EXPERIMENTAL WORK*

1. DETERMINATION AND TESTING OF STRYCHNOS POTATORUM LINN. f.
EXTRACTIVES

1.1 The Seed Extractives

The seeds were rendered into moderately coarse powder and the powder (50 g) was extracted successively with different solvents — petroleum ether (40-60°), benzene, ether, chloroform, acetone, ethanol and water. The per cent extractive was determined in each case (Table 2).

Tests for alkaloids, glycosides (and/or carbohydrates), sterols, tannins, proteins and free amino acids were carried out with each of the extractives.

Tests for Alkaloids:

A little of the residue from the respective extract obtained by evaporating the solvent, was rubbed with hydrochloric acid (2% w/w; 3 ml), filtered, and the filtrate tested

* The melting points (mp) reported are uncorrected. For column chromatography alumina, according to Brockmann (E. Merck), was used unless indicated otherwise. Thin-layer chromatography was carried on glass plates coated with silica gel G (250 μ) and activated at 110° for 30 min.
TABLE 2

Per cent Extractives of S. potatorum Seeds

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Period (hr)</th>
<th>Colour of the Extractive</th>
<th>Extractive (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40-60°)</td>
<td>22</td>
<td>Light yellow</td>
<td>2.26</td>
</tr>
<tr>
<td>Benzene</td>
<td>18</td>
<td>Dark brown</td>
<td>0.92</td>
</tr>
<tr>
<td>Ether</td>
<td>15</td>
<td>Greenish yellow</td>
<td>0.16</td>
</tr>
<tr>
<td>Chloroform</td>
<td>16</td>
<td>Brownish yellow</td>
<td>0.11</td>
</tr>
<tr>
<td>Acetone</td>
<td>16</td>
<td>Yellow</td>
<td>0.31</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20</td>
<td>Dark red</td>
<td>1.06</td>
</tr>
<tr>
<td>Water</td>
<td>36</td>
<td>Dark brown</td>
<td>2.04</td>
</tr>
</tbody>
</table>

with Mayer's reagent, Dragendorff's reagent, Wagner's reagent and Ammonium Reineckate Solution. A few drops of the aqueous extract were tested directly after acidification with dilute hydrochloric acid.

Tests for Glycosides and Carbohydrates:

Molisch's Test: A few milligrams of the residue from an extractive were taken up in ethanol. Two drops of a 20% w/v solution of α-naphthol in ethanol were added to it and concentrated sulphuric acid (1 ml) was allowed to flow down the side of the test tube. Appearance of a red-violet ring at the junction of two layers indicated the presence of glycosides and/or carbohydrates.
**Fehling's Test:** A few milligrams of the residue were taken up in distilled water (2 ml) and filtered. To the filtrate was added an equal amount of Fehling's solution and the contents were boiled. Appearance of a brick-red precipitate indicated the presence of reducing sugars. A few drops from the aqueous extract were tested directly.

**Tollen's Test:** A few milligrams of the residue were taken up in distilled water (2 ml) and filtered. To the filtrate were added a few drops of ammonical silver nitrate reagent, and kept in a boiling water-bath for a few minutes. Appearance of silver mirror along the side of the test tube indicated presence of reducing sugars.

**Tests for Sterols:**

**Salkowski Reaction**\(^73\): A few milligrams of the residue were dissolved in chloroform (1 ml) and an equal amount of sulphuric acid was added. Appearance of a blood-red colour indicated the presence of sterols.

**Liebermann's Reaction**\(^74\): A few milligrams of the residue were dissolved in acetic anhydride (1 ml) with the aid of heat. The contents were cooled and a few drops of concentrated sulphuric acid were added. Appearance of a blue colour was taken as a positive test for sterols.
Liebermann-Burchard Reaction: A few milligrams of the residue were dissolved in chloroform, a few drops of acetic anhydride were added followed by concentrated sulphuric acid. Transient colour developments indicated the presence of sterols.

Tests for Tannins:

A few milligrams of the residue were taken up in distilled water (2 ml), filtered, and the filtrate was tested with a 10% w/v lead acetate solution and ferric chloride solution. Appearance of a precipitate in the first case and a bluish green colour in the second would indicate the presence of tannins.

Tests for Proteins:

Millon's Test: A few milligrams of the residue were taken up in distilled water (2 ml), filtered, and to the filtrate were added a few drops of Millon's reagent. Aqueous extract was tested directly. Formation of a precipitate indicated the presence of proteins.

Tests for Free Amino Acids:

A few drops of the extract were spotted on a filter paper and allowed to dry. The paper was then sprayed with ninhydrin reagent and heated in an air oven for a few minutes. Appearance of a bluish-violet colour pointed towards the
presence of free amino acids.

The results are summarised in Table 3.

**TABLE 3**

Response of the Extractives of *S. potatorum* Seeds to Qualitative Tests for Different Organic Constituents

<table>
<thead>
<tr>
<th>Test</th>
<th>Pet. Ether Chloro- Acetone Ethanol Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer's reagent</td>
<td>- - - + + + -</td>
</tr>
<tr>
<td>Dragendorff's reagent</td>
<td>- + - + + + -</td>
</tr>
<tr>
<td>Wagner's reagent</td>
<td>- + - + + + -</td>
</tr>
<tr>
<td>Ammonium Reineckate Solution</td>
<td>- - - - + + -</td>
</tr>
</tbody>
</table>

For Glycosides and/or Carbohydrates

<table>
<thead>
<tr>
<th>Test</th>
<th>Pet. Ether Chloro- Acetone Ethanol Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molisch's test</td>
<td>- - - + + + +</td>
</tr>
<tr>
<td>Fehling's test</td>
<td>- - - + + + +</td>
</tr>
<tr>
<td>Tollin's test</td>
<td>- - - + + + +</td>
</tr>
</tbody>
</table>

For Sterols

<table>
<thead>
<tr>
<th>Test</th>
<th>Pet. Ether Chloro- Acetone Ethanol Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salkowski reaction</td>
<td>+ + + + + - -</td>
</tr>
<tr>
<td>Liebermann's reaction</td>
<td>+ + + + + - -</td>
</tr>
<tr>
<td>Liebermann-Burchard reaction</td>
<td>+ + + + + - -</td>
</tr>
</tbody>
</table>

(Contd.)
### TABLE 3 (Contd.)

<table>
<thead>
<tr>
<th>Test</th>
<th>Pet.</th>
<th>Ben-</th>
<th>Ether</th>
<th>Chloro-</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For Tannins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate solution</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferric chloride solution</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>For Proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millon's test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>For Free Amino Acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ninhydrin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ sign indicates positive response; - sign indicates negative response.

---

1.2 **The Bark Extractives**

The bark was reduced to moderately coarse powder and the powder (40 g) was extracted successively with different solvents and the percentage of extractives determined. The extractives were tested for alkaloids, glycosides and/or carbohydrates, sterols, tannins, proteins and free amino acids following the details as outlined under Sect. 1.1. The results are summarised in Tables 4 and 5.
TABLE 4
Per cent Extractives of S. potatorum Bark

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Period (hr)</th>
<th>Colour of Extractive</th>
<th>Extractive (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40-60°)</td>
<td>18</td>
<td>Light yellow</td>
<td>1.00</td>
</tr>
<tr>
<td>Benzene</td>
<td>16</td>
<td>Brown</td>
<td>0.38</td>
</tr>
<tr>
<td>Ether</td>
<td>15</td>
<td>Pale green</td>
<td>0.18</td>
</tr>
<tr>
<td>Chloroform</td>
<td>16</td>
<td>Dark brown</td>
<td>1.87</td>
</tr>
<tr>
<td>Acetone</td>
<td>18</td>
<td>Brown</td>
<td>0.38</td>
</tr>
<tr>
<td>Ethanol</td>
<td>18</td>
<td>Red</td>
<td>2.25</td>
</tr>
<tr>
<td>Water</td>
<td>28</td>
<td>Dirty brown</td>
<td>9.75</td>
</tr>
</tbody>
</table>

TABLE 5
Response of the Extractives of S. potatorum Bark to Qualitative Tests for Different Organic Constituents

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides and/or Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Free Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ sign indicates positive response; - sign indicates negative response.
1.3 The Leaf Extractives

The preliminary experiments as described under Sect. 1.1 were carried out using powdered leaves (100 g). The results are summarised in Tables 6 and 7.

**TABLE 6**
Per cent Extractives of *S. potatorum* Leaves

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Period (hr)</th>
<th>Colour of the Extractive</th>
<th>Extractive (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether (40-60°)</td>
<td>20</td>
<td>Dark green</td>
<td>4.75</td>
</tr>
<tr>
<td>Benzene</td>
<td>16</td>
<td>Green</td>
<td>1.25</td>
</tr>
<tr>
<td>Ether</td>
<td>16</td>
<td>Green</td>
<td>0.45</td>
</tr>
<tr>
<td>Chloroform</td>
<td>18</td>
<td>Dark green</td>
<td>9.20</td>
</tr>
<tr>
<td>Acetone</td>
<td>18</td>
<td>Green</td>
<td>1.20</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20</td>
<td>Green</td>
<td>8.08</td>
</tr>
<tr>
<td>Water</td>
<td>30</td>
<td>Dirty brown</td>
<td>8.50</td>
</tr>
</tbody>
</table>

**TABLE 7**
Response of the Extractives of *S. potatorum* Leaves to Qualitative Tests for Different Organic Constituents

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosides and/or</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Contd.)
TABLE 7 (Contd.)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Free Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ sign indicates positive response; 
- sign indicates negative response.

2. DETERMINATION OF ASH OF S. POTATORUM SEEDS

2.1 Total Ash

An accurately weighed amount of the air-dried powder was incinerated in a tared silica crucible to a constant weight. The results of three such experiments are summarised in Table 8.

TABLE 8

<table>
<thead>
<tr>
<th>Wt. of the Powder (g)</th>
<th>Wt. of the Ash (g)</th>
<th>Total Ash (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4418</td>
<td>0.0756</td>
<td>3.09</td>
</tr>
<tr>
<td>2.6624</td>
<td>0.0820</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Mean 3.08
2.2 Acid-insoluble Ash

The total ash obtained under 2.1 was boiled with dilute hydrochloric acid (25 ml) for 5 min. The insoluble matter was collected on an ashless filter paper, washed with warm distilled water and ignited in a tared silica crucible till free from carbon. The results are summed up in Table 9.

<table>
<thead>
<tr>
<th>Wt. of the Powder (g)</th>
<th>Wt. of the Acid-insoluble Ash (g)</th>
<th>Acid-insoluble Ash (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4418</td>
<td>0.0172</td>
<td>0.70</td>
</tr>
<tr>
<td>2.6624</td>
<td>0.0188</td>
<td>0.70</td>
</tr>
<tr>
<td>2.5580</td>
<td>0.0186</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.70</td>
</tr>
</tbody>
</table>

2.3 Water-soluble Ash

An accurately weighed amount of air-dried powder was ignited in a tared silica crucible to a constant weight. The ash obtained was boiled for 5 min with distilled water (25 ml), the insoluble matter collected on an ashless filter paper, washed with water and ignited in a tared silica crucible till free from carbon. The weight of the insoluble matter was subtracted from the weight of the total ash.
The results are summed up in Table 10.

### TABLE 10

**Water-soluble Ash of S. potatorum Seeds**

<table>
<thead>
<tr>
<th>Wt. of the Powder (g)</th>
<th>Wt. of the Ash obtained (g)</th>
<th>Wt. of the Water-insoluble Matter (g)</th>
<th>Wt. of the Water-soluble Ash (g)</th>
<th>Water-soluble Ash (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4938</td>
<td>0.0744</td>
<td>0.0650</td>
<td>0.0094</td>
<td>0.37</td>
</tr>
<tr>
<td>2.5388</td>
<td>0.0752</td>
<td>0.0648</td>
<td>0.0104</td>
<td>0.40</td>
</tr>
<tr>
<td>2.6364</td>
<td>0.0800</td>
<td>0.0698</td>
<td>0.0102</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Mean 0.38

3. **STUDY OF S. POTATORUM SEED OIL**

3.1 **Extraction of the Oil**

The seed powder (2.0 kg) was extracted with petroleum ether (60-80°) for about 18 hr in a Soxhlet unit. The extract was cooled and dried over anhydrous sodium sulphate. Filtration followed by removal of the solvent under vacuum yielded the oil (30 g).

3.2 **Determination of the Physical and Chemical Characteristics**

The following physical characteristics were determined:
Specific gravity at 25° 0.9135
Refractive index at 25° 1.4655
Optical rotation negligible

The chemical characteristics of the oil were found by the usual methods\textsuperscript{76,77} (Table 11).

TABLE 11

<table>
<thead>
<tr>
<th>Obs. No.</th>
<th>Iodine Value (ICl method)</th>
<th>Acid Value</th>
<th>Hydroxyl Value</th>
<th>Saponification Equivalent</th>
<th>Unsaponifiable Matter (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59.58</td>
<td>6.96</td>
<td>11.66</td>
<td>369.2</td>
<td>27.2</td>
</tr>
<tr>
<td>2</td>
<td>59.54</td>
<td>7.03</td>
<td>11.52</td>
<td>370.8</td>
<td>27.6</td>
</tr>
<tr>
<td>3</td>
<td>59.68</td>
<td>7.01</td>
<td>11.64</td>
<td>369.1</td>
<td>27.4</td>
</tr>
<tr>
<td>Mean</td>
<td>59.60</td>
<td>7.00</td>
<td>11.62</td>
<td>369.7</td>
<td>27.4</td>
</tr>
</tbody>
</table>

3.3 Separation of the Mixed Fatty Acids and Determination of Chemical Characteristics

The oil (50 g) was saponified by refluxing with 1 N ethanolic potassium hydroxide (200 ml) and the unsaponifiable fraction was separated.\textsuperscript{76} The aqueous layer was acidified with dilute hydrochloric acid and extracted with ether. The solvent was distilled and the traces of it removed under vacuum to give mixed fatty acids (MFA) (35.6 g). Iodine value\textsuperscript{76} and saponification equivalent\textsuperscript{77} of mixed fatty acids were determined. The data are given in Table 12.
TABLE 12

Chemical Characteristics of Mixed Fatty Acids (MFA) from *S. potatorum* Seed Oil

<table>
<thead>
<tr>
<th>Obs. No.</th>
<th>Iodine Value (IC1 method)</th>
<th>Saponification Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.2</td>
<td>275.9</td>
</tr>
<tr>
<td>2</td>
<td>65.8</td>
<td>276.5</td>
</tr>
<tr>
<td>3</td>
<td>65.7</td>
<td>275.0</td>
</tr>
<tr>
<td>Mean</td>
<td>65.9</td>
<td>275.8</td>
</tr>
</tbody>
</table>

3.4 Spectrophotometric Estimation of Unsaturated Fatty Acids

The unsaturated fatty acids were estimated spectrophotometrically by the method of Hilditch *et al.* described below:

Mixed fatty acids (about 0.1 g) were weighed accurately into a small glass capsule and then dropped into a loosely stoppered pyrex tube containing the alkali reagent (10 ml) maintained at 170° (± 0.2) in an oil bath. After 15 min, the tubes were rapidly cooled by immersion in a bath containing ice cold water. The contents were transferred quantitatively to a graduated flask and the volume made up to 250 ml with spectroscopic ethanol. A blank determination was carried out under identical conditions. The solutions were allowed to stand overnight at 0°, filtered and 10 ml each of the filtrate was diluted to 100 ml with spectroscopic ethanol. The absorbance
readings of the sample and blank were taken at 268 nm on Beckmann DB spectrophotometer. There was no absorption.

Another portion of the mixed fatty acids (about 0.1 g) was isomerised at 180° (± 0.2) for 60 min and the above experiment repeated. The absorbance readings were taken at 234 nm with 2 ml each of the filtrate diluted to 100 ml. % was found to be 100.2.

Mixed fatty acids were also examined in UV region for any preformed conjugation. No absorption was observed.

From the above data the total dienes as linoleic acid were determined using reference values of Hilditch et al. The total monoenees were calculated as oleic acid from the iodine value of mixed fatty acids (Sect. 3.3) after allowance had been made for the contribution of the dienes present. Saturated acid content was obtained by difference. The results are as follows:

- Total dienes as linoleic acid: 11.1%
- Total monoenes as oleic acid: 50.9%
- Saturated acids (by difference): 38.0%

3.5 Estimation of Total Saturated Acids by Bertram Oxidation

The total saturated fatty acids were estimated by Bertram oxidation method modified by Pelikan and von Mikusch described below:
The mixed fatty acids (about 5 g) were weighed accurately and saponified by refluxing with 0.5 N ethanolic potassium hydroxide (75 ml) for 30 min. The soap formed, after complete removal of ethanol, was diluted with water (200 ml) and treated, with continuous swirling and cooling, with a solution of potassium permanganate (35 g) in water (750 ml). The reaction mixture was allowed to stand overnight. It was then acidified with sulphuric acid (50% w/v) and the excess potassium permanganate was reduced with sodium bisulphite. The solution was boiled for 30 min, cooled and extracted with petroleum ether (40–60°C) (3 x 50 ml). The petroleum ether extract was washed with water and dried over anhydrous sodium sulphate. Removal of the solvent gave fatty acids. The fatty acids were warmed with dilute ammonia solution (25 ml) to give a clear homogenous solution. The solution was diluted with boiling water (200 ml), treated with a solution of ammonium chloride (10% w/v; 30 ml) and boiled. A solution of magnesium sulphate (15% w/v; 20 ml) was then added and the mixture boiled, cooled and filtered. The precipitate was transferred along with filter paper into a flask and to it was added sulphuric acid (50% w/v; 15 ml). It was heated on a water bath and swirled till the filter paper was completely disintegrated. The mixture was cooled in ice and made ammonical with strong solution of ammonia. The process of precipitation and filtration as outlined above was repeated, the magnesium salts of fatty acids were transferred back to the flask and decomposed.
with sulphuric acid and extracted with petroleum ether (40-60\(^\circ\)) (3 x 30 ml). The petroleum ether extract was washed with water and dried in a tared flask. The iodine value of isolated fatty acids was determined. The percentage of the saturated fatty acids (G) was calculated from the formula,

\[
G = \frac{100}{S} \left( W - \frac{IW}{90} \right)
\]

where, \( W \) = wt. of saturated fatty acids obtained
\( I \) = iodine value of the fatty acids
\( S \) = wt. of the sample used (MFA)
\( 90 \) = iodine value of oleic acid of which a small fraction passes off into the saturated acids.

The mean of three observations came out to be 37.1%.

3.6 Study of Fatty Acid Composition of the Oil

The mixed fatty acid fraction was converted to methyl esters. These were subjected to infrared and TLC studies before their final analysis by GLC.

3.6a Preparation of Methyl Esters

The fatty acids (10 g) were converted to methyl esters by boiling with methanol (50 ml) containing sulphuric acid (0.5 ml) for 2 hr, and removing the unesterified acids by washing the ether solution of esters with potassium carbonate solution. The ether solution was washed with water, dried over anhydrous sodium sulphate, and evaporated to yield the
methyl esters (8.1 g).

3.6b Spectral Study of Methyl Esters

The infrared spectrum of methyl esters showed bands at 2997 cm\(^{-1}\) (olefinic C—H stretching); 2918 and 2846 cm\(^{-1}\) (for CH\(_2\) and CH\(_3\)); 1740 cm\(^{-1}\) (C=O stretching); 1230, 1185, and 1160 cm\(^{-1}\) (C—O stretching for esters).

3.6c Chromatographic Study of Methyl Esters

i) Thin-layer Chromatography of the Methyl Esters: A petroleum ether solution of the methyl esters was spotted on silica gel G plates and developed using the system, petroleum ether-ether-acetic acid (70:30:1). The plates were sprayed with concentrated sulphuric acid and heated at 150° for 10 min. There was only one spot comparable to the non-oxygenated fatty acid ester of ground-nut oil, thereby indicating the absence of any oxygenated acid ester.

The unsaturated fatty acids were detected by TLC as silver ion complexes. A petroleum ether solution of methyl esters was spotted on silica gel G plates containing 12.5% silver nitrate and developed using the system, petroleum ether-ether-acetic acid (90:10:1). For comparison, methyl esters prepared from linseed oil were run side by side. The spots were detected by spraying with concentrated sulphuric acid and heating at 150° for 10 min. Three spots with R\(_f\) values 0.39, 0.57 and 0.73, respectively, were detected which...
compared with methyl linoleate (0.40), methyl oleate (0.57) and methyl esters of saturated fatty acids (0.73).

The saturated fatty acids, separated from mixed fatty acids by Bertram oxidation (Sect. 3.5), were converted to methyl esters. These were identified by reversed-phase partition-TLC. A petroleum ether solution of the methyl esters was spotted on silica gel G plates impregnated with 6% silicone oil (Dow Corning 200 Fluid) in ether using the system, acetonitrile-acetic acid-water-silicone oil (70:10:20:sat.). The methyl esters of palmitic, stearic, arachidic and behenic acid in equimolar mixture were used as reference standard. The $R_f$ values of the spots were 0.41, 0.29, 0.17, respectively, which compared with methyl palmitate (0.41), methyl stearate (0.29) and methyl arachidate (0.17). There was one spot just above the base line and that might have been due to methyl lignocerate. Spot corresponding to methyl behenate could not be detected.

ii) **Gas-liquid Chromatography of the Methyl Esters:**

The methyl esters of mixed fatty acids were analysed by GLC (Fig. 1) on a polyester column (20% diethylene glycol succinate on Chromosorb W, 8 ft. x 3/16 in., 215$^\circ$) with a thermal conductivity detector. The peak areas were measured by triangulation and the calculated composition was converted from weight to mole per cent. The results are given in Table 1 (under Results and Discussion).
FIG. 1
GAS CHROMATOGRAM OF METHYL ESTERS OF FATTY ACIDS FROM STRYCHNOS POTATORUM SEEDS
FIG. 2
LOG- PLOT OF METHYL ESTERS OF FATTY ACIDS
FROM S. POTATORUM SEEDS
petroleum ether fractions consisted of colourless oily hydrocarbons. Elution with petroleum ether-benzene (80:20) gave a residue (0.82 g) which was crystallised repeatedly from acetone to give silky needles, mp 184-186°C (Fraction A). It gave red violet colour when subjected to Liebermann-Burchard test. Further elution with petroleum ether-benzene (70:30) yielded a residue (0.21 g) which on crystallisation from acetone gave colourless needles, mp 218-220°C (Fraction B). The fraction gave red colour on Liebermann-Burchard test. Benzene-chloroform (90:10) eluted a residue (0.12 g) which crystallised from methanol, mp 137-138°C (Fraction C). It gave a green colour on Liebermann-Burchard test. Elution with benzene-chloroform (75:25) gave a residue (0.108 g) which on crystallisation from acetone gave small crystals, mp 186-188°C (Fraction D). The fraction gave a brown colour on Liebermann-Burchard test. Further elutions with solvents of increasing polarity gave oily residues.

4.2 Thin-layer Chromatography

The TLC study of fractions was done using silica gel G plates. The solvent systems used were: toluene-ethyl acetate (9:1), and benzene-ethyl acetate (7:3). The plates were sprayed with a solution of phosphomolybdic acid in ethanol (10% w/v) and the spots were detected after heating the plates at 80°C for 10 min. The 'Fractions A, B, C and D' each gave a single spot on TLC.
4.3 Study of 'Fractions A, B, C, and D'

4.3a 'Fraction A'

The infrared spectrum (Fig. 3) showed an alcoholic \( \text{OH} \) stretching (3400 cm\(^{-1}\)) and a band at 1044 cm\(^{-1}\) for \( \text{C} = \text{O} \) stretching.

The NMR spectrum (CDCl\(_3\)) (Fig. 4) showed a triplet at \( \delta 5.18 (J = 3 \text{ cps}) \) for vinylic proton (\( \text{CH} - \text{CH} = \text{C} \)), and a multiplet at \( \delta 3.28 \) for \( \text{CH} - \text{CH} = \text{C} \). Signals due to C-methyl groups appeared at \( \delta 0.8 \) (12 H), 0.96 (3 H), 1.01 (6 H) and 1.08 (3 H).

The mass spectrum (Fig. 5) showed a molecular ion peak at \( m/e \) 426 (11%), and other characteristic peaks at \( m/e \) 411 (2%), 218 (100%), 207 (6%), 203 (7%), 189 (6%) and 133 (8%).

4.3b 'Fraction B'

The infrared spectrum (Fig. 6) showed an alcoholic \( \text{OH} \) stretching (3300 cm\(^{-1}\)) and a band at 1033 cm\(^{-1}\) for \( \text{C} = \text{O} \) stretching.

The NMR spectrum (CDCl\(_3\)) (Fig. 7) showed multiplets at \( \delta 3.28 \) and 5.45. Signals due to C-methyl groups appeared at \( \delta 0.75, 0.80, 0.85, 0.97, 1.00 \) and 1.05.

The mass spectrum (Fig. 8) showed a molecular ion peak at \( m/e \) 426 (26%) and other characteristic peaks at \( m/e \) 220 (11%), 218 (99%), 207 (24%), 189 (35%) and 59 (100%).
FIG. 3
INFRARED SPECTRUM OF 'FRACTION A' (NUJOL)
RELATIVE INTENSITY
FIG. 6

INFRARED SPECTRUM OF 'FRACTION B' (NUJOL)
4.3c 'Fraction C'

The infrared spectrum (Fig. 9) showed bands at 3500 and 3400 cm\(^{-1}\) (associated O—H stretching), 1650 cm\(^{-1}\) (non-conjugated C=C stretching), 1035 cm\(^{-1}\) (C—O stretching) and a band at 980 cm\(^{-1}\) for trans-disubstituted double bond.

The NMR spectrum (CDCl\(_3\)) (Fig. 10) showed a multiplet at \(\delta 5.34\) for vinylic proton (\(>\text{C}=\text{CH}—\text{CH}_2—\)), and also at \(\delta 5.08\) for a trans-substituted double bond proton of stigmasterol. Singlets for C-18 and C-19 methyl groups appeared at \(\delta 0.69\) and 1.01, respectively.

The mass spectrum (Fig. 11) showed a molecular ion peak at m/e 414 (39%) and other peaks at m/e 399 (7%), 396 (10%), 381 (6%), 329 (7%), 303 (11%), 275 (3%), 273 (13%), 231(10%), 131 (23%) and 43 (100%). A peak at m/e 412 (26%) was indicative of molecular ion of stigmasterol.

4.3d 'Fraction D'

The infrared spectrum (Fig. 12) showed a band at 3450 cm\(^{-1}\) for O—H stretching and a band at 1060 cm\(^{-1}\) (C—O stretching).

The NMR spectrum (CDCl\(_3\)) (Fig. 13) showed signals for C-methyl groups at \(\delta 1.43, 1.31, 1.23, 0.98, 0.91\) and 0.83.

The mass spectrum (Fig. 14) showed a molecular ion peak at m/e 444 (15%) and other characteristic peaks at m/e
FIG. 9
INFRARED SPECTRUM OF 'FRACTION C' (NUJOL)
FIG. 2

MASS SPECTRUM OF ALKALOIDAL FRACTION C'

RELATIVE INTENSITY
TAKEN AS BASE PEAK
100
90
80
70
60
50
40
30
20
10
0

A
20 30 40 50 60 70

T T

H i l T

i i l

TT

FIG. 2

MASS SPECTRUM OF ALKALOIDAL FRACTION C'
FIG. 13

NMR SPECTRUM (600 MHz) OF FRACTION D' (CDCl₃)
FIG. 12

INFRARED SPECTRUM OF 'FRACTION D' (NUJOL)
429 (8%), 426 (33%), 411 (26%), 220 (2%), 207 (2%), 189 (11%) and 59 (100%).

5. STUDY OF THE ALKALOIDAL CONSTITUENTS OF **P. POTATORUM** SEEDS

5.1 Isolation of Total Alkaloids ('Alkaloidal Fraction A')

The total alkaloids were isolated by the following procedure:

The seed powder (250 g) was shaken with a mixture of two volumes of ether and one volume of chloroform (1 l) and allowed to stand for 10 min. Dilute solution of ammonia (100 ml) was then added and the mixture shaken continuously (mechanically) for 6 hr. The mixture was then transferred to a Soxhlet apparatus with the aid of more of the same solvent mixture and extracted for 2 hr. The extract was filtered and the filtrate shaken with 1 N sulphuric acid (5 x 200 ml) to effect complete extraction of the alkaloids. The acid extracts were combined, made alkaline with strong solution of ammonia, and extracted with chloroform till complete extraction of the alkaloids. The combined chloroform extract was washed with water and dried over anhydrous sodium sulphate. The chloroform extract was evaporated in a tared container. The residue was finally dried under vacuum and weighed (0.75 g).

The content of the total alkaloids ('Alkaloidal Fraction A') came out to be 0.30% w/w of the air-dried seed powder.
5.2 Chromatographic Study of 'Alkaloidal Fraction A' and Separation of 'Alkaloidal Fraction B'

5.2a Detection of the Constituents by Thin-layer Chromatography

The TLC study was carried out using silica gel G plates. The following solvent systems were used:

   [Strong solution of ammonia (17 ml) was diluted with water (83 ml) and the solution used].

B. Ethyl acetate-isopropanol-ammonia (80:15:5).
   (Strong solution of ammonia used).

C. Ethyl acetate-isopropanol-ammonia (10:2:1).
   (Strong solution of ammonia used).

D. n-Butanol-0.1 N hydrochloric acid-potassium ferrocyanide solution (100:15:34).
   [A 10% w/v aqueous potassium ferrocyanide solution (25 ml) was diluted with water (9 ml) and used].
   The mixture of the three components of the system was thoroughly shaken, allowed to separate into two layers, the bottom layer was run off and the top one was used in the tank for chromatography.

A solution of 'Alkaloidal Fraction A' in chloroform was spotted on the silica gel G plate and developed using solvent system A. For comparison authentic samples of common
Strychnos alkaloids were run side by side. The spots were detected by 2% ceric sulphate in 2 N sulphuric acid and heating at 105° for 30 min. The major spot with $R_f$ value 0.60 compared with diaboline (0.60). The other spots with $R_f$ values 0.65, 0.55, 0.41 and 0.39, respectively, compared with strychnine (0.66), brucine (0.55), strychnine N-oxide (0.42) and brucine N-oxide (0.39).

The co-chromatography of 'Alkaloidal Fraction A' with minor Strychnos alkaloids using solvent system D and detecting the spots with 2% ceric sulphate in 2 N sulphuric acid and Dragendorff's reagent revealed the spots of $R_f$ values 0.51, 0.37, 0.31, 0.20 and 0.03, respectively, which compared with pseudostrychnine (0.51), vomicine (0.37), icajine (0.32), novacine (0.20) and brucine (0.03). The spots corresponding to vomicine, icajine and novacine were in trace amounts.

The 'Alkaloidal Fraction A' was run with diaboline in solvent system B and the spots were detected with Dragendorff's reagent. A major spot with $R_f$ value 0.28 ran parallel to diaboline (0.28).

5.2b Gas-liquid Chromatographic Study

The 'Alkaloidal Fraction A' was analysed by GLC using Perkin-Elmer F11 apparatus on a 2 ft. x 1/8 in. column filled with 80-100 mesh Varaport 30 coated with 5% SE-52 with a hydrogen-flame ionisation detector. The carrier gas was nitrogen at 7 psi. The column temperatures were 250° and 225°.
GLC resolution at 250° (Fig. 15) detected diaboline as the main component with retention time relative to strychnine as 0.75. A peak close to diaboline was indicative of acetyl-diaboline. Strychnine and brucine were detected in small amounts. Several peaks with retention time shorter than diaboline were observed at a column temperature of 225° (Fig. 16), but it was not clear whether these were due to unknown alkaloids or to non-alkaloidal components.

5.2c Resolution of 'Alkaloidal Fraction B' by Preparative Thin-layer Chromatography

A preparative TLC of 'Alkaloidal Fraction A' (0.20 g) using solvent system A (Sect. 5.2a) was carried out and the major band gave a fraction (0.105 g). The material failed to crystallise or give the hydrochloride salt. The solution of the salt was basified with ammonia and the free base was extracted with chloroform. A check TLC of the recovered base in the system A showed the presence of a mixture. There were two spots, out of which the major one corresponded with diaboline and the other spot ran slightly behind the major one. The product was, therefore, subjected to a second preparative TLC and the major band gave a material (0.022 g; 'Alkaloidal Fraction B'). A check TLC in the same system again indicated that it was a mixture of two entities. Another TLC of 'Alkaloidal Fraction B' was run along with diaboline using chloroform-methanol (4:1). The major spot gave the $R_f$ value 0.13, which compared with diaboline (0.13). The second spot
FIG. 15
GAS CHROMATOGRAM OF 'ALKALOIDAL FRACTION A'
(COLUMN TEMPERATURE 250°)
FIG. 16
GAS CHROMATOGRAM OF 'ALKALOIDAL FRACTION A'
(COLUMN TEMPERATURE 225°)
which ran slightly behind diaboline pointed to acetyl-
diaboline.

5.3 Characterisation of 'Alkaloidal Fraction B'

The mass spectrum (direct insertion, 70 eV, temperature
235°) (Fig. 17) of 'Alkaloidal Fraction B' confirmed it to be
a mixture, and suggested the presence of not only diaboline
itself \([M^+ 352 (49\%); m/e 322 (8\%), 241 (33\%), 180 (22\%),
144 (taken as base peak), 143 (26\%) and 130 (27\%)\], but
also of acetyldiaboline \([M^+ 394 (42\%)\].

5.4 Separation of 'Alkaloidal Fraction C Hydrochloride'
from 'Alkaloidal Fraction A'

The 'Alkaloidal Fraction A' (1.2 g) was dissolved in
absolute ethanol and the solution made acidic by careful addi-
tion of ethanolic hydrochloric acid. To crystallise the base
hydrochloride, ethyl acetate was added dropwise to the solu-
tion till turbidity appeared, and the solution was kept for
sometime undisturbed. A brown resinous mass settled which
was separated by filtration. The filtrate was again treated
with ethyl acetate to bring turbidity and the solution again
yielded a resinous mass on keeping. The same process was
repeated with the filtrate several times till the salt crystal-
lised as small pellets (0.08 g; 'Alkaloidal Fraction C Hydro-
chloride'). The salt started turning brown at 220° but did not
melt up to 360° (lit\textsuperscript{21} diaboline hydrochloride, no definite
mp but shrinking and turning brown around 260°; lit\textsuperscript{66} more
than 360°).
5.5 Characterisation of 'Alkaloidal Fraction C Hydrochloride'

A TLC of 'Alkaloidal Fraction C Hydrochloride' on silica gel G plate using n-butanol-acetic acid-water (5:1:4) system revealed it to be a single entity.

The infrared spectrum of 'Alkaloidal Fraction C Hydrochloride' (Fig. 18) showed bands at 3300 cm\(^{-1}\) (O—H stretching); 2915 cm\(^{-1}\) (C—H stretching); 2500 cm\(^{-1}\) (N—H stretching, amine salt); 1653 cm\(^{-1}\) (amide C==O stretching); 1600 cm\(^{-1}\) (aromatic C==C stretching).

5.5a Separation of 'Alkaloidal Fraction C' from 'Alkaloidal Fraction C Hydrochloride'

A solution of 'Alkaloidal Fraction C Hydrochloride' in water was basified with ammonia and the free base ('Alkaloidal Fraction C') was extracted with chloroform.

5.5b Characterisation of 'Alkaloidal Fraction C'

5.5b.i Chromatographic Study

Thin-layer Chromatography: TLC of 'Alkaloidal Fraction C' was carried out on silica gel G plates in systems A and B (Sect. 5.2a) and in chloroform-methanol (4:1). An authentic sample of diaboline was run side by side. In all the three systems the spot of 'Alkaloidal Fraction C' compared with diaboline.

Gas-liquid Chromatography: GLC of 'Alkaloidal Fraction C' at 270° (conditions as described in Sect. 5.2b)
Figure 18

Infrared Spectrum of Alkaloidal Fraction C Hydochloride (Nujol)
showed the retention time relative to strychnine as 0.75 which was identical to that of authentic diaboline.

5.5b.ii Spectral Study

The infrared spectrum of 'Alkaloidal Fraction C' (Fig. 19) showed bands at 3390 cm\(^{-1}\) (O—H stretching); 2910 cm\(^{-1}\) (C—H stretching); 1655 cm\(^{-1}\) (amide C==O stretching); and 1600 cm\(^{-1}\) (aromatic C=C stretching).

The NMR spectrum (CDCl\(_3\)) (Fig. 20) of 'Alkaloidal Fraction C' showed the aromatic part as a three-protons multiplet at \(\delta 7.27 - 6.87\). A diffused multiplet appeared at \(\delta 7.88\) for C-4 proton. The C-22 and C-12 protons appeared at \(\delta 5.73\) and 5.26, respectively. The signal integrating for three protons of \(>\text{N—CO—CH}_3\) appeared at \(\delta 2.33\). A signal at \(\delta 3.61\) appeared for OH.

The mass spectrum (Fig. 21) of the 'Alkaloidal Fraction C' showed the molecular ion peak at m/e 352 (43%). Other significant peaks appeared at m/e 322 (7%), 241 (5%), 180 (92%), 144 (taken as base peak), 143 (23%) and 130 (44%). Accurate mass determination on the molecular ion using high resolution mass spectrometer gave the value 352.1783 (Calc. for \(\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_3\) : 352.1781).
INFRARED SPECTRUM OF ALKALOID FRACTION C (KBr)

FIG. 19

[Graph showing absorptions and wavelengths]
NM R SPECTRUM (100 MHz) OF ALKALOIDAL FRACTION C

(\text{CDCl}_3)

FIG 20
M A S S S P E C T R U M O F 'F R A C T I O N

R E L A T I V E I N T E N S I T Y

M A S S S P E C T R U M O F 'F R A C T I O N

R E L A T I V E I N T E N S I T Y
6. STUDY OF THE ALKALOIDAL CONSTITUENTS OF S. POTATORUM BARK

6.1 Isolation of Total Alkaloids ('Alkaloidal Fraction D')

The bark powder was subjected to isolation of total alkaloids following the procedure described under Sect. 5.1. The content of the total alkaloids ('Alkaloidal Fraction D') came out to be 0.091% w/w of the air-dried bark powder.

6.2 Chromatographic Study of 'Alkaloidal Fraction D'

6.2a Detection of the Constituents by Thin-layer Chromatography

The TLC was carried out using silica gel G plates. The solvent systems A, B, C and D outlined under Sect. 5.2a were used.

A solution of the 'Alkaloidal Fraction D' in chloroform was spotted on the silica gel G plates and developed using system A. For comparison authentic samples of common Strychnos alkaloids were run side by side. The spots were detected by 2% ceric sulphate in 2 N sulphuric acid and heating the plates at 105° for 30 min. There were four spots, the main spot with \( R_f \) value 0.60 compared with diaboline (0.60). The other spots with \( R_f \) values 0.43 and 0.39, respectively, compared with strychnine \( N \)-oxide (0.43) and brucine \( N \)-oxide (0.39).

The 'Alkaloidal Fraction D' was run along with minor Strychnos alkaloids using system D. The spots were detected with Dragendorff's reagent and 2% ceric sulphate in 2 N
sulphuric acid. The \( R_f \) values of the spots were 0.50, 0.37 and 0.31, respectively, which compared with pseudostrychnine (0.51), vomicine (0.37) and icajine (0.32). Vomicine and icajine were in trace amounts only.

In system B, using Dragendorff's reagent for detection, the 'Alkaloidal Fraction D' gave the spots with \( R_f \) values 0.63, 0.49 and 0.13, respectively, which compared with pseudostrychnine (0.63), pseudobrucine (0.50) and diaboline (0.13). The spot corresponding to diaboline was the major one.

6.2b Gas-liquid Chromatographic Study

The 'Alkaloidal Fraction D' was analysed by GLC (Fig. 22) as described under Sect. 5.2b using the column temperature of 275°. Diaboline was detected as the major component. A peak close to diaboline was indicative of acetyldiaboline. A few peaks other than those of diaboline and acetyldiaboline were also observed which could be either due to unknown alkaloids or to non-alkaloidal components.

7. STUDY OF THE ALKALOIDAL CONSTITUENTS OF S. POTATORUM LEAVES

7.1 Isolation of Total Alkaloids ('Alkaloidal Fraction E')

The powdered leaves were processed as described under Sect. 5.1 to isolate the total alkaloids. The content of the total alkaloids ('Alkaloidal Fraction E') came out to be 0.096% w/w of the air-dried material.
ACETYLDIABOLINE

DIABOLINE

UNIDENTIFIED

FIG. 22

GAS CHROMATOGRAM OF 'ALKALOIDAL FRACTION D'
7.2 **Chromatographic Study of 'Alkaloidal Fraction E'**

7.2a **Detection of the Constituents by Thin-layer Chromatography**

The TLC study of the 'Alkaloidal Fraction E' was carried out as described under Sect. 5.2a.

A solution of the 'Alkaloidal Fraction E' in chloroform was spotted on the silica gel G plates and developed using system A. The authentic samples of common *strychnos* alkaloids were run side by side. The spots were detected by 2% ceric sulphate in 2 N sulphuric acid and heating the plates at 105°C for 30 min. The major spot with \( R_f \) value 0.59 compared with diaboline (0.60). The other spots had \( R_f \) values 0.43 and 0.39, respectively, which compared with strychnine N-oxide (0.43) and brucine N-oxide (0.39).

The 'Alkaloidal Fraction E' was co-chromatographed with minor *strychnos* alkaloids using systems D and B. The \( R_f \) values of the spots in system D were 0.50, 0.37 and 0.31, respectively, which compared with pseudostrychnine (0.51), vomicine (0.37) and icajine (0.32). The spots comparing with vomicine and icajine were in trace amounts. In system B the \( R_f \) values were 0.63, 0.49 and 0.14, respectively, which compared with pseudostrychnine (0.63), pseudobrucine (0.50) and diaboline (0.13).

7.2b **Gas-liquid Chromatographic Study**

The 'Alkaloidal Fraction E' was analysed by GLC (Fig. 23)
FIG. 23

GAS CHROMATOGRAM OF 'ALKALOIDAL FRACTION E'
as described under Sect. 5.2b using the column temperature of 275°. Diaboline was found to be the major alkaloid. An adjacent peak was of acetyldiaboline. The other few peaks could not be identified.

8. PHARMACOLOGICAL STUDY OF 'ALKALOIDAL FRACTION A'
(TOTAL ALKALOIDS OF THE SEEDS)

The total alkaloids of the seeds of \textit{s. potatorum}, 'Alkaloidal Fraction A', obtained as described under Sect. 5.1 were submitted to pharmacological studies.

8.1 Preparation of Suspension

A suspension (1%) with gum acacia (1%) in water was made, which was suitably diluted further. For isolated tissue experiments dilutions were made with physiological solutions.

8.2 Effect on Central Nervous System

8.2a Effect on Behavioural Pattern and Acute Toxicity

Mongrel dogs (4-8 kg), albino rabbits (2-3 kg), rats (150-200 g), and Swiss mice (20-25 g) were employed for the studies. Gross observations were made after intravenous injection (cephalic vein in dogs, ear vein in rabbits, and tail vein in rats), and intraperitoneal injections (in rats and mice) in graded doses of 'Alkaloidal Fraction A'. For both intravenous and intraperitoneal routes and at each dose level three animals of each species were employed.
There was no apparent behavioural or autonomic effect on dog, rabbit and rat up to 2 mg/kg intravenous injection of 'Alkaloidal Fraction A'. A dose of 3.5-4 mg/kg produced restlessness, irritability and tremors followed by convulsions of tonic type all over the body. Convulsions of full severity started within 30 ± 5 sec of injection of the alkaloid. Head was stretched backwards, the back inverted and the legs were extended. The animal could respire with great difficulty. After that the animal recovered back gradually or died within 3.5 ± 0.5 min time. In mice and rats the 'Alkaloidal Fraction A' injected intraperitoneally produced similar effects in doses of 70-100 mg/kg. A dose of 80 mg/kg was found to be LD$_{50}$, while 100 mg/kg was LD$_{100}$.

8.2b Effect on Barbiturate Sleeping Time

In a series of mice, 25 mg/kg of pentobarbitone sodium was injected intraperitoneally and the sleeping time noted as the time lapse between the loss and return of the righting reflex. In another series of mice, the same dose of pentobarbitone sodium was followed after 2 min by the injection of 'Alkaloidal Fraction A' and the effect on the sleeping time noted. The 'Alkaloidal Fraction A' in the dose of 70 mg/kg markedly decreased the sleeping time.

8.2c Effect of Known Anticonvulsants on the Convulsive Dose of 'Alkaloidal Fraction A' in Rats

Diphenylhydantoin sodium (suspended in 2% Tween 80) was administered subcutaneously (100 mg/kg), 2 hr prior to
the administration of the convulsive dose (70 mg/kg) of 'Alkaloidal Fraction A'. In another series of the experiment, troxidone (suspended in 2% Tween 80) was administered in a single dose of 500 mg/kg intraperitoneally followed after 2 hr by the convulsive dose (70 mg/kg) of the 'Alkaloidal Fraction A'. In both the experiments, the control animals were injected with 2% Tween 80 in normal saline. The dose of diphenylhydantoin sodium did not show any protection against the convulsions induced by the alkaloid. The single dose of troxidone protected 60% rats against convulsions.

8.2d Effect of Prior Administration of Chlorpromazine on the Convulsive Dose of 'Alkaloidal Fraction A' in Rats

Chlorpromazine diluted with normal saline was injected intraperitoneally (5 mg/kg) followed after 45 min by the convulsive dose of the 'Alkaloidal Fraction A'. The animals showed rigidity and slight extension of hind limbs, but were protected against full severity of convulsions.

8.2e Effect of Prior Administration of 'Alkaloidal Fraction A' on Subconvulsive Doses of the Known Convulsants in Rats

Subconvulsive dose (50 mg/kg) of 'Alkaloidal Fraction A' was administered intraperitoneally in three groups of rats followed after 5 min by subconvulsive doses of strychnine hydrochloride (2 mg/kg intraperitoneally) in the first group, leptazol (80 mg/kg subcutaneously) in the second group and electroshock by current delivered through corneal electrodes
(strength 120 ma for a period of 0.2 sec) in the third group. Convulsions were observed in all three groups of animals, but synergism was most marked between 'Alkaloidal Fraction A' and strychnine hydrochloride.

8.2f Effect on Amphetamine Aggregate Toxicity in Mice

The dose of amphetamine sulphate which increases the locomotor activity (but not lethal) was determined and found to be 8 mg/kg intraperitoneally. Amphetamine sulphate was administered intraperitoneally in 8 mg/kg dose level followed after 5 min by the subconvulsive dose (50 mg/kg) of 'Alkaloidal Fraction A' and the effect noted. In control group amphetamine sulphate was injected alone. The 'Alkaloidal Fraction A' administered after amphetamine sulphate resulted in 80% mortality in aggregated mice whereas control group did not show any mortality.

8.3 Action on Blood Pressure and Respiration

Mongrel dogs of either sex (weighing 5-10 kg) were anaesthetized with quinalbarbitone sodium (30-35 mg/kg intraperitoneally). The right femoral vein was exposed and cannulated. A mid line incision was made on the ventral side of the neck, trachea was exposed and a transverse incision made. A tracheal cannula was inserted and held in place by tying with threads. One end of the cannula was connected to a Marcy's tambour and through the other air entry was regulated. The right common carotid artery was exposed and connected to
a mercury manometer for the record of blood pressure. The blood pressure tracings and respiratory movements were recorded on a moving drum. The administration of drugs was done through cannulated femoral vein. The experiments were repeated after complete atropinisation (atropine sulphate 2 mg/kg), ganglionic blockage (hexamethonium bromide 5 mg/kg), adrenergic blockage (tolazoline hydrochloride 7.5 mg/kg), after administration of antihistaminic (antazoline hydrochloride 10 mg/kg), and in bilaterally vagotomised dogs.

The effect was studied in three different dose level of 'Alkaloidal Fraction A' (2 mg/kg, 3 mg/kg and 4 mg/kg). There was very slight fall of blood pressure with 2 mg/kg dose. The injections of 3 mg/kg and 4 mg/kg dose produced a marked fall (80-100 mm/Hg) with slow recovery to a level 20-30 mm/Hg below normal (Fig. 24 and 25). A decrease in amplitude and rate of pulse was also observed. Subsequent doses of 'Alkaloidal Fraction A' caused very little or no fall of blood pressure. Doses higher than 4 mg/kg caused lethal fall of blood pressure. The effect of 'Alkaloidal Fraction A' was not altered after complete atropinisation, ganglionic blockage, adrenergic blockage, and in bilaterally vagotomised animal. Fall of blood pressure was, however, reduced to some degree by antihistamine. Strychnine hydrochloride in equivalent dose caused very little fall of blood pressure and of very less duration as compared with that of 'Alkaloidal Fraction A' with severe stimulation of respiration.
FIG. 24

EFFECT OF 'ALKALOIDAL FRACTION A' (TOTAL ALKALOIDS OF THE SEEDS) ON BLOOD PRESSURE OF DOG (DOSE 3 mg/kg).
FIG. 25
EFFECT OF 'ALKALOIDAL FRACTION A' (TOTAL ALKALOIDS OF THE SEEDS) ON BLOOD PRESSURE OF DOG (DOSE 4 mg/kg).
It also caused severe convulsions while the 'Alkaloidal Fraction A' did not show any such effect in anaesthetised animal.

There was no remarkable effect on respiration of 'Alkaloidal Fraction A'.

8.4 Electrocardiographic Records

Electrocardiograms using conventional bipolar lead II were recorded from anaesthetised dogs before and after the administration of 'Alkaloidal Fraction A' (3 mg/kg intravenously) at slow rate. The record revealed decrease in amplitude of P and R waves and there was marked increase in PR and RR interval.

8.5 Action on Isolated Rat Heart

The effect of 'Alkaloidal Fraction A' was studied on isolated rat heart according to Langendorff's technique as described by Burn. The animal was stunned and bled to death by cutting neck vessels. The chest was opened and the heart removed quickly with a small portion of ascending aorta. The heart was dipped into Ringer's solution. It was gently squeezed to remove blood from the aorta. The aorta was freed from its attachment to the pulmonary artery and cannulated. A bent entomological pin was fixed in the tip of the ventricle and perfusion was begun with the oxygenated fluid maintained at 37°C. A record was obtained of the amplitude of ventricular
contractions. The rate was counted. The outflow was measured for 1 min before and after the administration of the alkaloid.

'Alkaloidal Fraction A' in doses of 100 µg or above caused transitory initial stimulation followed by marked depression of heart. Both heart rate and coronary outflow decreased. The response is shown in Fig. 26.

8.6 Action on Isolated Guinea-pig Ileum

Terminal portions of ileum (3-5 cm) were taken from a freshly killed guinea-pig and suspended in oxygenated Tyrode solution maintained at 37° in an isolated organ bath of 25 ml capacity. The preparation was left for 30 min, a dose of acetylcholine chloride (0.1 µg/ml) was allowed to act for 20 sec and the contraction recorded. The tissue was washed twice at 1 min interval for subsequent doses. When repeated doses of acetylcholine chloride produced the same height of contractions, 'Alkaloidal Fraction A' in graded doses was added to the bath and response recorded. Antispasmodic effect of the alkaloid was tested against spasms produced by acetylcholine chloride (0.1 µg/ml), histamine acid phosphate (0.2 µg/ml) and barium chloride (0.1 mg/ml).

There was no apparent effect of 'Alkaloidal Fraction A' on the guinea-pig ileum up to 20 µg/ml dose. Against acetylcholine chloride, histamine acid phosphate, and barium chloride the alkaloid did not show any effect up to 10 µg/ml dose. A dose of 20 µg/ml caused about 25% check against
FIG. 26

EFFECT OF 'ALKALOIDAL FRACTION A' (TOTAL ALKALOIDS OF THE SEEDS) ON ISOLATED RAT HEART.
acetylcholine chloride and histamine acid phosphate.

8.7 **Effect on Blood Sugar of Rabbit**

Blood sugar of rabbit was determined according to Bose's simplified method before and after the intravenous administration of the subconvulsive dose of 'Alkaloidal Fraction A'. The fraction did not show any effect on blood sugar.

8.8 **Analgesic Action**

Analgesic action was tested on rats by using a Techno-analgesimeter. Time taken by the animals to withdraw their tails from the hot nichrome wire was recorded before and after the intraperitoneal administration of 'Alkaloidal Fraction A' (50 mg/kg). The 'Alkaloidal Fraction A' did not exhibit any analgesic action.

8.9 **Diuretic Effect**

It was carried out on unanaesthetised rats according to the method of Burns. The 'Alkaloidal Fraction A' did not show any effect on diuresis.

9. **PHARMACOLOGICAL STUDY OF 'ALKALOIDAL FRACTION C HYDROCHLORIDE' (DIABOLINE HYDROCHLORIDE)**

The 'Alkaloidal Fraction C Hydrochloride' obtained as described under Sect. 5.4 and characterised to be diaboline hydrochloride (Sect. 5.5) was submitted to pharmacological studies on cardiovascular system of rat.
9.1 Preparation of Solution

An aqueous solution (1%, pH 6-6.5) of 'Alkaloidal Fraction C Hydrochloride' was prepared which was suitably diluted further.

9.2 Action on Blood Pressure of Rat

Healthy albino rats (weighing 250-300 g) were anaesthetised with urethane (1.2 g/kg intraperitoneally). For intravenous administration of drugs the jugular vein was exposed and cannulated. The carotid artery was exposed and connected to a mercury manometer for recording the arterial blood pressure.

'Alkaloidal Fraction C Hydrochloride' in the doses below 4 mg/kg did not show any effect on blood pressure. A dose of 4 mg/kg produced slight gradual fall, but a marked gradual fall of blood pressure was produced with 8 mg/kg and 12 mg/kg of the alkaloid (Fig. 27).

9.3 Action on Blood Pressure of Spinal Rat

Rats (weighing 250-300 g) were anaesthetised with urethane (1 g/kg intraperitoneally). The jugular vein was exposed and cannulated for intravenous administration. Both the carotid arteries were exposed, one of them was tied off and the other left for recording blood pressure. The trachea was cannulated and artificial respiration was restored. A needle was passed through the eye orbit to destroy the spinal
FIG. 27

EFFECT OF 'ALKALOIDAL FRACTION C HYDROCHLORIDE' (DIABOLINE HYDROCHLORIDE) ON BLOOD PRESSURE OF RAT (DOSE 12 mg/kg).
cord, and the destruction was considered complete when there was abolition of reflexes after extension of the hind limbs. The carotid artery was cannulated and connected to a mercury manometer for the record of blood pressure. The response of the animal was checked with a dose of noradrenaline (250 ng).

'Alkaloidal Fraction C Hydrochloride' in dose of 12 mg/kg produced a slight rise in blood pressure which soon came to normal. A subsequent dose (12 mg/kg) also produced the same type of effect. The recordings are shown in Fig. 28.

9.4 Action on Blood Vessels

The action of 'Alkaloidal Fraction C Hydrochloride' on blood vessels was studied on hind quarters preparation of rats as described below:

Rats (weighing 250-300 g) were anaesthetised with urethane (1.2 g/kg intraperitoneally) and heparin (500 IU) was administered intravenously. The abdomen was cut open and aorta below the renal artery was exposed and cannulated. The cannula was connected to a perfusion pump through a mercury manometer, and oxygenated Locke's solution at 37°C was perfused at a constant rate. The upper half body was cut off. The pressure in the manometer which is a function of resistance of the blood vessels in hind quarters was allowed to stabilize for 20 min. The pressure changes in the manometer after injection of noradrenaline (250 ng), adrenaline (250 ng), 'Alkaloidal Fraction C Hydrochloride'...
FIG. 28

EFFECT OF 'ALKALOIDAL FRACTION C HYDROCHLORIDE' (DIABOLINE HYDROCHLORIDE) ON BLOOD PRESSURE OF SPINAL RAT (DOSE 12 mg/kg).
FIG. 33
EFFECT OF 'ALKALOIDAL FRACTION E' (TOTAL ALKALOIDS OF THE LEAVES) ON BLOOD PRESSURE OF DOG (DOSE 10 mg/kg).
(3 mg) and normal saline were recorded on a moving smoked kymograph paper (Fig. 29).

The alkaloid did not show any effect on the blood vessels.

9.5 Action on Isolated Rat Heart

The details of the method are given under Sect. 8.5.

'Alkaloidal Fraction C Hydrochloride' (4 mg) caused a transitory stimulation of amplitude of cardiac contractions. There was decrease in heart rate and cardiac outflow. The response is shown in Fig. 30.

10. PHARMACOLOGICAL STUDY OF 'ALKALOIDAL FRACTION D'
(TOTAL ALKALOIDS OF THE BARK)

The total alkaloids of *S. potatorum* bark, 'Alkaloidal Fraction D', obtained as under Sect. 6.1 were submitted to pharmacological studies.

10.1 Preparation of Suspension

A suspension (1%) of 'Alkaloidal Fraction D' was prepared in the same way as described under Sect. 8.1.

10.2 Effect on Central Nervous System

The effect of 'Alkaloidal Fraction D' on behavioural pattern and its acute toxicity were studied on rats and mice.
FIG. 29

EFFECT OF 'ALKALOIDAL FRACTION C HYDROCHLORIDE' (DIABOLINE HYDROCHLORIDE)
ON BLOOD VESSELS (RAT HIND QUARTERS PREPARATION).
FIG. 30

EFFECT OF 'ALKALOIDAL FRACTION C HYDROCHLORIDE' (DIABOLINE HYDROCHLORIDE) ON ISOLATED RAT HEART.
Albino rats (150-200 g) and Swiss mice (20-25 g) were employed. Gross observations were made after intraperitoneal injection in graded doses of 'Alkaloidal Fraction D'. At each dose level three animals of each species were employed. There was no apparent behavioural or autonomic effect on rats or mice up to 150 mg/kg intraperitoneal injection of 'Alkaloidal Fraction D'. A dose of 200-250 mg/kg produced restlessness, irritability and tremors followed by convulsions of tonic type. There was extension of hind limbs and the animal could respire with great difficulty. After that the animal recovered back or died due to respiratory failure. A dose of 250 mg/kg was found to be LD$_{50}$ in mice.

10.3 Action on Blood Pressure and Respiration

The details of the method are described under Sect. 8.3.

The effect of 'Alkaloidal Fraction D' was studied with different doses (3 mg, 6 mg, and 10 mg/kg body weight). A dose of 10 mg/kg administered intravenously produced marked and persistent fall of blood pressure with a slow recovery to a level 20-30 mm/Hg below normal (Fig. 31). A decrease in amplitude and rate of pulse was also observed. Subsequent doses of 'Alkaloidal Fraction D' caused very little or no fall at all. No remarkable effect was observed on respiration.

10.4 Action on Isolated Rat Heart

The details of the method are given under Sect. 8.5.
FIG. 31

EFFECT OF 'ALKALOIDAL FRACTION D' (TOTAL ALKALOIDS OF THE BARK)
ON BLOOD PRESSURE OF DOG (DOSE 10mg/kg).
The 'Alkaloidal Fraction D' was injected into the perfusion fluid in graded doses (100 µg, 300 µg, 500 µg and 1 mg). No appreciable effect was observed with 100 µg dose. The alkaloid in doses of 300 µg-1 mg caused transitory initial stimulation followed by depression of the heart. Both heart rate and coronary outflow decreased. The response is shown in Fig. 32.

10.5 Action on Isolated Guinea-pig Ileum

The method is outlined under Sect. 8.6.

The 'Alkaloidal Fraction D' did not exhibit any effect on guinea-pig ileum with 20 µg/ml and 40 µg/ml doses. A dose of 40 µg/ml caused about 25% check against acetylcholine chloride (0.1 µg/ml) and histamine acid phosphate (0.2 µg/ml).

10.6 Effect on Blood Sugar of Rabbit

The 'Alkaloidal Fraction D' did not show any effect on blood sugar when tested following the procedure mentioned under Sect. 8.7.

10.7 Analgesic Action

The method is outlined under Sect. 8.8.

The alkaloidal fraction did not show any analgesic action.
FIG. 32
EFFECT OF 'ALKALOIDAL FRACTION D' (TOTAL ALKALOIDS OF THE BARK) ON ISOLATED RAT HEART.
10.8 Diuretic Effect

There was no effect on diuresis of 'Alkaloidal Fraction E' when the test was carried out on unanaesthetised rats according to the method of Bum.91

11. PHARMACOLOGICAL STUDY OF 'ALKALOIDAL FRACTION E' (TOTAL ALKALOIDS OF THE LEAVES)

The total alkaloids of J. potatorum leaves, 'Alkaloidal Fraction E', as obtained under Sect. 7.1, were submitted to pharmacological studies.

11.1 Preparation of Suspension

A suspension (1%) of 'Alkaloidal Fraction E' was prepared in the same way as described under Sect. 8.1.

11.2 Effect on Central Nervous System

The effect of 'Alkaloidal Fraction E' on behavioural pattern and its acute toxicity were studied on rats and mice.

The 'Alkaloidal Fraction E' was injected intraperitoneally in graded doses in albino rats and Swiss mice and gross observations were made. At each dose level three animals of each species were employed. There was no behavioural or autonomic effect on rats or mice up to 200 mg/kg intraperitoneal injection of the alkaloid. A dose of 250-300 mg/kg produced restlessness, irritability and tremors followed by tonic type convulsions. The hind limbs were extended and there was difficulty in breathing. After
that the animal recovered back or died. A dose of 280 mg/kg was found to be LD$_{50}$ in mice.

11.3 Action on Blood Pressure and Respiration

The details of the method are outlined under Sect. 8.3.

A number of experiments were conducted with different doses. A dose of 10 mg/kg produced a marked fall of blood pressure which persisted for a long time (Fig. 33). A decrease in amplitude and pulse rate was also observed. Subsequent administration of the same dose caused very little or no fall at all of blood pressure. No remarkable effect was observed on respiration.

11.4 Action on Isolated Rat Heart

The details of the method are given under Sect. 8.5.

The alkaloid in doses of 500 µg-1 mg when injected into the perfusion fluid caused a marked depression of amplitude and rate of cardiac contractions after an initial transitory stimulation. A decrease in cardiac outflow was also observed. The response is shown in Fig. 34.

11.5 Action on Isolated Guinea-pig Ileum

The method is outlined under Sect. 8.6.

The 'Alkaloidal Fraction B' did not show any effect on guinea-pig ileum up to the dose of 40 µg/ml. A check of
Fig. 34

EFFECT OF 'ALKALOIDAL FRACTION E' (TOTAL ALKALOIDS OF THE LEAVES) ON ISOLATED RAT HEART.
about 25-30\% was observed against acetylcholine chloride
(0.1 \mu g/ml) and histamine acid phosphate (0.2 \mu g/ml) with
40 \mu g/ml dose of 'Alkaloidal Fraction 2'.

11.6 Effect on Blood Sugar of Rabbit

The 'Alkaloidal Fraction 2' did not show any effect on
blood sugar when tested following the method mentioned under
Sect. 8.7.

11.7 Analgesic Action

The method is outlined under Sect. 8.8.

The alkaloidal fraction did not show any analgesic action.

11.8 Diuretic Effect

It was carried out on unanaesthetised rats according
to the method of Lum. The 'Alkaloidal Fraction 2' had
no effect on diuresis.