-50°C, when it was filtered at the water pump. The precipitate was suspended in alcohol and hydrogen sulphide added (slight excess). This was warmed to expel hydrogen sulphide and filtered. The clear light straw colour filtrate on keeping deposited a crop of crystals M.P. 211-212°C.

The filtrate (from the cooled solution) on the addition of water gave colourless gelatinous precipitate.

This was collected on a buchner funnel and dried. On ignition, it left a considerable residue, which on analysis gave the presence of Aluminium and Iron.

The filtrate did not seem to contain anything further.

This substance M.P. 211-212°C is sparingly soluble in warm ethyl alcohol, but readily soluble in warm methyl alcohol and develops a deep pink when an alcoholic solution of this substance is added to a solution of Ferrous sulphate (freshly prepared).
Examination of the Product B.

The product was recrystallised from alcohol in which it was very sparingly soluble and gave M.P. 165-166°C. The mother liquor deposited a further crop of crystals, but it appeared from the appearance that they were of different form. Repeated attempts to separate these two forms of crystals only resulted in raising the M.P. to 166-167°C. It appears that either the quantity of one of them is very small or else they are different forms of the same substance with very close melting points, a point which could not be decided in the present investigation.

The combustion of the product gave the following values.

Found C. 39.23%, H, 7.45%

A determination of the molecular weight by boiling point method gave for it the value 186.

The above values and the melting point suggest the compound to be a hexahydric alcohol and probably mannitol.

A little of the substance (0.32 g) was then acetylated with acetic anhydride (freshly distilled -20 c.cs.) and a pinch of Zinc chloride (anhydrous) by warming on a water bath for a couple of hours. It was thereafter poured into water and well shaken where upon it gave a colourless product. This was collected and recrystallised from alcohol and had M.P. 121°C. From the above it is clear that the compound is mannitol and occurs in the seeds in 0.92% yields.
Treatment of the thoroughly defatted and alcohol extracted seeds with water.

The exhausted seeds (100g) were taken in water (500 c.c.) and boiled on a sand bath for an hour and then filtered. The filtrate was then mixed up with acidulated alcohol (1 litre). A gelatinous precipitate was obtained. This was again taken in water (200 c.c.s.) and dissolved by boiling, and reprecipitated with acidulated alcohol (400 c.c.s.). The precipitate obtained was dried and weighed. (Yield 1'1g).

A solution of this product in water gave precipitates with copper sulphate and lead nitrate, but no precipitates with ferric chloride and barium chloride.

Water extract of the fresh seeds.

The powdered seeds (2 lbs.) were exhausted with boiling water (twice; in two lots of 4 litres). On cooling it deposited a brown crystalline mass. This was filtered on a piece of cloth and washed with cold water and dried. It yielded a brown product (about 23-25 g). This was taken up in large quantity of alcohol (by warming) and then allowed to cool. The colour of the solution was brown and thick and it deposited a solid on standing. This was filtered off. The clear brown solution was then treated drop by drop with lead acetate (alcoholic) with constant shaking and gave a reddish-yellow ill-defined precipitate. The precipitate obtained with the first few c.c.s. of Lead acetate was filtered off. This precipitate when in the usual manner tested for a tannin failed in the vital tests to show its presence. Further drop wise addition of the Lead acetate solution gave reddish yellow precipitate. From this solution in the manner
described earlier (alcoholic extract gelatinous mass) two and only two products M.P. 211-212°C and M.P. 245-246°C could be obtained. In fact the behaviour of the solution about gelatinisation was the same as in the case of alcoholic extract (gelatinous mass).

Supernatant liquid from the steam distillation of the seeds also behaved in the manner described above and gave the same two products M.P. 211-212°C and M.P. 245-246°C.

The yield of these two products was practically identical with that obtained in the case of alcoholic extract of the defatted seeds. The only difference was that in this case mannitol and other water soluble products were lost.

If the seeds are very finely powdered and then water extracted, much oily product finds its way with the solid product.

Examination of the product M.P. 245-246°C.

The compound was analysed for Carbon and Hydrogen values and gave C, 50.84% and H, 4.78%.

Hydrolysis of the product.

The product (5.40g) was hydrolysed with hydrochloric acid (acid 100 c.cs. and water 900 c.cs). The hydrolysis went smoothly and gave a coloured precipitate (2.12g) which twice recrystallised from alcohol had M.P. 327-328°C (Perkin, loc.cit. Luteolin M.P. 327-329°C; Kostanecki and collaborators Ber, 1900, 33, 3410; Luteolin M.P. 327°C). This on combustion gave carbon 62.89% and hydrogen 3.73% while Luteolin C₁₅H₁₀O₆ requires carbon 62.94% and hydrogen 3.49%.
Acetylation of the product M.P. 327-328°C.

The product (0.31g) was taken in freshly distilled acetic anhydride (30°C c. c.) with a few crystals of Zinc chloride (anhydrous) and refluxed for a couple of hour, and the product so obtained poured into water and allowed to cool. A white solid separated and was filtered. This was recrystallised from alcohol and gave M.P. 218-219°C (Perkin, loc.cit., Tetra-acetyl luteolin M.P. 221-223°C; Herzig, Ber, 1896, 29, 1013, tetraaceetyl luteolin M.P. 225-227°C).

Methylation of the product M.P. 327-328°C.

The product (0.250g taken in 2g caustic soda dissolved in 10 c.c.s. methyl alcohol) was refluxed with methyl iodide (15 c.c.s. excess) for thirty hours (Perkin's method). The excess methyl iodide and some methyl alcohol were then recovered by distillation and the remaining product poured into water. The precipitate that was formed was filtered and taken into ether and washed with dilute alkali (0.5%). The ether solution was washed free of the alkali and dehydrated over sodium sulphate (anhydrous), gave on removal of ether, a crystalline product which on recrystallisation from ether had M.P. 160-161°C (Perkin, loc.cit., luteolin trimethylether M.P. 161-163°C).

Bromination of the product M.P. 327-328°C.

A little of the product was made into a paste with acetic acid (glacial) in a pestle and mortar and transferred to a small beaker with the help of a little more acid and treated with bromine (dropwise), till a permanent red colour was obtained. This was kept over the week-end, whereupon lemon yellow (colour) crystals separated which
were recrystallised from alcohol (absolute) and melted at 237°C (Perkin, J.C.S. 1896, 209, dibromomelitrate M.P. 303°C). The mother liquors on removal of the solvent gave a solid product (reddish yellow) which on recrystallisation from alcohol (absolute) gave M.P. 286°C.

Nitration of the product M.P. 327-328°C.

The nitration of this product yielded only oxalic acid (Roehedler, Zeitsch. fur Chem, 1836, 602).

Alkaline fusion of the product M.P. 327-328°C.

The alkaline fusion of the stuff gave two products one melting at 197-198°C and the other at 208-209°C, identical (mixed melt) with protocatechuic acid and phloroglucinol respectively. The product M.P. 197-198°C was obtained in some quantity while the other product was in small amount.

Examination of the filtrate from the hydrolysis of the product M.P. 245-246°C.

The filtrate from the hydrolysis of the product M.P. 245-246°C readily reduced both Fehling and ammonial Silver nitrate solutions, and yielded an osazone M.P. 206-7°C, identical with glucosazone (mixed melt 206°C).

Partial hydrolysis of the product M.P. 245-246°C.

A regulated hydrolysis of this product, with sulphuric acid (0.5%) and half an hour’s heating on a water bath and leaving overnight yielded a very pale yellow crystalline product which recrystallised from alcohol gave M.P. 256-257°C.

The filtrate readily reduced Fehling’s solution and on separation of the acid through lead acetate and concentration under vacuum gave a syrup. This syrup was taken in water and added to phenylhydrazine (1g) in acetic acid (5 c.c)
and gave on warming (half hour) an osazone which recrystallised had M.P. 205-206°C and was identical with glucosazone.

From the above work on the product M.P. 245-246°C it will appear that the analytical values correspond to its being a diglucoside of luteolin crystallising out with one molecule of water, which is not separable by heating at 110-130°C. It gives C, 50.84%; H, 4.78%; luteolin diglucoside C_{27}H_{30}O_{16}.H_{2}O, requires C, 51.59%; H, 5.09%. The hydrolysis of the product gives luteolin, confirmed by its melting point, combustion values, formation of tetra-acetyl luteolin, luteolin trimethyl ether and the products of alkaline fusion. However, it gives two bromides M.P. 237°C and M.P. 286°C, and none of these bromides correspond in melting point to luteolin dibromide as recorded by Perkin (loc. cit. M.P. 305°C), though the bromination was done according to his method (J.C.S. 1896, 206). It appears that in the present case the bromination has taken a different course to that of Perkin. This point is being further investigated.

Partial hydrolysis of the product (repeated twice) yielded a glucoside M.P. 256-257°C which on further hydrolysis gave glucose (confirmed through its Osazone) and luteolin M.P. 327-328°C.

So far as could be ascertained, there is no mention of a diglucoside of luteolin in literature, and therefore, the product M.P. 245-246°C, obtained from karaffs seeds and shown in the present work to be luteolin diglucoside is a new compound. The diglucoside residue on the analogy of apiin, appears to be attached in position 7, as shown below:
Luteolin-7-diglucoside.  Luteolin-7-glucoside

The partial hydrolysis of this diglucoside yields a glucoside, the melting point of which is 256-257°C. The only known glucoside of luteolin, galuteolin, from Galega officinalis (Barger and White, Biochem. J. 1924, 17, 836) and from Equisetum arvense L. (Nakamura and Nukuti, J. Pharm. Soc. Japan 60, 449-53 (in English, 179-180) (1940) has been given the constitution of luteolin 5-glucoside and melts at 260-3°C and not at 256-257°C, as the present glucoside. This further lends support to the attachment of the diglucoside residue in position 7 in the molecule.

Examination of the product M.P. 211-212°C.

The product after drying at 125-130°C for a couple of hours was analysed and gave C, 55.22%; 53.33%, H, 4.80%, 4.61%; Apin, C11H6O3 requires C, 53.60%, H, 5.15%.

Regulated hydrolysis of the product M.P. 211-212°C.

A regulated (partial) hydrolysis of the product (1'0g) according to Van Gerichten (loc. cit. 0.5% sulphuric acid and half an hour's refluxing) was made and the mixture left overnight, deposited a yellowish product (about 0.7g). This was recrystallised from alcohol and obtained as slightly yellowish, short needles, M.P. 234-235°C.

The filtrate reduced Fehling's solution and gave positive Molisch test and therefore the above hydrolysis was repeated with a large quantity (4'5g) of the stuff. It again gave the same product M.P. 234-235°C, which after usual
purification and drying at 125-130°C gave on analysis, 
C,55'69%,H,4'52%, glucoapsigenin C_{21}H_{20}O_{10}.H_{2}O requires 
C,56'00%,H,4'88%.

The filtrate was concentrated to a syrup, after 
removing the acid by baryta solution, under reduced pressure, 
acid and added to phenylhydrazine in dil.acetic with a little 
water. This was warmed on a water bath for a couple of hours. 
On cooling it deposited a few crystals M.P. 144-147°C which 
after two recrystallisations gave M.P. 153-155°C (Von 
Hydrolysis of the product M.P. 234-235°C.

The product (1g) was taken in water (boiling 200 c.cs.) 
and acid hydrochloric (20 c.cs) added to it. The whole was 
refluxed on a sand bath for three hours. The hydrolysis went 
smoothly and gave a yellow crystalline product, which on 
recrystallisation gave M.P. 343°C and was identical with 
apigenin (mixed melt)
Total hydrolysis of the product M.P. 211-212°C.

The product (0'20g) was dissolved in boiling water 
(250 c.cs) and hydrochloric acid (30 c.cs.in 20 c.cs.water) 
added to it and refluxed on a sand bath. After 15 minutes 
refluxing, straw coloured silky needles, made their appear-
ance. As the refluxing was continued the crystals went on 
multiplying. The refluxing was discontinued (after about 
three hours) when it was found that no more crystals were 
coming. This crystalline mass was filtered through a weighed 
gooch and after washings and drying of the crystals, the 
gooch was weighed ag.in. The yield of the product was
0.083 g. This was recrystallised and gave M.P. 343°C. Yield 89.4%.

This hydrolysis was repeated several times with larger quantities and every time, the hydrolysis went smoothly and gave the product M.P. 343°C (Found C, 66.33%; H, 3.56%. Spigenin C₁₅H₂₁O₅ requires C, 66.66%; H, 3.70%).

The filtrate gave all the tests of reducing sugars and yielded an osazone which on two recrystallisations from alcohol gave M.P. 206-207°C and was identical with glucosazone (mixed melt).

Methylation of the product M.P. 343°C Spigenin.

A little of the product taken in its weight of Caustic potash and mixed with methyl alcohol and methyl iodide (excess) was refluxed for 36 hours (Perkin loc. cit.). After the recovery of methyl iodide (excess) and some methyl alcohol, the residue on treatment in the usual manner gave a yellow crystalline product. This on recrystallisation from alcohol yielded pale yellow needles M.P. 170-171°C (Perkin loc. cit. 171-172°C).

Alkaline fusion of the product M.P. 211-212°C.

The alkaline fusion of the stuff gave two products one melting at 209-210°C and the other at 208-209°C identical (mixed melt) with p-hydroxybenzoic acid and phloroglucinol respectively.

From the above account it will be seen that the product M.P. 211-212°C gives on analysis values for carbon and hydrogen, identical with those obtained by previous investigators for apiin and as required by the formula C₂₆H₂₃O₁₄H₂O. This one molecule of water can not be got rid
of by heating the substance to 110-130°C or by dehyration of
the substance over Calcium chloride. Hydrolysis of the product
with dilute acids gave apigenin in 89.4% yields as against
the yield in 40% only of apigenin by Perkin (loc.cit.) from
his purified apiin, and to which the melting point 228°C has
been attributed. Apigenin from this hydrolysis is unaccompa­
nied with any brown product. Perkin (loc.cit.) obtained
apigenin with a considerable amount of a brown product. This
apigenin (M.P. 343°C) has been confirmed by its analysis and
the formation of its dimethylester. Further the compound
M.P. 211-212°C yields on alkaline fusion Phloroglucinol
and P-hydroxy benzoic acid only and no other product.

Regulated (i.e., partial) acid hydrolysis of the
product M.P. 211-212°C gives (repeatedly) the substance
M.P. 234-235°C. The analytical values obtained for carbon
and hydrogen on this substance are identical with those
found for glucose-apigenin by Power and Browning (loc.cit.)
and closely agree with the values for carbon and hydrogen
required by the formula C₂₁H₂₀O₁₁H₂O. It appears that in
this series of compounds, the substances, crystallise out
with varying molecules of water. Some of this water could
be removed by dehydration over calcium chloride or by
heating the substance to 110-130°C, but one molecule of
water is so associated that it can not be removed without
rupturing the molecule. The experience of all the previous
investigators on these compounds is similar.

The filtrate from the hydrolysis, gives an osazone
which is presumably the osazone of apiose.

When this glucose-apigenin is completely hydrolysed
it yields apigenin and glucose.

From the above work it is evident that the compound M.P.211-212°C is the crystalline apin, obtainable repeatedly and in definite crystalline form. It is very sparingly soluble in warm alcohol. When obtained in the crystalline form it does not show great tendency of coming down in the gelatinous form (at least from alcohol solutions).

This work also shows that the product M.P.234-235°C is glucose-apigenin, and is a product with a definite melting point, crystalline form and obtainable repeatedly.

During the course of the present work, it has been found that if from the alcoholic solution of the gelatinous mass the product M.P. 245-246°C is not carefully removed, apin, which has a great tendency to come down in the gelatinous form carries with it the higher melting constituents (in the present case a diglucoside of luteolin) when the method given by the earlier investigators is adopted to obtain it, and, this strangely, is a product melting round about 228°C. Further from this product both the products M.P. 245-246°C and M.P.211-212°C have been separated.

If the above higher melting point product has been carefully separated, the remaining solution, if proceeded with, in the manner of earlier investigators yields products M.P. 178-180°C, M.P.191-196°C and M.P. 202-205°C but these products on recrystallisation from alcohol or boiling water are not reproducible. As it has been found that organic compounds with metallic residues are not fully removed by lead acetate, when the alcoholic solution of the gelatinous mass is treated with this reagent; and that some of these
invariably pass on to this stage, the product, M.P.178-180°C, M.P.191-196°C and M.P.202-208°C seem mixtures of apiin with varying amounts of these salts, which do not materially affect the analytical values of these substances, but hinder the separation of apiin in the pure state.
CONCLUSIONS.

The following conclusions from the work shown in this thesis may be drawn:-

1. The essential oils in the karaff seeds amount to 2.25\%.
   This oil carries 79.0\% distillable matter and is made up of Limonene (presumably d,l forms), 45.4\% of the total oil. Selinene also occurs in the oil in some quantity.

2. Fixed oils occur in the karaff seeds to the amount of 9\% and consist of solid acids 52.20\% and liquid acids 47.80\%.
   These solid and liquid acids are made up of palmitic, petroselinic, oleic, linoleic acids.

3. Mannitol occurs in the seeds to the amount of 0.93\%.

4. Glucose occurs free in the karaff seeds and is obtainable through alcohol extraction.

5. Resins occur in the seeds in 4.2\% yields.

6. Two glucosides M.P. 245-246°C and M.P. 211-212°C are found in the seeds in about equal quantities.

7. The glucoside M.P. 245-246°C is luteolin-7-diglucoside and is a new diglucoside, so far as known to the investigator.

8. The glucoside M.P. 211-212°C is the reputed 'Apiin' and therefore, it is for the first time, that this compound has been obtained in a pure and crystalline form. The correct melting point of Apiin is 211-212°C and not 228°C. This Apiin when once obtained in a pure condition is very sparingly soluble in alcohol and does not show the same great tendency of coming down in gelatinous form.

9. Glucoscapiigenin is a well-defined, crystalline product M.P. 234-235°C and not the product M.P. 215-220°C.
10. A clear and well connected method for obtaining the various constituents of the karaff seeds has been developed and the quantities of most of these constituents determined.

11. Both the glucosides on partial hydrolysis behave similarly and give monosaccharose derivatives, with definite crystalline form and melt melting points.

12. Pectins occur in the karaff seeds in 1 1% yields.

13. The unsaponifiable matter (2.5%) could be separated into a hydrocarbon M.P. 68-70°C and a product which yields an acetyl derivative M.P. 123-124°C.

14. Organic compounds carrying metallic residues are found in the karaff seeds in some quantity.

15. The karaff seeds yield an ash (12.0%) which shows the presence of calcium, magnesium, iron and aluminium.
EXPERIMENTAL.
EXPERIMENTAL.

The Moisture and the Ash contents of the Seeds.

The seeds (10g) were taken in a crucible and heated at 110°C in an air oven for a couple of hours, with occasional stirring of the seeds, and the loss of weight was noted (0.25g). This gave moisture content 2.5%.

The above seeds (9.75g) were then gradually heated on a blow pipe flame, when they first burnt with a lot of smoke and finally left an ash (colourless, 1.17g). This gave an ash value of 12% in the seeds.

The ash on analysis showed the presence of Iron, Aluminium, Calcium and Magnesium.

Extraction of the seeds with Organic solvents.

The seeds (200 g) were taken in a two litre round bottom, long neck flask and covered with freshly distilled ether (500 cc) and refluxed under a 12" double surface condensor, for ten hours. The extract was then poured out, and the seeds washed with another fresh portion of ether (300 c.c.). The washings and the extracts were mixed together and filtered. The solvent on recovery gave a thick brown product (28.6g, i.e.14.3% yield).

The seeds were similarly extracted with Petroleum ether (60-80°), Carbon tetrachloride and Alcohol (95%) and gave the following yields.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
<td>14.3%</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>11.5%</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>14.8%</td>
</tr>
<tr>
<td>Alcohol (95%)</td>
<td>18.0%</td>
</tr>
</tbody>
</table>
These extracts were green in colour and except for the one from petroleum ether, were acidic to litmus. The ether and the carbon tetrachloride extracts, on keeping, separated into two layers, while the alcoholic extract deposited a gelatinous solid on keeping.

Steam distillation of the seeds.

The seeds (500g) were taken in a specially constructed round bottom, short neck, tinned inside, copper flask and steam (superheated 120-125°C) distilled till a clear distillate (6 hours) was obtained. The distillate was collected in a long neck flask and distilled water added to bring the liquid column to the middle of the neck of the flask. On standing, the oil floated on the top of the liquid and was drawn off by means of a pipette and the liquid (distillate) extracted with ether (500 c.c., in two portions). The ethereal layers were collected and added to the oil. The ethereal solution of the oil was dried over sodium sulphate (anhydrous). The solvent on recovery left an oil, which weighed (11'25, yield 2'25%). This extraction was made several times and each time the yield approximated to 2'5%.

The density of the oil was then determined.

(i) The weight of the pyknometer and water at 25°C = 26'8046g
(ii) The weight of pyknometer and oil at 25°C = 25'1850g
(iii) The weight of pyknometer and therefore D₂₅ = 16'3000g

The oil (13'58g) was fractionally distilled into the following three fractions, at 2 m.m. pressure (atmospheric pressure 74'65 cm).
The residue in the flask was acidic to litmus and was not further worked up.

Fraction 1, when redistilled at atmospheric pressure (74.65 cm.) came over at 174-175°C.

Its specific gravity was determined by the pyknometer method and found to be \( d_{4}^{18} 0.8495 \).

**The Formation of a hydrochloride.**

The oil \((1g)\) was taken in a boiling test tube with glacial acetic acid \((10 c.c.)\) and cooled in a freezing mixture to \(2^\circ\). Dry hydrogen chloride was led into it at the bottom, through a glass tube, at a very slow rate. When the mixture has assumed brown colour, the passage of hydrochloric acid gas was stopped. The product was then evaporated in a vacuum desiccator at the water pump. A semi-solid mass was obtained which was taken in amyl alcohol, concentrated and kept over night deposited small shining, needle shape, crystals. These were recrystallised from amyl alcohol and had m.p. 48°C.

**Bromination.**

The oil \((1g)\) was taken in chloroform \((25 c.c.)\) and cooled to \(0^\circ\), Bromine \((1g)\) in ether \((35 c.c.)\) cooled in a freezing mixture was then added drop wise (one drop added every
half a minute) by a narrow glass tube, till the mixture retained permanent colour of bromine. The mixture was kept in an ice bath for another half an hour. Ether was then allowed to evaporate gradually by keeping the beaker in the open. After a little time colourless crystals began to deposit. These crystals were filtered, washed with a little chloroform, recrystallised (thrice) from absolute alcohol, gave m.p. 105°C. The yield was 0.55g.

The filtrate when evaporated to dryness in a vacuum desiccator at the water-pump gave a brown resinous mass which was fractionally crystallised from absolute alcohol. A small quantity of needle shape crystals first separated and then a further crop of crystals in glistening plates (1g) were deposited. The former, on recrystallisation from absolute alcohol had m.p. 105°C and later had m.p. 103°C. Three recrystallisations of the later did not improve the melting point. Molecular weight of the oil fraction No.1.

The oil (0.1400g) was taken with 5 c.c. of alcohol (b.p. 78°C at 75.35 cms) in the inner tube of the Landsberger apparatus and alcohol vapours from boiling alcohol in a conical flask (150 c.c. of alcohol) were led into it. The passage of alcohol vapours was discontinued immediately the alcohol from the boiling tube began to distil at a constant rate, and the temperature was noted (78.23°C). After removing the thermometer and the inlet tube the volume of alcohol was recorded (13.6 c.c.) at the room temperature.
The amount of the oil taken 0'1400g
The boiling point of alcohol 78'0°C
The volume of alcohol with the substance used 12'6 c.c.
Rise in the boiling point 0'23°C
Molecular weight of the oil 134'9

Combustion of the oil fraction No. 1.

(i) The weight of the boat 8'6744g
  " " " " " a substance 9'9163g
  \[8'2424g\]

(ii) The weight of calcium chloride tube before experiment 39'4546g
The weight of calcium chloride tube after experiment 39'7040g
\[0'2494g\]

(iii) The weight of sofnolite tube before experiment 36'9450g
The weight of sofnolite tube after experiment 37'7234g
\[0'7784g\]

round, C, 37'56%; H, 11'43%.

The second fraction redistilled at atmospheric pressure (74'65 cms.) came over a range of 6°C from 263-269°C and was apparently a mixture. This oil on redistillation gave the main fraction at 268-270°C and left a small residue.

Bromination.

The oil (0'85g) in chloroform (10 c.c.), and bromine (0'5 c.c. in 35 c.c. ether) were separately cooled in a freezing mixture. After cooling to 0°C bromine was added dropwise to the oil till it retained the permanent colour of bromine. The mixture was kept in the cold bath for another
half an hour and then taken off. Ether was gradually evaporated by keeping it in the open. No crystalline product came. The mixture was then evaporated to dryness at the water pump, when syrupy mass was left, but all efforts to obtain a crystalline product from this mass resulted in failure.

Preparation of the hydrochloride.

The oil (0.55g) was placed in a test tube with glacial acetic acid (10 c.c.). Dry hydrogen chloride was slowly led into the oil solution at 0°C, till it developed a slightly brownish colour when the addition of the gas was stopped.

Acetic acid was then removed at the water pump and the resulting brownish syrupy mass was taken in amyl alcohol. The solution was concentrated and kept aside. After a few hours crystals began to separate. These crystals were filtered, and the filtrate on further concentration gave another crop of crystals. These crystals (0.23g) were recrystallised from amyl alcohol (thrice) and had melting point 72°C.

Molecular weight of the Oil fraction II (By Landesberger's method)

<table>
<thead>
<tr>
<th>The amount of oil taken</th>
<th>0.2016g</th>
</tr>
</thead>
<tbody>
<tr>
<td>The boiling point of the alcohol used</td>
<td>78.0°C</td>
</tr>
<tr>
<td>Barometric pressure</td>
<td>75.85 (c.m.s)</td>
</tr>
<tr>
<td>The rise in boiling point</td>
<td>0.12°C</td>
</tr>
<tr>
<td>The volume of alcohol in the main tube</td>
<td>16.5 c.c.</td>
</tr>
<tr>
<td>Found Molecular weight of the oil fraction II</td>
<td>208</td>
</tr>
</tbody>
</table>
Combustion of the oil fraction II.

(a) The weight of the boat 8'6742g
   " " " " & the substance 8'7838g
   \[\text{Total} = 8'7786g\]
   0'1146g

(b) The weight of calcium chloride tube before experiment 38'5694g
   The weight of calcium chloride tube after experiment 38'6974g
   \[\text{Total} = 77'2668g\]
   0'1280g

(c) The weight of sofrolite tube before experiment 35'7848g
   The weight of sofrolite tube after experiment 36'1508g
   \[\text{Total} = 71'9356g\]
   0'3660g

Found, C, 87'77%; H, 11'53%.

(i) The weight of the boat 8'6743g
   The weight of the boat and substance 8'8775g
   \[\text{Total} = 8'8818g\]
   0'2032g

(ii) The weight of calcium chloride tube before experiment 38'7942g
    The weight of calcium chloride tube after experiment 39'0068g
    \[\text{Total} = 77'8010g\]
    0'2126g

(iii) The weight of sofrolite tube before experiment 34'2046g
    The weight of sofrolite tube after experiment 34'8568g
    \[\text{Total} = 79'0614g\]
    0'6522g

Found, C, 87'72%; H, 11'61%.

The third fraction being very small could not be distilled at atmospheric pressure and attempts to obtain derivatives from it also did not succeed.
Petroleum ether extraction of the seeds.

Fresh ground seeds (300g) were treated with boiling petroleum ether (1½ litre, B.P. 60-80°C) for ten hours in a round bottom flask (3 l), fitted with a double surface condenser (12 inches), and filtered on a Buchner funnel. The filtrate was green coloured solution and neutral to litmus. The residual seeds were once more exhausted with petroleum ether. The extracts were mixed and the solvent recovered. This gave a greenish oil (34.6g) which was exhausted in a vacuum desiccator and then shaken with a paste of zinc chloride (obtained by keeping zinc chloride in an open dish over night) in a separating funnel and allowed to settle. Zinc chloride carried with it green resinous matter leaving a clear brownish upper layer. This layer of oil was separated and was washed free of zinc chloride with distilled water. The oil (free from zinc chloride) was treated with dilute solution of caustic soda (one percent, 200 c.c.) and was washed free of alkali by distilled water. An almost colourless transparent oil was obtained which was then treated with alcohol (rectified spirit distilled once over lime) in which the fatty oil appeared insoluble, but the essential oil dissolved. The fatty oil (thus separated from essential oil) was heated on a water bath to remove alcohol. The oil was then dried over sulphuric acid in a vacuum desiccator. The yield of the fatty oil was 26.2g i.e., 8.7%.

The alcoholic layer was cooled in an ice bath for about an hour and then decanted. The vessel was rinsed with a little cold alcohol. Some oily droplets were found sticking to the side of the vessel. These were taken in ether and after removing
ether the fatty oil (0.8g) was obtained.

The alcoholic layer was then saturated with sodium chloride and kept overnight. An oily layer was found to float at the top. This was separated and after drying in the usual way the oil (1.5g) was obtained. From its odour and character it appeared to be the essential oil (mentioned earlier). Several such operations were carried out and fatty oil (350g) free from essential oil was obtained.

Examination of the fatty oil.

Refractive Index: (Butyro Refractometer).

Butyro refractive reading at 40°C, 81.0

The corresponding refractive Index on Abbe Scale, 1.4789

Specific gravity: (By Pyknometer).

The weight of oil at 18°C 18.5240g
The weight of water at 18°C 19.9244g

and therefore \( d_{18} \) 0.9275

Iodine value of the fatty oil (By Hubbs method).

Experiment No.1.

The oil (0.3472g) was taken with chloroform (20 c.c., freshly distilled) in an Erlenmeyer flask (glass stoppered) and 20 c.c. of a mixture (prepared 48 hours before) of equal volumes of Iodine solution (25g iodine in 500 c.c., 95% alcohol) and mercuric chloride (30g mercuric chloride in 500 c.c. 95% alcohol) 20 c.c. potassium iodide (10g. in 10 c.c. water) solution was also added to the oil mixture.

A blank with equal volumes of these reagents was simultaneously performed. Both these flask were kept in a cool place for twenty four hours and after adding...
distilled water (150 c.c.) to each of the flasks, the excess iodine was titrated by standardised sodium thiosulphate solution (sodium thiosulphate 25g, water 15)

(i) Sodium thiosulphate solution required for the oil 23.0 c.c.
(ii) Sodium thiosulphate solution required for the blank 31.3 c.c.

(1 c.c. thiosulphate by titration against standard potassium dichromate solution = 0.0115g of iodine)

Therefore the iodine value of the oil 95.4

Experiment No. 2

(i) The amount of the oil taken 0.5130g
(ii) Sodium thiosulphate solution required for the oil 23.5 c.c.
(iii) Sodium thiosulphate solution required for the blank 65.0 c.c.

Iodine value found 95.3

Saponification value of the oil.

The oil (0.2052g in 20 c.c., 95% alcohol) was refluxed in a conical flask (350 c.c.) and alcoholic potassium hydroxide (20 c.c., N/2.04) solution for three quarter of an hour. After the saponification was completed another 20 c.c. alcohol (95%) was added and the excess of alkali titrated by hydrochloric acid solution (N/5.49).

A blank experiment was simultaneously performed

(i) The amount of the oil taken 0.2052g
(ii) The amount of hydrochloric solution used for the titration of the alkali in the blank 54.0 c.c.
(iii) The amount of hydrochloric solution used for the titration of the alkali with the oil 43.2 c.c.
Therefore, the saponification value of the oil

\[
\frac{(54.48.2)}{3.49} \times \frac{56}{1000} \times \frac{1000}{0.2082} = 288.3
\]

Preparation of mixed fatty acids from the fatty oil.

The fat (300 g) was then refluxed with alcoholic potassium hydroxide (alcohol-1½ litre, potassium hydroxide 180g). The alcohol used was fusel oil free (95% alcohol) and was obtained through treatment with potassium hydroxide and distillation over lime.

After refluxing for six hours, most of the alcohol was removed by distillation on a water bath. The soap thus obtained was dissolved in water (excess) and the unsaponifiable matter removed by continuous ether extraction method.

Removal of the unsaponifiable matter.

This was obtained from the soap solution by continuous ether extraction method of Hilditch (loc. cit.) as shown below.

Ether.

Soap solution.

The extraction was continued for twenty four hours. The ethereal layer (solution) so obtained, was dried over sodium sulphate (anhydrous) and ether recovered. The unsaponifiable
matter was dried over sulphuric acid in a vacuum desiccator. The yield was 7.95g.

**Liberation of fatty acids.**

The soap solution after the separation of the unsaponifiable matter was acidified with dilute sulphuric acid (40%), avoiding excess of the acid, and gently warmed to ensure complete decomposition of the soaps. The liberated fatty acids floated on the surface of aqueous layer and extracted with ether. The aqueous layer was again extracted (twice) with ether and the ether of all the extracts was united, washed free of mineral acid, dried over sodium sulphate (anhydrous) and then recovered by distillation.

The mixed fatty acids were then heated on a steam bath (for half an hour) and finally dried over sulphuric acid (conc.) in a vacuum desiccator. The yield of mixed acids being 250.4g i.e. 83.3%.

**Separation of the fatty acids.**

The mixed fatty acids (250g) were taken in 95% alcohol (1L), free from fusel oil, boiled, and mixed with a boiling solution of lead acetate (140g) in 95% alcohol (1L), containing 5% glacial acetic acid (50 c.c.). The mixture was boiled for three quarter of an hour. It was first cooled to room temperature and then to 15°C by placing it in an ice bath. The white crystalline lead salt was filtered next morning and washed with alcohol (cold) until the washings on dilution with water gave no turbidity. The washing were united with the filtrate. Thus the mixed fatty acids were separated into soluble and insoluble lead salts of the fatty acids.
Decomposition of the lead salts.

(a) The insoluble lead salts were transferred to a porcelain dish with successive portions of concentrated hydrochloric acid and boiling water. Hydrochloric acid (50%, 200 c.c.) was added and the mixture warmed for some time when a clear layer of fatty acids floated on the surface of the aqueous layer. After cooling, the layer of the fatty acids was transferred to a separating funnel, and the aqueous layer was decanted into another separating funnel. The lead chloride was extracted repeatedly with ether and the ether was transferred to the separating funnel, containing the aqueous layer. More ether was added and the aqueous layer was extracted thrice with ether.

All these ethereal extracts were added to the first separating funnel containing the fatty acids and was washed free of mineral acid by shaking with distilled water. The ethereal extract was dried over sodium sulphate (anhydrous), the ether recovered, and the fatty acids dried first by warming for some time on a water bath and then over sulphuric acid in a vacuum desiccator, gave an yield of solid acids (130.4g) was obtained.

(b) The soluble lead salts, after removal of the solvent (alcohol) were warmed with hydrochloric acid when a clear oily layer was formed at the top of aqueous layer. The lead chloride precipitate and the aqueous layer were washed with ether in the manner mentioned earlier, and the washings were added to the separating funnel containing the fatty acids. The ethereal solution after washing free of the mineral acid, was dried over (anhydrous) sodium sulphate, ether recovered, and the fatty oil (liquid acids), thus obtained was dried in the
manner mentioned earlier (the yield being 119'5g).

The solid acids on keeping for some time deposited a colourless crystalline product. This was separated, and recrystallised several times from absolute alcohol and amyl alcohol and gave m.p. 61-62°C.

**Molecular weight of the product m.p. 61-62°C.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The amount of the substance taken</td>
<td>0'3000g</td>
</tr>
<tr>
<td>Boiling point of the alcohol</td>
<td>73'2°C</td>
</tr>
<tr>
<td>Barometer reading</td>
<td>76'0 cm</td>
</tr>
<tr>
<td>Rise in the boiling point of alcohol with the substance</td>
<td>0'2°C</td>
</tr>
<tr>
<td>Volume of alcohol</td>
<td>9'1 c.c.</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>268</td>
</tr>
</tbody>
</table>

**Combustion of the product m.p. 61-62°C.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The weight of the boat</td>
<td>3'6436g</td>
</tr>
<tr>
<td>The weight of the boat &amp; substance</td>
<td>3'7594g</td>
</tr>
<tr>
<td></td>
<td>0'1158g</td>
</tr>
<tr>
<td>The weight of calcium chloride tube before experiment</td>
<td>35'4258g</td>
</tr>
<tr>
<td>The weight of calcium chloride tube after experiment</td>
<td>35'5498g</td>
</tr>
<tr>
<td></td>
<td>0'1240g</td>
</tr>
<tr>
<td>The weight of the sofnolite tube before experiment</td>
<td>37'5826g</td>
</tr>
<tr>
<td>The weight of the sofnolite tube after experiment</td>
<td>37'9000g</td>
</tr>
<tr>
<td></td>
<td>0'3174g</td>
</tr>
</tbody>
</table>

Found, C, 74'7%; H, 11'89%. 
Preparation of methyl ester.

The solid acids (100g) were taken in methyl alcohol (500 c.c.) distilled over potassium hydroxide and refluxed for nearly twelve hours in presence of concentrated sulphuric acid (3 c.c.). The excess of methyl alcohol was recovered by distilling on water bath and the resulting ester was dissolved in ether. The ethereal solution after washing free of mineral acid was shaken with dilute sodium carbonate solution and then washed free of alkali. After drying over anhydrous sodium sulphate, ether was removed. The ester thus obtained was dried over sulphuric acid in a vacuum desiccator and kept in the inert atmosphere of Nitrogen. The yield was 96.5g.

The liquid fatty acids (100g) were similarly converted into their methyl esters, and after the usual purification were stored in an atmosphere of nitrogen.

Solid Acids.

The methyl esters (5g) were taken in alcohol (400 c.c. 95%) and alcoholic potassium hydroxide (N/2, 40'0 c.c.) was added to it and the mixture refluxed for two hours. After removing the alcohol the soap was taken in water (300 c.c.). The soap solution was cooled (below 4°8 C.) in an ice bath and freshly prepared alkaline potassium permanganate was added to it (5 c.c. after every two minutes) with gentle stirring until the solution retained the colour of the permanganate. A thick brown precipitate was formed. The mixture was kept in the ice bath for another half an hour. Sulphurous acid was then added till a piece of litmus paper showed the solution was distinctly acidic. The brown precipitate dissolved
and a colourless flocculent precipitate appeared. The precipitate was filtered and boiled for a short time with hydrochloric acid (5%). After filtering and drying in an air oven, the precipitate was refluxed with petroleum ether (B.P. 45-50°C). The precipitate (m.p. 110-113°C) was crystallized from absolute alcohol (three times) and then from amyl alcohol and had m.p. 121-122°C. (This melting point of the derivative of solid fatty acid corresponded with the dihydroxy stearic acid of Petroselenic acid found by Hilditch and Christian - Bio. Chem. J. 1929).

Liquid acida.

The methyl esters of the liquid acids on treatment as above detailed gave first a product m.p. 115-117°C and from this through absolute alcohol and partial concentration two products were finally obtained (on repeated recrystallization) m.p. 170°C and m.p. 128°C.

These melting points are the same as obtained by Hilditch and Christian (loc. cit) for tetra and dihydroxy stearic acids for the presence of linolic and oleic acids in celery seeds.

Examination of the unsaponifiable matter.

The unsaponifiable matter (7.5g), isolated from the fatty oil by continuous ether-extraction method mentioned earlier, was dissolved in methyl alcohol (cold) in which it was totally soluble. The solution was cooled at -5°C in a freezing mixture when a colourless flocculent precipitate was obtained. It was noticed that the precipitate so formed dissolved in the solvent when it attained room temperature. The solution was again cooled to -2°C and allowed to stand in the
freezing mixture till the precipitate formed settled down when the supernatant liquid was carefully decanted, and the precipitate washed with a little cold alcohol. This alcohol was added to the decanted liquid. The decanted liquid was cooled again to -2°C and gave a further precipitate which was allowed to settle down. It was quickly filtered at very low temperature on a Buchner funnel. The two lots of precipitates combined and on several recrystallisation from alcohol gave m.p. 69-70°C.

This product was soluble in ether, benzene, chloroform, petroleum ether, acetone, alcohol and solution was neutral to litmus. It gave no test for hydroxyl, carboxyl, ketone and aldehyde groups.

Combustion of the product m.p. 69-70°C.

(i) The weight of the boat 3'6460g
The weight of the boat and the substance. 3'7866g
0'1426g

(ii) The weight of the calcium chloride tube before expt. 41'7116g
The weight of the calcium chloride tube after expt. 41'9000g
0'1884g

(iii) The weight of the sofnolite tube before experiment. 40'2914g
The weight of the sofnolite tube after experiment. 40'7357g
0'4443g

Found, C, 84'9%; H, 14'6%.

The filtrate from the above product was evaporated to dryness in a desiccator and the brown sticky mass obtained was dissolved in alcohol (95%, 50 c.c.). A solution of digitonin
(1g) in 95% alcohol (100 c.c.) was prepared, warmed to boiling and while hot was gradually added to the above solution till no more precipitate was formed. On allowing the mixture to stand for a couple of hours, the precipitate settled down and was then filtered on a weighed Gooch crucible. It was washed well with alcohol and after drying at 100°C was weighed (3.8012 g).

Preparation of acetyl derivative of the above product.

The digitonide so prepared was refluxed for more than two hours with acetic anhydride (25 c.c.) and a few fine crystals of aluminium chloride (anhydrous). A portion (5 c.c.) of this mixture was poured into a beaker containing water (200 c.c.). No definite product could be obtained.

The rest of the above mixture was transferred to a conical flask and cooled to the room temperature. A crop of white crystalline product was formed, which was completely separated by allowing to stand overnight. The crystals were separated, re-crystallised a number of times from alcohol and gave m.p. 123-124°C.

The filtrate from the above on further close examination gave only a little more quantity of the above product (m.p. 123-124°C).

Carbon tetrachloride Extraction of the seeds.

The seeds (200 g) thoroughly defatted by petroleum ether, were refluxed with carbon tetrachloride (750 c.c.) for ten hours, filtered and again refluxed with more carbon tetrachloride (500 c.c.). They were filtered and washed with a fresh quantity (400 c.c.) of the solvent. The filtrates and the washing were united and after recovering the solvent (carbon tetrachloride) the extract was dried over concentrated sulphuric acid in a vacuum desiccator and weighed. A resinous brown product 8.4g
The brown resinous mass was acidic to litmus. This was soluble in cold ether, benzene, toluene, xylene, acetone, chloroform methyl and ethyl alcohols, but insoluble in water and petroleum ether, but no definite product could be obtained through any of the above solvents.

The resinous mass (3.5g) was dissolved in caustic potash (M/2) solution, and thrice extracted with ether. The ethereal extract was washed free of alkali and after drying over sodium sulphate (anhydrous), ether recovered, when it gave a brown resinous product neutral to litmus. This was treated with different solvents, one by one, but no definite product could be obtained.

The alkali solution, after removal of the above neutral resinous matter, was acidified with dilute hydrochloric acid. The turbid solution was extracted (thrice) with ether. The ethereal solution after usual washing, drying and recovery of ether, gave a brown resinous product which was acidic to litmus. Attempts to get any crystalline product by treatment with various solvents have not so far materialized. Apparently the brown resinous product obtained through carbon tetrachloride is made up of two parts, one neutral and the other acidic.

**Alcohol extraction of the seeds:**

The seeds (500g) thoroughly exhausted with petroleum ether and carbon tetrachloride were taken in a round bottom (3L) flask and alcohol (1.5L) was added to it. The whole was refluxed for eight hours and left overnight. It was then filtered. The filtrate was reduced in bulk at the water pump
and the seeds were again taken in alcohol (1L) and refluxed for another eight hours. Fresh alcohol (recovered from the above extract, 500 c.c.) was added during the course of this refluxing. On allowing it to stand overnight the filtrate was added to the first extract and the bulk partially reduced till crystallisation appeared in the distillation flask, when it was poured out in a conical flask and left overnight. This gave a gelatinous mass and was filtered on a Buchner funnel at the pump. It was washed with a little fresh alcohol and well pressed to drain out all solvent.

The filtrate on standing gave a further deposit of the gelatinous mass. This operation was continued till no more gelatinous deposit was obtained. All the fractions (thus obtained) of the gelatinous mass were united and dried at 100°C and weighed. It was yellow coloured solid mass and was labelled (A) and weighed 14.7g.

The filtrate from the above solid was further concentrated and allowed to stand for a few days, when it deposited a white crystalline product which was separated and termed B. The melting point of this product (B) was found to be 162-63°C. The filtrate was gradually concentrated and kept till it gave no more of the product (B). All the portions of the product (B) were united and dried at 100°C, desiccator and weighed (4.68g). The filtrate after the separation of the product (B) was dark red in colour and was concentrated a little further at the water pump, and left for crystallisation. It gave a syrup, which was sweet in taste and readily reduced Fehling and ammomiacal silver nitrate solutions. It also gave positive Molisch's test. It was left over a few days when crystallisation
appeared. The crystalline product was sticky and on washing with absolute alcohol gave colourless crystals which melted at 146°C.

The crystals were dissolved in water and a few drops of phenylhydrazine in acetic acid added, where upon warming on water bath for about twenty minutes brownish yellow crystals appeared. They were allowed to grow and then filtered and recrystallised gave m.p. 265-206°C. A mixed melt of this with a genuine sample of glucosazone gave no depression in the melting point.

Repeated attempts were made by leaving the remaining syrup in absolute alcohol to separate completely glucose by crystallisation, but only partial separation occurred. The syrup did not dissolve in benzene, petroleum ether or ether at this stage and therefore the whole lot of this syrup was taken and phenylhydrazine (5 c.c.) and acetic acid (30 c.c.) with a little water added to it and the whole warmed on a water bath. After a little time yellow crystalline product began to separate when the separation appeared completed, the product was filtered while still warm and recrystallised gave m.p. 205-206°C. It weighed about 2.5g.

The filtrate from the above was well shaken first with benzene and then with ether and concentrated under vacuum, but no further crystalline product separated. The residue when analysed gave the presence of Nitrogen (in some quantity) and phosphorus and sulphur. The quantity of Nitrogen appeared large compared to the other two elements, when the syrup was tested before the addition of phenylhydrazine also.
Alcohol extraction of the seeds was repeated several times to get a quantity of the products. It was found that 2.5 litres of alcohol and sixteen hours period of refluxing was enough for complete extraction.

Examination of the gelatinous mass.

A little of this gelatinous mass was ignited on a nickel spatula when it first softened and then burnt with a smoky flame and finally left a residue. A further quantity (1g) was ignited in a crucible and the ash analysed, showed, the presence of Iron and Aluminium. Calcium and Magnesium were also indicated but they appeared in traces only.

The entire lot (13g) of the gelatinous mass was then dissolved in boiling alcohol and filtered. The quantity of alcohol was so arranged that on cooling no precipitation occurred. A warm dilute alcoholic solution of lead acetate was added (drop by drop) to the alcoholic solution of the gelatinous mass with constant stirring. With the first few c.c's of lead acetate solution, a brown sticky precipitate (1) was formed, which was filtered and washed with a little fresh alcohol. This precipitate (1) was suspended in alcohol and hydrogen sulphide passed into it to precipitate lead as sulphide. The filtrate from lead sulphide was boiled free of hydrogen sulphide and on keeping gave a gelatinous precipitate. Nothing definite could be obtained from it. This gelatinous precipitate on ignition showed the presence of Iron and Aluminium.

The filtrate together with the washings from the above sticky precipitate, were treated again with alcoholic lead acetate solution (drop by drop and with constant stirring) until the supernatant liquid gave a reddish brown colour with a fresh
solution of ferric sulphate. The deep yellow (with distinct red tinge) precipitate (ii) thus formed was filtered on a Buchner funnel and washed with fresh alcohol. This precipitate was suspended in alcohol, and hydrogen sulphide passed into it. It was slightly warmed and filtered quickly. The lead sulphide precipitate was washed with minimum quantity of warm alcohol. The washing was added to the filtrate which was boiled free of hydrogen sulphide and kept aside. As it gradually cooled yellow needle shaped clusters of crystals began to separate. The crystalline product was filtered next morning, washed with cold alcohol and dried on a porous tile had m.p. 240-242°C. The washings were added to the filtrate which was partially concentrated and on keeping overnight gave a further quantity of the above crystalline product. By gradual concentration of the filtrate further crops of this product were obtained till a stage was reached when a gelatinous precipitate appeared. This gelatinous precipitate had no definite melting point. It was redissolved in alcohol, cooled and added to the filtrate. The above yellow product was collected and dried at 110°C, cooled in a vacuum desiccator and weighed gave 3.6g of the product.

This yellow crystalline product was recrystallised several times from alcohol (distilled over lime) and had m.p. 245-246°C.

The filtrate was treated in a like manner with lead acetate till the supernatant liquid gave blood red colour with a freshly prepared solution of ferrous sulphate. The precipitate obtained now was yellow in colour but distinct from the colour of the precipitate earlier described.
The precipitate was suspended in alcohol and decomposed by hydrogen sulphide as in previous case. The filtrate after removing excess hydrogen sulphide and on standing gave silky, long, needle shape crystals. These crystals on repeated recrystallisation did not give any product of a definite melting point. Their melting points varied from 190°C to 232°C with the crystals taken from different portions of the mass.

However these crystals taken in alcohol and reprecipitating fractionally by alcoholic lead acetate finally gave only a little of the product m.p. 245-246°C. Alcoholic solution of the above product of indefinite melting point gave with water a white gelatinous precipitate. The precipitate was collected redissolved in alcohol and reprecipitated by water. These operations were repeated several times but no product of a definite melting point was obtained.

The filtrate and the washings from the above precipitate were united and treated with alcoholic lead acetate (dropwise and constant stirring) till the supernatant liquid was almost colourless, and it appeared that the addition of more lead acetate did not increase the quantity of the precipitate. This light yellow precipitate was filtered, washed, and suspended in alcohol and decomposed by hydrogen sulphide. After warming the lead precipitate was filtered quickly and the filtrate on cooling deposited practically colourless short needles which melted at 209-210°C. These on recrystallisation (twice) from absolute alcohol gave melting point 211-212°C. Further recrystallisations did not improve the melting point.

The filtrate and the washings after the separation of the product m.p. 211-212°C were concentrated by distilling it
at the water pump till a solid appeared. The concentrate along with the deposited solid was transferred to an open dish and cooled to 40-50°C. A white gelatinous precipitate was obtained. This gelatinous precipitate was quickly filtered on a buchner funnel and washed. It was taken in alcohol and hydrogen sulphide was passed into it (avoiding large excess). On warming, the lead sulphide precipitate was filtered. After removing the excess hydrogen sulphide, the filtrate was kept aside. As it cooled, white short needles made their appearance, which had m.p. 208-9°C, and on several recrystallisations had m.p. 211-212°C.

The filtrate and the washings from the above gave on the addition of water white gelatinous precipitate. This precipitate was filtered on a buchner funnel at the water pump. A little of the precipitate on the spatula burnt and left an ash which gave the presence of Iron and Aluminium.

The filtrate was then evaporated to dryness by distillation under reduced pressure. A little quantity of a solid mass was obtained which on ignition left a residue. This residue gave feeble tests for Calcium and Magnesium.

Examination of the product 9.

This product (4g) was dissolved in alcohol (rectified spirit distilled over lime) in which it was very sparingly soluble. The alcoholic solution was partially concentrated and allowed to cool. After some time, the solution deposited white feathery crystalline product. This product was separated and had melting point 163-165°C. Repeated recrystallisations from methyl alcohol gave a product with m.p. 166-167°C.
The mother liquor was further concentrated and allowed to stand overnight. The above feathery crystals, accompanied with distinct needle shaped crystals were deposited. The former product was in larger amount than the later. This mixed crystalline product was recrystallised alternately from ethyl and methyl alcohols to separate them, but with no success. After several recrystallisations from methyl alcohol the product had m.p. 166-167°C.

The above crystalline product m.p. 166-167°C (0'2g) was treated with different solvents and found sparingly soluble in hot methyl and ethyl alcohols, acetone; insoluble in ether, benzene, carbon tetrachloride and petroleum ether but highly soluble in water.

The analysis of the compound gave the following values for Carbon and Hydrogen.

(1) The weight of the boat 8'7644g
   The weight of the boat and the substance 8'9790g
   Balance weight of the boat 0'2146g

(ii) The weight of the calcium chloride tube before expt. 38'1794g
     The weight of the calcium chloride tube after expt. 38'3264g
     Balance weight of the calcium chloride tube 0'1470g

(iii) The weight of the sofnolite tube before experiment 40'5264g
      The weight of the sofnolite tube after experiment 40'8348g
      Balance weight of the sofnolite tube 0'3084g

Found, C,39'22%; H,7'45%.

The substance was sweet in taste and its solution in alcohol
and in water was neutral to litmus.

**Molecular weight of the product m.p. 166-167°C.**
(by Landsberger method).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance taken</td>
<td>0.4022g</td>
</tr>
<tr>
<td>Boiling point of alcohol used</td>
<td>77.9°C</td>
</tr>
<tr>
<td>Boiling point of alcohol with the substance</td>
<td>78.1°C</td>
</tr>
<tr>
<td>Volume of alcohol immediately constant rate of boiling is attained</td>
<td>12.5 c.c.</td>
</tr>
<tr>
<td>Molecular weight of the substance</td>
<td>186.</td>
</tr>
</tbody>
</table>

**Acetyl derivative of the product m.p. 166-167°C.**

The substance (0.8174g) was taken in acetic anhydride (20 c.c. redistilled, B.P. 137°C) with a few crystals of zinc chloride (anhydrous) in a round bottom flask (100 c.c.) fitted with an air condenser. The mixture was first heated on a water bath with constant, gentle shaking until the substance was completely dissolved (10 minutes). Then the flask was transferred to a sand bath and heated to the boiling point of acetic anhydride. After three quarters of an hour the mixture was poured into a beaker containing cold water (200 c.c.) whereupon an oily mass separated. This was stirred and as the mixture cooled, a white precipitate began to deposit. After a couple of hours the precipitate was filtered and washed well with distilled water. The precipitate after drying at 100°C, was weighed (1.30g).

The precipitate was taken in alcohol (warm, 50 c.c.), the solution concentrated and left overnight, white rhombic crystals. This crystalline product was recrystallised from absolute alcohol and had m.p. 121°C.
The exhausted seeds (100g) were taken in water (500 c.c) and boiled on a sand bath for an hour and then filtered. Acidulated alcohol (1 litre) was added to the filtrate. A gelatinous precipitate was obtained which was filtered, redissolved in hot water (200 c.c., 70-80°C) and reprecipitated by acidulated alcohol (400 c.c.). The precipitate was dried and weighed. Yield 1'1g. The following tests were performed with the substance.

A little of the substance was dissolved in water and was neutral to litmus. It gave precipitates with copper sulphate and lead nitrate but no precipitate with ferric chloride and barium chloride.

The product (0'5g) was taken with water (20 c.c.) in a distilling flask. To the above mixture was added caustic soda solution (5 c.c., 10% in water). After gently agitating, the flask was corked and left for a few minutes. Then sulphuric acid (5 c.c., 5%) was added, the mixture distilled and the distillate (17 c.c.) was collected. This distillate on warming with a little p-nitrobenzoyl chloride gave a solid product which was crystallised from alcohol and had m.p. 96°C (a characteristic test for methyl alcohol).

The substance (0'2g) was distilled with hydrochloric acid (15 c.c., 12%). The distillate reduced Fehling's solution showing the presence of furfural. Water extract of the fresh seeds.

The powdered seeds (2 lbs.) were exhausted with boiling water (twice, in two lots of 4 litres) and filtered hot, on a
piece of cloth. The filtrate on cooling deposited a gelatinous mass. This was filtered on a piece of cloth, washed with cold water, and dried. A brown solid product (23-25g) was obtained. This was taken up in a large quantity of alcohol (3 litres, warm) and then allowed to cool. The colour of the solution was brown and thick and it deposited a solid on standing. This was filtered off. The clear brown solution was then treated with alcoholic lead acetate solution (drop by drop and with constant shaking) and a reddish yellow precipitate was obtained. The precipitate formed by the first few cubic centimeters of the lead acetate solution was filtered off. This precipitate was suspended in water and decomposed by hydrogen sulphide. The water solution after removal of the excess hydrogen sulphide gave some of the tests for tannin but failed in the vital tests for tannin.

Further drop wise addition of lead acetate solution gave reddish yellow precipitate. This precipitate was filtered and decomposed with hydrogen sulphide and proceeded with in the manner described earlier (alcoholic extract, gelatinous mass) and gave the product m.p. 245-246°C.

The filtrate from the above product on further treatment with alcoholic lead acetate solution gave a yellow precipitate which was worked up as mentioned earlier, and gave the product m.p. 211-212°C.

The supernatant liquid from the steam distillation of the seeds for obtaining the essential oils, was filtered while hot on a piece of cloth. The filtrate, on cooling, gave a gelatinous mass. This was filtered on a piece of cloth and washed with cold water and well pressed. This precipitate was proceeded with as earlier described and gave the same two
products m.p. 245-246°C and m.p. 211-212°C.

Examination of the product m.p. 245-246°C.

The above product (0'1g) was dissolved in warm alcohol. A little of this solution was taken in a test tube and a few drops of Ferrous sulphate solution (Freshly prepared) were added. A dark blue colour was produced which on standing gave dark blue precipitate. Similar behaviour was observed with ferric chloride solution. Water solution of the above substance gave no precipitate with Nickel sulphate chromium oxalate, Manganese chloride, cobalt-nitrate and Zinc chloride.

Combustion of the product m.p. 245-246°C.

(i) The weight of the boat 3'6440g
   The weight of the boat & the substance 3'6494g
   0'2054g
(ii) The weight of the calcium chloride tube before expt. 41'3486g
    The weight of the calcium chloride tube after expt. 41'4370g
    0'0884g
(iii) The weight of the sofnolite tube before experiment 36'6548g
    The weight of the sofnolite tube after experiment 37'0377g
    0'3829g

Found, C, 50'84%; H, 4'78%.

Hydrolysis of the product m.p. 245-246°C.

Hydrochloric acid (100 c.c. in water 900 c.c.) was boiled in a round bottom flask (3 litres) carrying a condenser on a sand bath and the product (5'40g) was dropped into it, where upon a clear yellow solution was obtained. This was boiled
when within half an hour's refluxing a light yellow thread-like crystals made their appearance in the flask. The precipitate went on increasing as the boiling continued. When it appeared that no more precipitate was coming (4 hours), the mixture was filtered while still hot, on a buchner funnel. The precipitate was washed free of hydrochloric acid, dried and weighed (2'3g). This yellow precipitate was dissolved in warm alcohol 150 c.c., and filtered. After adding water (20 c.c.) the filtrate was concentrated and kept overnight. Yellow small needle-shaped crystals were formed. This product was recrystallised from alcohol and had m.p. 327-328°C.

The product m.p. 245-246°C was hydrolysed in the manner described above. The substance (3'55g) and hydrochloric acid (150 c.c. in water 1350 c.c.) were employed and the hydrolysis was continued for a longer period (3-9 hours). Like the previous hydrolysis, a clear yellow solution was obtained, which gave a crystalline precipitate within half an hour's boiling though the reaction appeared completed in about four hour's boiling. The precipitate deepened in colour as the boiling was continued. The mixture was cooled and the precipitate filtered, washed, dried and weighed (4'75g).

This precipitate was dissolved in alcohol where upon brownish solution was obtained. It was treated with cold lead acetate solution (alcoholic, dropwise) till the supernatant liquid was clear yellow in colour. The lead precipitate (small in quantity) was filtered and any unreacted lead acetate was removed by hydrogen sulphide and the solution after the addition of 30 c.c. water was concentrated. This concentrate on allowing to stand gave yellow crystalline product which was
recrystallised from alcohol and had m.p. 327-328°C (yield 4.34g). 

In subsequent hydrolysis of the product m.p. 245-246°C, four (4) hour's boiling was maintained.

This product was dried at 160°C in an air oven for three quarters of an hour and analysed for carbon and hydrogen values. 

**Combustion of the product m.p. 327-328°C.**

(i) The weight of the boat: 3.6440g

The weight of the boat and the substance: 3.8650g

0.2210g

(ii) The weight of the calcium chloride tube before expt.: 40.7966g

The weight of the calcium chloride tube after expt.: 40.8708g

0.0742g

(iii) The weight of the sofnolite tube before experiment: 36.6326g

The weight of the sofnolite tube after experiment: 37.1425g

0.5099g

**Found, C, 62.89%; H, 3.73%.**

**Acetylation of the product m.p. 327-328°C.**

The product (0.31g) was taken in freshly distilled acetic anhydride (30°c c.c.) with a few crystals of zinc chloride (anhydrous) in a round bottom flask (100 c.c.). The mixture was first gently heated with continuous shaking until the substance was completely dissolved.

The solution was refluxed on a sand bath and after a couple of hours was poured into cold water. A white precipitate was obtained when the mixture was cooled. This precipitate was filtered, washed with distilled water, recrystallised from absolute alcohol gave m.p. 218-219°C.
Methylation of the product m.p. 327-328°C.

The product (0.250g) was taken in methyl alcohol containing caustic soda (2g) in a 100 c.c. round bottom flask and refluxed with methyl iodide (15 c.c., excess) on a sand bath. After refluxing for thirty hours (Perkin method), the excess methyl iodide and some methyl alcohol was recovered and the remaining product was poured into water. A reddish precipitate was formed. After filtering, the precipitate was taken into ether and washed with caustic soda solution (0.5%) and dried over sodium sulphate (anhydrous). On removing ether a very light yellow crystalline product was obtained. It was recrystallised first from alcohol and then from absolute alcohol and had m.p. 160-161°C.

Bromination of the product m.p. 327-328°C.

A small quantity of the product was made into a paste with a little quantity of acetic acid (glacial in pestle and mortar. The paste after transferring to a small beaker with the help of a little more acid, was treated with bromine solution (bromine in acetic acid) drop by drop until the mixture retained permanent colour of bromine. The mixture was kept aside for forty eight hours. A lemon yellow colour precipitate was obtained. This was filtered washed free of excess bromine, and recrystallised from absolute alcohol had melting point 237°C. The mother liquors were concentrated in a vacuum desiccator at the water pump. A reddish yellow sticky solid was obtained. It was taken in absolute alcohol and kept aside. After seven days a few light yellow crystals appeared. These crystals were separated and washed with a small quantity of alcohol and had m.p. 236°C.
Nitration of the product m.p. 327-328°C.

The product (1g) was taken in a mixture of nitric acid (15 c.c.) and acetic acid (5 c.c.) in a conical flask. A red solution was formed with evolution of heat. The above red solution was warmed on a water bath with constant shaking till the evolution of nitrous fumes ceased.

The mixture was kept for some time but no solid product separated. Thereupon a little of the mixture was diluted with water and extracted with ether, benzene and petroleum ether, but nothing could be obtained.

After treatment with the above solvents, the aqueous layer was concentrated in a vacuum desiccator (water pump). When completely evaporated a crop of white crystals was obtained, m.p. 100°C. This was identified as oxalic acid.

The nitration of the product was repeated several times with varying concentrations of nitric acid and in different media such as sulphuric acid, acetic acid, and water but no definite products except oxalic acid or the unreacted material could be obtained.

Alkali fusion of product m.p. 327-328°C.

The substance (2g) was put into a boiling solution of potassium hydroxide (10 g, 10 c.c. water) in a nickel crucible and heated in an oil bath at 180-200°C until a drop of the solution taken in water gave no precipitate with hydrochloric acid two (2) hours.

The fused mass was then taken in water (100 c.c.) and neutralised by hydrochloric acid. A clear brown solution was obtained. This solution was then slightly acidified whereupon
a turbidity appeared. This turbid solution was extracted with ether, the red ethereal layer was washed with water and on recovery of ether a brown crystalline mass was obtained. This crystalline stuff was taken in sodium bicarbonate solution (0.2%) and carbon dioxide was passed into it for some time and then extracted with ether. The ether layer was washed with water, dried over sodium sulphate (anhydrous) and on recovery of ether a few needle shaped crystals m.p. 208-209°C were obtained. Water solution of this product gave no colour with ferric chloride.

The aqueous layer was acidified by dilute hydrochloric acid, extracted with ether, the ethereal layer dried in the usual way, ether recovered and a brown syrup was obtained. This syrup on standing deposited a crystalline product which was drained on porous tile and long white needles m.p. 197-198°C were obtained. The water solution of this product gave with ferric chloride a deep blue colour, and reduced ammoniacal silver nitrate solution.

Examination of the filtrate from the hydrolysis of the m.p. 245-246°C.

A little of the above filtrate was neutralised with ammonia and found to reduce Fehling's solution and ammoniacal silver nitrate solution and to give positive Molisch test.

The filtrate from the hydrolytic product of m.p. 245-246°C was treated with lead acetate until no more precipitate was formed. This lead chloride precipitate was filtered and the filtrate was concentrated by distillation under reduced pressure (water pump). A brown syrup was obtained. This was extracted with ether, benzene and petroleum ether but no result was obtained. The syrup was partially soluble in alcohol. The syru
was then taken in a little water, mixed with an excess of phenyl hydrazine in acetic acid and warmed for some time. Yellow needle shape crystals of osazone separated. This was recrystallised from pyridin and had m.p. 206-207°C. When mixed melt with glucosazone it gave m.p. 206-207°C.

Partial hydrolysis of the product m.p. 245-246°C.

The product (2g) was dropped in a boiling solution of sulphuric acid (½%) and the boiling was continued for half an hour. The clear yellow solution so formed was left overnight. A yellow flocculent precipitate was formed, the precipitate filtered, washed free of mineral acid, and recrystallised from absolute alcohol had m.p. 256-257°C.

The filtrate from the product m.p. 256-257°C gave the tests of reducing sugar. It was then treated with lead acetate to remove sulphuric acid. After separating the lead sulphate precipitate, the filtrate was concentrated to a syrup. This syrup was taken in water, added to phenyl hydrazine (1g) in acetic acid (5 c.c.) and warmed on a water bath for about 20 minutes when yellow crystals separated. These on recrystallisation from absolute alcohol had m.p. 205-206°C, and were identical with glucosazone (mixed melt).

Examination of the product m.p. 211-212°C.

Water as well as alcoholic solution of the above product produced with ferrous sulphate solution (freshly prepared) blood red colouration, while with ferric chloride solution dark blue colour. Highly concentrated alcoholic solution of this product gelatinised and its water solution even at a lower concentration readily gelatinised.
Combustion of the product m.p. 211-212°C.

The product was dried in an air oven at m.p. 125-130°C for a couple of hours and then analysed.

(i) The weight of the boat and the substance

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6438</td>
</tr>
<tr>
<td>3.7794</td>
</tr>
<tr>
<td>0.1356</td>
</tr>
</tbody>
</table>

(ii) The weight of the calcium chloride tube before expt.

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.8022</td>
</tr>
<tr>
<td>34.8606</td>
</tr>
<tr>
<td>0.0584</td>
</tr>
</tbody>
</table>

(iii) The weight of sofnoilite tube before experiment

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.3074</td>
</tr>
<tr>
<td>43.0720</td>
</tr>
<tr>
<td>0.2646</td>
</tr>
</tbody>
</table>

Found, C, 53.22%; H, 4.30%.

(i) The weight of the boat and the substance

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.7740</td>
</tr>
<tr>
<td>8.9662</td>
</tr>
<tr>
<td>0.1922</td>
</tr>
</tbody>
</table>

(ii) The weight of calcium chloride tube before expt.

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.7142</td>
</tr>
<tr>
<td>34.7950</td>
</tr>
<tr>
<td>0.0308</td>
</tr>
</tbody>
</table>

(iii) The weight of sofnoilite tube before experiment

<table>
<thead>
<tr>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.3628</td>
</tr>
<tr>
<td>42.7420</td>
</tr>
<tr>
<td>0.3792</td>
</tr>
</tbody>
</table>

Found, C, 53.33%; H, 4.61%.
Regulated hydrolysis of the product m.p. 211-212°C.

The product (1.0g) was put into boiling diluted sulphuric acid (½%, 150 c.c.). A clear yellow solution was immediately produced. The boiling of this solution was continued for half an hour. On cooling, the solution deposited yellowish flocculent precipitate. After standing overnight, the precipitate was filtered and washed free of mineral acid by cold water. On recrystallisation from alcohol the above product gave slightly yellowish, short needles m.p. 234-235°C. The yield was about 0.7g.

The filtrate from the above product gave faint yellow colour with sodium carbonate, and readily reduced Fehling's solution, ammoniacal silver nitrate solution and gave positive Molisch's test.

The above partial hydrolysis of the product m.p.211-212°C was repeated with the substance (4.5g) and dilute sulphuric acid (½%, 750 c.c.). On half an hour's boiling and cooling overnight, the light yellow precipitate (3.4g) was obtained. This product recrystallised from alcohol had m.p. 234-235°C.

This light yellow crystalline product was heated in an air over at 125-130°C for about an hour and analysed (combustion).

Combustion of the product m.p. 234-235°C.

(i) The weight of the boat 3.7740g

The weight of the boat and the substance 8.9146g

0.1406g

(ii) The weight of calcium chloride tube before experiment 30.1130g

The weight of calcium chloride tube after experiment 30.1752g

0.0572g
(iii) The weight of sofnolite tube before experiment 36'5824g

The weight of sofnolite tube after experiment. 36'8695g

Found, C, 55'62%; H, 4'52%.

Total hydrolysis of the product m.p. 211-212°C.

The filtrate from the product m.p. 234-235°C was neutralised with barium hydroxide solution and the filtrate was concentrated by distillation under reduced pressure. The concentrated a yellowish syrup was taken in a little water, mixed with an excess of phenyl hydrazine (2 c.c.) in acetic acid(6 c.c.) and warmed on a water bath for an hour, when on cooling yellow crystalline product was obtained. It was filtered, washed free of phenyl hydrazine by dilute acetic acid and melted at 144-147°C. This was recrystallised twice from alcohol and pyridine and had m.p. 153-155°C.

A little of the filtrate from the product m.p. 343°C was neutralised and was found to reduce Fehling's solution,
ammoniacal silver nitrate solution.

The hydrolysis of the product m.p. 211-212°C was repeated with larger quantity of the substance (8.52 g) and hydrochloric acid (10%, 1000 c.c. 4 hours). The yield of the product was 3.6 g. This product was recrystallised from alcohol and had m.p. 343°C.

During the course of this work large quantities of the product m.p. 211-212°C, had to be hydrolysed and it was found that neither prolonged refluxing (ten or twenty hours) nor very large concentration of the acid is necessary. In fact the reaction goes well with hydrochloric acid (5%) and four (4) hours refluxing. The only change that could be marked on prolonged heating was in the colour of the product, which slightly deepened and became brownish.

Combustion of the product m.p. 343°C.

(i) The weight of the boat
The weight of the boat and the substance 8.9212 g

(ii) The weight of the calcium chloride tube before expt. 38.5246 g
The weight of the calcium chloride tube after expt. 33.5718 g

(iii) The weight of the sofnolite tube before experiment 41.0848 g
The weight of the sofnolite tube after experiment 41.4428 g

Found, C, 66.33%; H, 3.56%.
Treatment of the filtrate from the total hydrolysis of the product 211-212°C.

The filtrate from the total hydrolysis of the product 211-212°C gave all the reduction tests of a reducing sugar and positive Molisch's test. It was treated with lead acetate to get rid of hydrochloric acid as lead chloride and thereafter with hydrogen sulphide to separate lead and then concentrating under reduced pressure to a syrup. This syrup was taken in water (10 c.c.) and treated with phenyl hydrazine (2g in 3 c.c. glacial acetic acid) in the usual way, whereupon it gave a yellow water insoluble osazone. The osazone on recrystallisation came in yellow fine needles m.p. 206-207°C, identical with glucosazone (mixed melt).

Methylation of the product m.p. 345°C.

The product (0.5g) was taken with caustic potash (0.5g) in methyl alcohol (15 c.c.) in a round bottom flask (150 c.c.) and refluxed with methyl iodide (10 c.c.) for thirty six hours on a water bath. The excess methyl iodide and some methyl alcohol was then distilled off. This gave a brown solid mass. This was taken in water, extracted with ether and the ethereal layer, washed with dilute alkali (0.5% caustic soda solution). The alkali was washed with distilled water and then ethereal solution dried over sodium sulphate (anhydrous). On recovery of ether a pale yellow crystalline product was obtained. This product was recrystallised (twice) from absolute alcohol and had m.p. 170-171°C. It was a pale yellow product in shining needles.
Alkali fusion of the product m.p. 343°C.

The product (10g) was taken with caustic potash (1g) and water (5 c.c.) in a nickel dish. The mixture—a yellow liquid, was then heated at 150-170°C in an oil bath. The yellow liquid became gradually brown and after one hour's heating it was cooled and the fused mass dissolved in water (100 c.c.). A little of this solution gave no precipitate with mineral acid showing the complete decomposition of the product m.p. 343°C. The above solution was acidified (little excess) with dilute sulphuric acid and extracted with ether. The ethereal layer was washed and the ether recovered. A brown sticky crystalline mass was obtained. This was taken in dilute solution of sodium bicarbonate and thoroughly extracted with ether. The ethereal layer was washed with water and on removing ether a brown oily product was obtained. This was again taken in a little water, concentrated in a vacuum desiccator, and a colourless crystalline product obtained. This was filtered and recrystallised from alcohol. This product melted at 209-210°C, and when mixed with phloroglucinol showed no melt depression. The sodium bicarbonate solution after extraction with ether was acidified with dilute sulphuric acid and extracted with ether. The ethereal layer, after washing free of sulphuric acid was evaporated to dryness and a brown crystalline product was obtained. This was taken in benzene which on gradual concentration gave colourless needles m.p. 203-209°C and was identical with p-hydroxy benzoic acid (mixed melt).

Nitration of the product m.p. 343°C.

The substance (0.5g) was taken in concentrated nitric acid (10 c.c.) and warmed on a water bath with constant shaking till
no more nitrous fumes were evolved. The solution was evaporated in a vacuum desiccator at the water pump. A crystalline product was obtained. This was oxalic acid (m.p. 100°C).

**Acetylation of the product m.p. 343°C.**

The product (0.4g) was refluxed with freshly distilled acetic anhydride (10 c.c.) and a few crystals of zinc chloride (anhydrous), in a round bottom flask (100 c.c.) on a sand bath. After an hour the liquid in the flask was poured into cold water. A turbid solution was obtained. On cooling it gave a white precipitate, which was filtered, washed with water and recrystallised from alcohol. Thus a white crystalline product m.p. 213-214°C was obtained, which did not correspond in melting point to triacetyl apigenin (m.p. 181-182°C).

**Hydrolysis of the product m.p. 234-235°C.**

The product (1g) was taken in boiling water (200 c.c.) and hydrochloric acid (20 c.c.) in a round bottom flask and refluxed for four hours on a sand bath. A light yellow precipitate was obtained which was filtered, washed free of mineral acid and crystallised from alcohol. This product melted at 343°C and when mixed with apigenin showed no melt depression.

**Alkali fusion of the product m.p. 211-212°C.**

The product (2g) was heated with potassium hydroxide (10g in 10 c.c. water) at 180-200°C for two hours. The fused mass was then treated in the manner earlier described (alkali fusion of the substance m.p. 343°C) and two crystalline products one melting at 209-210°C and the other at 208-209°C were obtained. These compounds were found identical (mixed melt) with p-hydroxy benzoic acid and phloroglucinol respectively.