The seeds, bark and leaves of *Strychnos potatorum* Linn. f. used for the present investigation were supplied by Messrs. United Chemical and Allied Products, Calcutta (West Bengal, India).

1. PRELIMINARY STUDIES ON *Strychnos potatorum*

The different plant parts were extracted with various solvents, the percentage of extractives were estimated, and tests made on the extractives for the presence of alkaloids, glycosides/carbohydrates, sterols, proteins and free amino acids (*Sect. 1*). The benzene, chloroform, acetone and ethanol extractives of the seeds showed positive response to the alkaloidal reagents. The alkaloids were also present in chloroform, acetone and ethanol extractives of the bark and leaves. The acetone, ethanol and water extracts from seeds gave positive response to Molisch's test and also indicated the presence of reducing sugars. The acetone, ethanol and water extracts from bark and leaves also showed positive response to Molisch's test. The petroleum ether, benzene, ether, chloroform and

* Pertains to the Experimental part.
acetone extractives of the seeds indicated the presence of sterols/triterpenoids. The presence of sterols/triterpenoids was also shown by the petroleum ether and benzene extractives of the bark and leaves. The presence of proteins was indicated in acetone, ethanol and water extractives of the seeds. The water extract from bark, and ethanol and water extracts from leaves also gave positive test for proteins. The presence of free amino acids was detected in ethanol and water extracts from the seeds and water extracts from the bark and leaves.

The powdered seeds of *S. potatorum* yielded 3.08% of total ash, 0.70% of acid-insoluble ash and 0.38% of watersoluble ash (Sect. 2).

2. SEED OIL AND ITS FATTY ACID COMPOSITION

The oil was separated through extraction with petroleum ether. The physical and chemical characteristics of the oil were studied. The chemical characteristics of the separated fatty acids were also determined.

On spectrophotometric estimation, the seed oil revealed the absence of fatty acids like linolenic acid, and was found to contain 11.1% of total dienes calculated as linoleic acid. The total monoenes were found to be 50.9% as determined utilizing iodine value of the mixed fatty acids after making allowance for the contribution of total dienes present. The content of saturated fatty acids (found by difference) came out to be 38.0% (Sect. 3.4). The total saturated fatty acids
were also estimated by Bertram oxidation method and were found to be 37.1% (Sect. 3.5). For a detailed study of the fatty acid composition of the oil, methyl esters of the fatty acids were prepared and submitted to spectral, thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) studies (Sect. 3.6). Help of log-plot method was taken to further determine the identity of minor peaks. Table 1 lists the fatty acid contents in terms of per cent (wt) and per cent (mole) as estimated by GLC of the methyl esters.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Per cent (wt)</th>
<th>Per cent (mole)</th>
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</thead>
<tbody>
<tr>
<td>Lauric acid</td>
<td>Traces</td>
<td></td>
</tr>
<tr>
<td>Myristic acid</td>
<td>Traces</td>
<td></td>
</tr>
<tr>
<td>15 : 0 acid</td>
<td>Traces</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>27.2</td>
<td>29.4</td>
</tr>
<tr>
<td>Hexadecenoic acid</td>
<td>1.5</td>
<td>1.6</td>
</tr>
<tr>
<td>17 : 0 acid</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>4.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>49.4</td>
<td>48.5</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>11.3</td>
<td>11.1</td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Behenic acid</td>
<td>Traces</td>
<td></td>
</tr>
<tr>
<td>Lignoceric acid</td>
<td>3.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>
The seed oil of *S. nux-vomica* is reported to contain 62.0% oleic, 9.2% linoleic, 12.6% palmitic, 6.6% stearic and 7.0% arachidic acid as the major components as determined by ester fractionation method. *Buddleia davidii* also belonging to family Loganiaceae is reported to contain oleic and linoleic acids in the proportions of 22 and 59%, respectively.

### 3. UNSAPONIFIABLE MATTER OF THE SEED OIL

The unsaponifiable matter obtained by saponification of the seed oil was chromatographed on alumina (E. Merck) (Sect. 4.1). Material eluted with petroleum ether consisted of colourless oily hydrocarbons. Further elution was done with solvents of increasing polarity and all fractions were monitored by TLC. Elution with petroleum ether-benzene (80:20) gave a white sticky residue which on repeated crystallisations from acetone yielded silky needles, mp 184-186\(^\circ\), designated as 'Fraction A'. The fraction was found to be single entity by TLC. It gave red violet colouration on Liebermann-Burchard test, indicating the fraction to be a triterpenoid. Petroleum ether-benzene (70:30) eluted a residue which on crystallisation from acetone gave colourless needles, mp 218-220\(^\circ\), 'Fraction B'. The fraction, found to be single component by TLC, also gave a red colouration on Liebermann-Burchard test. Further elution with benzene-chloroform (90:10) gave a residue which crystallised from methanol, mp 137-138\(^\circ\), designated as 'Fraction C'. The fraction appeared to be single
component by TLC. It gave a green colour on Liebermann-Burchard test, thus indicating it to be a sterol. Elution with benzene-chloroform (75:25) gave a residue which crystallised from methanol, mp 186-188°, designated as 'Fraction D'. The fraction was also found to be a single entity by TLC and gave a brown colour on Liebermann-Burchard test.

The fractions obtained were subjected to infrared, nuclear magnetic resonance (NMR) and mass spectral studies (Sect. 4.3). The infrared spectrum of 'Fraction A' showed a band at 3400 cm⁻¹ due to alcoholic O—H stretching. The NMR spectrum indicated vinylic proton at δ 5.18, and multiplet appeared at δ 3.28 for —CH₂—CH≡C<. Signals for C-methyl protons appeared at δ 0.80 (12 H), 0.96 (3 H), 1.01 (6 H) and 1.08 (3 H). The spectrum was indicative of a pentacyclic triterpenoid. The mass spectrum showed a molecular ion peak at m/e 426 and other fragmentation peaks at m/e 411 (M⁺—CH₃), 218, 207, 203, 189 and 133. The molecular ion peak and the fragmentation peaks strongly suggested that the compound was of amyrin type (49). The peak at m/e 218 is due to a characteristic fragment (a) produced by retro-Diels-Alder reaction in ring C of pentacyclic triterpenes and is indicative of the presence of a 12-13 double bond. The peaks at m/e 203 and 133 are due to the fragments (b) and (c) produced from species (a). The peak at m/e 207 is due to another characteristic fragmentation product (d) of
The spectral studies are, thus, indicative of 'Fraction A' being an entity related to amyrin series of pentacyclic triterpenoids.
The infrared spectrum of 'Fraction B' showed a band at 3300 cm\(^{-1}\) due to \(\text{O-H}\) stretching. The NMR spectrum showed multiplets at \(\delta 3.28\) and 5.45. The information from integration about vinylic protons (\(\delta 5.45\)) was not conclusive. 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 showed a molecular ion peak at \(m/e\) 426. Other important fragments appeared at \(m/e\) 220, 218, 207 and 189. The peak at \(m/e\) 189 is characteristic of a fragmentation product (e) of pentacyclic triterpene of lupene series (50). The other two characteristic peaks at \(m/e\) 218 and 220 are also due to the fragments (f) and (g) of
a pentacyclic triterpene of lupene series.

The infrared spectrum of 'Fraction C' showed bands at 3500 and 3400 cm\(^{-1}\) for associated O—H stretching and at 1650 cm\(^{-1}\) for nonconjugated C=C stretching. A band at 980 cm\(^{-1}\) was indicative of a trans disubstituted double bond of stigmasterol. The NMR spectrum showed a multiplet at \(\delta 5.34\) for vinylic proton, and also at \(\delta 5.08\) for trans disubstituted 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 showed a parent ion at m/e 414 and other fragmentation ions at m/e 399 (\(M^+—\text{CH}_3\)), 396 (\(M^+—\text{HOH}\)), 381 (\(M^+—\text{CH}_3—\text{HOH}\)), 329, 303, 275, 273 (\(M^+—\text{side chain}\)), 231 and 131 (\(M^+—283\)). The fragmentation pattern is consistent with the structure of \(\beta\)-sitosterol (51). A peak at m/e 412 in the mass spectrum of 'Fraction C' was indicative of the presence of stigmasterol in the fraction. The natural \(\beta\)-sitosterol is commonly associated with stigmasterol, having the trans 22-ene system (see a review by Singh, Kapoor, and Chawla65).
The infrared spectrum of 'Fraction D' showed a band at 3450 cm⁻¹ for O—H stretching. The NMR spectrum did not reveal any unsaturation in the molecule. Signals due to C-methyl appeared at δ 1.43, 1.31, 1.23, 0.98, 0.91 and 0.83. The mass spectrum showed a molecular ion peak at m/e 444. Other fragmentation peaks appeared at m/e 429 (M⁺—CH₃), 426 (M⁺—HOH), 411 (M⁺—CH₃—HOH), 220, 207 and 189. The peak at m/e 207 may be due to the fragment (h) with an isopropyl function and a hydroxymethylene group in ring E from pentacyclic triterpene. The peaks at m/e 220 and 207 suggest

the molecule to be a pentacyclic triterpene. On the basis of these indications the 'Fraction D' may be considered to be a diol (52) of lupane series of pentacyclic triterpenoids.
The occurrence of pentacyclic triterpenes along with sterols in the genus Strychnos gives a support to the theory of a common biogenetic origin for the triterpenes and sterols. The hydrocarbon squalene which is biosynthesised from mevalonate in plants, and present in a number of seed oils is considered to be precursor of sterols and triterpenes in higher plants. The occurrence of a diol in S. potatorum is in accord with the recent studies which revealed that synthesis of monols in plants is followed by their hydroxylation to corresponding diols.

4. ALKALOIDAL CONSTITUENTS OF THE SEEDS

The total alkaloids ('Alkaloidal Fraction A') of the S. potatorum seeds isolated by the described procedure (Sect. 5.1) came out to be 0.30% w/w of the air-dried material. The TLC and GLC studies (Sect. 5.2) done on the total alkaloidal fraction revealed it to contain diaboline (17) as the major component together with brucine (2), brucine N-oxide (53), strychnine (1), strychnine N-oxide (54) and pseudostrychnine (6). Vomicine (10), icajine (9) and novacine (11) were identified in minor amounts. A simultaneous study by Bisset and Phillipson using TLC and GLC techniques indicated the presence of diaboline as the major component of the alkaloidal fraction from leaves, seeds and bark of S. potatorum.

The major fraction, 'Alkaloidal Fraction B' was obtained by preparative TLC of the 'Alkaloidal Fraction A' and was
shown to contain an acetyldiaboline together with diaboline

by TLC and mass spectral studies. The mass spectrum of
'Alkaloidal Fraction B' showed peaks at m/e 394 (M+ acetyl-
diaboline) and m/e 352 (M+ diaboline). It is not easy to
suggest from the spectrum which of the isomeric acetyl-
diabolines, jobertine (21) or henningsamine (19), may be
present. However, TLC run in the system chloroform-methanol
(4:1) suggested that the second component ran slightly behind
diaboline itself and it points to henningsamine rather than
jobertine. In the same solvent system henningsamine is
reported to run close to diaboline while jobertine travels
noticeably behind diaboline.\textsuperscript{26,69} The 'Alkaloidal
Fraction A' on careful handling yielded a crystalline salt
'Alkaloidal Fraction C Hydrochloride' (Sect. 5.4) which was
identified as diaboline hydrochloride (Sect. 5.5) on the
basis of the following evidence. The infrared spectrum of
the salt in nujol was found to be identical with diaboline
hydrochloride. The liberated base 'Alkaloidal Fraction C'
from the salt was found to be identical with diaboline on comparison with the authentic sample by TLC and GLC. The infrared spectrum of the liberated base, 'Alkaloidal Fraction C' 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 of the base showed a diffused multiplet at \(\delta\) 7.88 for C-4 proton and another multiplet at \(\delta\) 7.27 - 6.87 for C-1, 2, 3 protons of aromatic part. The C-22 and C-12 protons appeared at \(\delta\) 5.73 and 5.26, respectively. The signal integrating for three protons of >N—CO—CH\(_3\) appeared at \(\delta\) 2.33, and a signal at \(\delta\) 3.61 appeared for —OH. Further confirmation of the 'Alkaloidal Fraction C' being diaboline came from its mass spectrometric analysis. The mass spectrum of the base showed a molecular ion peak at m/e 352. Other significant peaks appeared at m/e 322, 241, 180, 144 (taken as base peak), 143 and 130. The peaks at m/e 130 and 144 are due to the characteristic fragments (i) and (j) of indole alkaloids.\(^{70}\) The genesis of other significant peaks at m/e 241 and m/e 180 is due to

\[
\begin{align*}
(i) & \quad m/e 130 \\
(j) & \quad m/e 144
\end{align*}
\]
the fragments \( k \) and \( l \) produced from diaboline.\textsuperscript{71,72}
The accurate mass determination on the molecular ion of the free base using a high resolution mass spectrometer gave the value 352.1783 in support of the composition \( C_{21}H_{24}N_2O_3 \) (Calc. 352.1781).

5. ALKALOIDAL CONSTITUENTS OF THE BARK

The bark powder was worked according to the procedure mentioned (Sect. 5.1) and the total alkaloids, designated as 'Alkaloidal Fraction D', came out to be 0.091% w/w of the air-dried material. Diaboline was identified as the major alkaloid together with acetyldiaboline, pseudostrychnine, pseudobrucine, strychnine N-oxide and brucine N-oxide by TLC and GLC (Sect. 6.2). Vomicine and icajine were detected in trace amounts.

6. ALKALOIDAL CONSTITUENTS OF THE LEAVES

The leaves were found to contain 0.096% w/w of the total alkaloids designated as 'Alkaloidal Fraction E' and isolated by the procedure described (Sect. 5.1). The TLC and GLC studies (Sect. 7.2) identified diaboline as the major alkaloid. Acetyldiaboline, pseudostrychnine, pseudobrucine, strychnine N-oxide, brucine N-oxide, vomicine and icajine were identified in minor amounts.

7. PHARMACOLOGICAL STUDIES

The general pharmacological actions of 'Alkaloidal Fraction A' (total alkaloids of the seeds) (Sect. 8), 'Alkaloidal Fraction D' (total alkaloids of the bark) (Sect. 10) and 'Alkaloidal Fraction E' (total alkaloids of the leaves)
(Sect. 11) were studied. The pharmacological studies of 'Alkaloidal Fraction C Hydrochloride' (diaboline hydrochloride) on cardiovascular system were also carried out (Sect. 9).

The 'Alkaloidal Fraction A' when injected parenterally in different species of animals produced restlessness, irritability and tremors followed by convulsions of tonic type. The fraction, when injected 2 min after the phenobarbitone injection, markedly decreased the sleeping time in mice. Single dose of troxidone protected (prevention of hind limb extension was considered criterion for protection) 60 per cent rats against drug induced convulsions. Prior administration of chlorpromazine did not protect the rats against convulsive dose of drug. The synergism was shown between the 'Alkaloidal Fraction A' and the known convulsants in rats but was most marked with strychnine hydrochloride. The fraction administered after amphetamine sulphate resulted in 80 per cent mortality in aggregated mice whereas control group did not show any mortality. In all probability it is suggested that the effect of alkaloids is partly spinal but mainly at a higher level. The alkaloids produced no effect on rabbit blood sugar, hence convulsions due to hypoglycaemia may be ruled out. The LD$_{50}$ of the 'Alkaloidal Fraction A' in mice was 80 mg/kg.

The 'Alkaloidal Fraction A' showed a marked fall in blood pressure of anaesthetised dogs which did not return to normal for 2 hr. Subsequent doses of the drug caused very
little fall or no fall at all in blood pressure. The effect of total alkaloids on blood pressure was not altered after complete atropinisation, ganglionic block, and in bilaterally vagotomised animal. Fall in blood pressure was, however, reduced to some extent after administration of antazoline hydrochloride. There were no convulsions in the anaesthetised dog which suggests that there may be some physiological antagonism of the alkaloids with the barbiturate. On the isolated heart the fraction showed transitory initial stimulation followed by marked depression of heart. In the light of these observations it appears that the fall in blood pressure is in part due to central action and in part due to depressant action on heart. The total alkaloids had no effect on the smooth muscles of guinea-pig ileum, nor any anti-spasmodic action as tested against acetylcholine, histamine and barium chloride. Slight check revealed against these spasmogens in higher doses may be attributed to its toxic action.

Similar results were obtained with 'Alkaloidal Fraction D' and 'Alkaloidal Fraction E'. The LD_{50} for the two fractions were 250 mg and 280 mg/kg, respectively.

The pharmacological studies of 'Alkaloidal Fraction C Hydrochloride' (diaboline hydrochloride) were carried out on cardiovascular system of rat (Sect. 9). The alkaloid showed a marked fall in blood pressure in rats anaesthetised with urethane. In spinal rat the administration of the alkaloid
produced a slight rise in blood pressure which soon came to normal. The alkaloid did not show any effect on the blood vessels. On isolated heart the alkaloid showed a transitory stimulation. The slight transitory rise in blood pressure in spinal rat may, thus, be due to the transitory stimulation of heart, and as there is no fall in blood pressure (in spinal rat) it suggests that the alkaloid exhibits its hypotensive action through central action.

It appears that the hypotensive action of the total alkaloidal fractions of the seeds, bark and leaves of *S. potatorum* is mainly due to the presence of diaboline in them, while the minor alkaloids present potentiate the action.