CHAPTER III

ISOLATION AND STRUCTURAL STUDY OF NOVEL SESQUITERPENE
LACTONE; 15-HYDROXY DEHYDROCOSTUS LACTONE FROM THE
ROOTS OF SAUSSUREA LAPP A C.B. CLARKE.*

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the Journal of Institution of Chemists, Calcutta.
SAUSSUREA LAPPAC.B.CLARKE\textsuperscript{1-3} belongs to compositae family and is commonly known as kuth or costus.

As a medicine, the root is considered as carminative and stimulent in China and also useful in cough, cholera and asthma especially of vagotonic type\textsuperscript{3}. The plant is used against cancer\textsuperscript{4}. Many workers\textsuperscript{5-12} have already reported the sesquiterpene lactone in Sassurea lappa C.B. Clarke. Some of the lactone fractions have been found to exhibit the hypotensive, spasmyloytic and bronchodilatory effects\textsuperscript{13}.

The alkaloid Saussurine has depressant action on the vagus centre in the medulla as well as on the involuntary muscle to fibres of the bronchicles and gasteroientestinal tract. It also produces a slight rise in blood pressure.

**ISOLATION OF SESQUITERPENE LACTONE**

Saussurea lappa C.B. Clarke was procured from Lahoul Kuth growers co-op. Ltd. Manali (H.P.) and authenticated by Botany Department. A herbarium specimen (No. XXII) has been deposited at room no. 36 of Chemistry Department.

The roots were dried, powdered (3 kg.) and extracted with 95\% ethanol. The ethanotic extract was
concentrated under reduced pressure to a viscous mass (.201%). It was successively extracted with pet. ether, benzene, chloroform, ethylacetate, acetone and finally with methanal.

STUDY OF ACETONE SOLUBLE FRACTION

The acetone soluble fraction on the removal of solvent gave light yellow coloured amorphous mass (0.096%) which showed three spots on TLC examination using ethyl acetate: chloroform : acetone (70:20:10) as solvents. The fraction was therefore subjected to column chromatography with eluents; ethylacetate:acetone (13:7 v/v). The eluents were of same Rf values hence combined. Removal of the solvents yielded a colour less crystalline compound Sg (0.087%).

STUDY OF THE COMPOUND (Sg)

The compound Sg was soluble in ethylacetate, methanol and ethanol. It analysed for molecular formula C\textsubscript{15}H\textsubscript{19}O\textsubscript{3} m.p. 60\textdegree C and M\textsuperscript{+} 247 (EIMS).

It gave all the characteristic colour reactions\textsuperscript{14} of sesquiterpene lactone. Its lactonic nature was also confirmed by positive hydroxamic acid test\textsuperscript{15}.

UV SPECTRUM OF THE COMPOUND Sg

It showed strong absorption at EtOH 207 nm indicating the presence of \(\alpha\)-methylene-\(\gamma\)-lactone ring\textsuperscript{16}. 


IR SPECTRUM OF THE COMPOUND Sq

The significant peaks obtained in the IR spectrum (Fig-I) of Sq and structural units made with the help of available literature\textsuperscript{17-20} are recorded in the Table-1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Absorption (cm\textsuperscript{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3370</td>
<td>-OH group (s)</td>
</tr>
<tr>
<td>2.</td>
<td>2860</td>
<td>Methoxy group</td>
</tr>
<tr>
<td>3.</td>
<td>1762, 1750</td>
<td>(\alpha)-methylene(-)lactone</td>
</tr>
<tr>
<td>4.</td>
<td>1712</td>
<td>Acetoxy group</td>
</tr>
<tr>
<td>5.</td>
<td>1640</td>
<td>Unsaturation</td>
</tr>
<tr>
<td>6.</td>
<td>890</td>
<td>-C=CH\textsubscript{2} group</td>
</tr>
</tbody>
</table>

GUAINANE SKELETON OF THE COMPOUND Sq

The IR spectrum of Sq showed peak at \(\gamma\)\textsubscript{KBr} at 3370 cm\textsuperscript{-1} indicating the presence of -OH group in it. Sq on dehydration with conc. H\textsubscript{2}SO\textsubscript{4} at very high temperature yielded a colour less compound Sq\textsubscript{1}, m.p. 58.6\degree C, molecular formula C\textsubscript{15}H\textsubscript{18}O\textsubscript{2} and M\textsuperscript{+} 230 (EIMS), which showed absence of any peak in the -OH group region in its IR spectrum, thereby confirmed that hydroxy group has been converted into exomethylene group.
Sg₁ when reduced with sodium borohydride yielded a crystalline compound Sg₂ molecular formula $C_{15}H_{22}O_2$ m.p. 109-112° and M⁺ 234 (EIMS). Sg₂ on Huang-Minlon reduction followed by dehydration of the resulting product Sg₈ by potassium hydrogen sulphate, gave compound Sg₃ molecular formula $C_{15}H_{26}$ b.p. 114°. Sg₃ was then subjected to hydrogenation in acetic acid using Pt-catalyst when it gave a colourless liquid Sg₄, b.p. 126-129°, molecular formula $C_{15}H_{28}$, which was identified as guaiane (by superimposable spectra). Formation of Sg₄ confirmed the guaiane skeleton of Sg₄ (FIG.II).

$$\text{Sg} \xrightarrow{\text{Sod. Borohydride}} \text{Sg}_1$$
$$\text{Sg}_1 \xrightarrow{\text{Huang-Minlon reduction}} \text{Sg}_2$$
$$\text{Sg}_2 \xrightarrow{\text{Dehydration/KHSO}_4} \text{Sg}_3$$
$$\text{Sg}_3 \xrightarrow{\text{Pt-catalyst}} \text{Sg}_4$$

Thus the Sg may tentatively be represented as:
PRESENCE OF DOUBLE BOND (S)

Peaks at $\nu_{\text{max}}^{\text{KBr}}$ 1640 and 890 cm$^{-1}$ in the IR spectrum of Sg showed the presence of exocyclic unsaturation in it.

On catalytic hydrogenation with palladium black, Sg yielded a liquid Sg$_5$, molecular formula C$_{15}$H$_{23}$O$_3$, b.p. 130-132$^\circ$ which is tetrahydroderivative of Sg and indicated the presence of two double bonds in Sg.

\[
\text{Sg} \xrightarrow{\text{Catalytic hydrogenation}} \quad \text{Sg}_5 \quad \text{(tetrahydroderivative)}
\]

\[
\begin{array}{c}
\text{(C}_{15}^\text{H}_{19}^\text{O}_3) \\
\text{Pd-black}
\end{array} \rightarrow \begin{array}{c}
\text{(C}_{15}^\text{H}_{23}^\text{O}_3)
\end{array}
\]

NATURE OF DOUBLE BONDS

Peaks in the IR spectrum of Sg (as mentioned above) confirmed the presence of exomethyleneic double bonds in it.

PRESENCE OF EXOMETHYLENE GROUP (S)

Absorption in the IR spectrum of Sg at $\nu_{\text{max}}^{\text{KBr}}$ 890 and 1640 cm$^{-2}$ showed the presence of exocyclic methylene group (s) in it, whereas IR peaks at $\nu_{\text{max}}^{\text{KBr}}$ 1762 and
1750 cm⁻¹ confirmed the presence of additional exomethylene group in the lactone ring²³.

POSITION OF EXOCYCLIC METHYLENE GROUP (S)

On chromic acid oxidation²² Sg₂ (obtained as described on page 50) yielded a colourless liquid Sg₆ molecular formula C₁₅H₂₀O₂ b.p. 154-157° which is dihydroderivative of Sg₁ in which the methylene group, present in conjugation with lactone carbonyl in Sg, would have undergone reduction to -CH-CH₃ group and thus confirmed its position in Sg at C-13, because this is the only possibility for the position of exomethylene group.

¹HNMR spectrum of Sg (Fig-III) displayed low field doublets at δ 5.4 and δ 6.12 which further confirmed the C-13 position of this exocyclic methylene group.

The position of the other exocyclic methylenic group was established by ¹HNMR spectrum (Fig-III) of Sg which showed signal at δ 4.90 (d, J=3 Hz) showing the only possibility for the presence of exocyclic methylene group at C-14.

The tentative structure of Sg may be represented as:

![structure diagram]
PRESENCE OF LACTONE RING

IR spectrum of Sg showed peaks at $KBr_{max}$ 1762 and 1750 cm$^{-1}$ indicating the presence of $\alpha$-methylene $\gamma$-lactone in it$^{23}$.

POSITION OF LACTONE RING

Sg$_1$ (obtained as described on page 49) on reduction with sodium borohydride yielded a crystalline compound Sg$_2$, molecular formula C$_{15}H_{22}O_2$, m.p. 109-112$^\circ$, and M$^+$.234 (EIMS).

\[
\begin{align*}
\text{Sg}_1 & \xrightarrow{\text{Reduction}} \text{NaBH}_4 \xrightarrow{} \text{Sg}_2 \\
(C_{15}H_{18}O_2) & \quad (C_{15}H_{22}O_2)
\end{align*}
\]

IR spectrum of Sg$_2$ showed significant peak at $\nu_{KBr}^{max}$ 3485 cm$^{-1}$ for -OH group and $\nu_{KBr}^{max}$ 2722 cm$^{-1}$ for -CHO group. Of course no absorption band at $\nu_{KBr}^{max}$ 1762 and 1750 cm$^{-1}$ for $\alpha$-methylene-$\gamma$-lactone ring was observed in Sg$_2$. The IR spectrum of Sg$_2$ was found to be superimposable with the IR spectrum of the lactol, thereby leading to its identity as 'lactol' and fixing the position of lactone ring at C$_6$ and C$_7$. 
The above conclusion about the position of the \( \alpha \)-methylene-\( \gamma \)-lactone is also supported by the \( ^1 \)HNMR spectrum (Fig-III) of Sg due to the fact that double doublet was observed for \( C_6^-H \) while multiplet was observed for \( C_7^-H \).

Sg\(_2\) on Huang-Minlon reduction yielded a liquid Sg\(_7\), molecular formula \( C_{15}^1 H_{24} O \), b.p. 120°. The IR spectrum of Sg\(_7\) (Fig-IV) was found to be superimposable with the IR spectrum of Monol.

\[
\begin{align*}
\text{Sg}_2 & \quad \text{H.M. Reduction} \quad \rightarrow \\
(C_{15}^1 H_{22} O_2) & \quad \text{Sg}_7 \\
(C_{15}^1 H_{24} O) &
\end{align*}
\]

Above facts further supported the position of lactone ring at \( C_6^-C_7 \).

Based on the above discussion the compound Sg may tentatively be represented as.
IR SPECTRUM OF \( S_9 \)

FIG. IV
Now the only part left with the structure of Sg is to study the functional nature of remaining oxygen atom in it.

**PRESENCE OF HYDROXYL GROUP (S)**

A peak at $\nu_{\text{max}}^{KBr} 3370 \text{ cm}^{-1}$ in the IR spectrum of the Sg showed the presence of $-\text{OH}$ group (s) in it.

The number of OH group (s) in Sg were estimated by its acetylation with $\text{Ac}_2\text{O/pyridine}$ to get an acetylated product $\text{Sg}_9$, molecular formula $\text{C}_{17}\text{H}_{21}\text{O}_4$, m.p. 145-148°C, $M^+ = 289$ (EIMS) indicating the presence of only one acetylatable hydroxy group (14.87%).

\[
\begin{align*}
\text{Sg} & \xrightarrow{\text{Ac}_2\text{O/Py}} \text{Sg}_9 \text{ (acetylated product)} \\
(C_{15}H_{19}O_3) & \quad (C_{17}H_{21}O_4)
\end{align*}
\]

IR spectrum of $\text{Sg}_8$ showed peak at $\nu_{\text{max}}^{KBr} 1719 \text{ cm}^{-1}$ and did not exhibit any peak of $-\text{OH}$ group there by showing complete acetylation of the hydroxyl group.

Sg on methylation with $\text{K}_2\text{CO}_3$ in acetone yielded a methylated product $\text{Sg}_{10}$ m.p. 131-133°C, molecular formula $\text{C}_{16}\text{H}_{21}\text{O}_3$ and $M^+ 261$ (EIMS).

\[
\begin{align*}
\text{Sg} & \xrightarrow{\text{dry} \text{K}_2\text{CO}_3} \text{Sg}_{10} \\
(C_{15}H_{19}O_3) & \quad \text{In acetone} \quad (C_{16}H_{21}O_3)
\end{align*}
\]
The IR spectrum of Sg showed peak at $\nu_{\text{max}}^{\text{KBr}}$ 2862 cm$^{-1}$ for -OCH$_3$ group and did not exhibit any peak of -OH group there by showing complete methylation of hydroxyl group. Estimation of the methoxy group by the Weisels method$^{25}$ confirmed the presence of only one methoxy group (11.87%) in Sg.

**POSITION OF HYDROXYL GROUP**

The position of hydroxyl group in Sg was confirmed by its dehydration with 75% conc. H$_2$SO$_4$ at 136-140$^\circ$C, when it yielded a compound Sg$_1$, molecular formula C$_{15}$H$_{16}$O$_2$, m.p. 58$^\circ$, and M$^+$ 230 (EIMS) in which -CH$_2$OH group was converted into C=CH$_2$ group.

The $^1$HNMR spectrum (Fig-II) of Sg showed double doublet at $\delta$ 4.18 indicating the position of hydroxyl group at C-15.

$^1$HNMR spectrum of Sg$_1$ showed signals at $\delta$ 5.30 and $\delta$ 5.08 (2H) for exocyclic methylene group at C-15 in it, which were absent in the $^1$HNMR spectrum of Sg, thereby confirming the reduction of -CH$_2$OH group into C=CH$_2$ group at C-15 position.

Based on above discussion, the final structure of the compound Sg was assigned as (I), which is; 15-hydroxy dehydrocostus lactone and explained all
the reactions as described in the Scheme - I.

![Chemical Structure](image)

The identity of the compound Sg was further confirmed by $^1$HNMR, $^{13}$C NMR and Mass spectral studies.

**$^1$HNMR SPECTRUM OF THE COMPOUND Sg**

$^1$HNMR spectrum of compound (Sg) was found to be in complete agreement with the structure. The important signals obtained in the $^1$HNMR spectrum of Sg and structural assignment made with the help of available literature are given in Table-2.
**TABLE - 2**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value $\delta$</th>
<th>Pattern</th>
<th>J value (H$_2$)</th>
<th>No. of protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6.12</td>
<td>d</td>
<td>3</td>
<td>1</td>
<td>C$_{13}$-H</td>
</tr>
<tr>
<td>2.</td>
<td>5.48</td>
<td>d</td>
<td>3</td>
<td>1</td>
<td>CH$_3$-H</td>
</tr>
<tr>
<td>3.</td>
<td>4.90</td>
<td>d</td>
<td>3</td>
<td>2</td>
<td>C$_{14}$H$_2$</td>
</tr>
<tr>
<td>4.</td>
<td>4.50</td>
<td>dd</td>
<td>11</td>
<td>1</td>
<td>C$_6$-H</td>
</tr>
<tr>
<td>5.</td>
<td>4.18</td>
<td>dd</td>
<td>11</td>
<td>-</td>
<td>CH$_2$OH</td>
</tr>
<tr>
<td>6.</td>
<td>4.08</td>
<td>dd</td>
<td>11</td>
<td>1</td>
<td>C$_5$H</td>
</tr>
<tr>
<td>7.</td>
<td>3.90</td>
<td>t</td>
<td>10</td>
<td>2</td>
<td>C$_9$-H$_2$</td>
</tr>
<tr>
<td>8.</td>
<td>3.86</td>
<td>t</td>
<td>10</td>
<td>1</td>
<td>C$_1$H</td>
</tr>
<tr>
<td>9.</td>
<td>3.67</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>C$_8$H$_2$</td>
</tr>
<tr>
<td>10.</td>
<td>3.49</td>
<td>m</td>
<td>-</td>
<td>1</td>
<td>C$_7$H</td>
</tr>
<tr>
<td>11.</td>
<td>3.40</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>C$_3$H$_2$</td>
</tr>
<tr>
<td>12.</td>
<td>3.30</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>C$_2$H$_2$</td>
</tr>
</tbody>
</table>

**13C NMR OF THE COMPOUND Sg**

The $^{13}$C NMR spectrum of Sg showed significant signal which are recorded in the Table-3 and further confirmed the identity of Sg as 15-hydroxy dehydrocostus lactone.
### TABLE - 3

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>Value (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>45.4</td>
</tr>
<tr>
<td>2.</td>
<td>30.6</td>
</tr>
<tr>
<td>3.</td>
<td>31.1</td>
</tr>
<tr>
<td>4.</td>
<td>150.9</td>
</tr>
<tr>
<td>5.</td>
<td>51.9</td>
</tr>
<tr>
<td>6.</td>
<td>85.7</td>
</tr>
<tr>
<td>7.</td>
<td>48.2</td>
</tr>
<tr>
<td>8.</td>
<td>32.9</td>
</tr>
<tr>
<td>9.</td>
<td>36.8</td>
</tr>
<tr>
<td>10.</td>
<td>182.1</td>
</tr>
<tr>
<td>11.</td>
<td>140.1</td>
</tr>
<tr>
<td>12.</td>
<td>119.9</td>
</tr>
<tr>
<td>13.</td>
<td>109.8</td>
</tr>
<tr>
<td>14.</td>
<td>112.1</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM$^{29}$ OF THE COMPOUND (Sg)**

The important fragmentation pattern obtained in the electron impact mass spectrum of the compound (Sg) has been given below which further confirmed the structure I for Sg. The various fragments obtained are described in scheme-II. M/e 247, 154, 97, 56.
Scheme II
EXPERIMENTAL

The plant Saussurea lappa C.B. Clarke was supplied by Lahoul Kuth grower co-op Ltd. Manali (H.P.) and authenticated by Botany Department of this University.

Air dried and powdered roots (3 kg.) were extracted with 95% ethanol in 10 lit. R.B. flask which was fitted with a water condenser and extraction continued for several days. The extract was concentrated under reduced pressure to a viscous mass (6.70 gm). It was successively extracted with pet. ether, benzene, chloroform, ethyl acetate, acetone and finally with methanol.

STUDY OF ACETONE SOLUBLE PART

The acetone soluble part (250 ml.) was concentrated under reduced pressure to get light yellow viscous mass (3.20 gm.) which showed three spots on TLC examination using ethylacetate : chloroform : acetone (70:20:10). The fraction was then subjected to column chromatography. The observation and results are recorded in the Table-4 and 5.
TABLE - 4

1. Length of column - 100.0 cm.
2. Diameter of column - 3.0 cm.
3. Weight of silica gel - 80.0 gm.

TABLE - 5

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluent collected</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-18</td>
<td>ethylacetate : acetone (13:7)</td>
<td>Yielded Compound (Sg)</td>
</tr>
<tr>
<td>2</td>
<td>19-29</td>
<td>(7:13)</td>
<td>Negligible</td>
</tr>
<tr>
<td>3</td>
<td>30-42</td>
<td>(10:10)</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

Eluents from 19-29 and 30-42 were of same Rf values hence combined separately and on removal of solvents yielded very small amount of viscous mass which were insufficient for any substantative studies, so were rejected.

STUDY OF THE FRACTION (1-18)

Fractions 1-18 were of same Rf values so these were mixed, which on removal of solvent yielded a colourless crystalline compound (2.90 gm.).
STUDY OF THE COMPOUND (Sg)

The compound was soluble in methanol, ethyl acetate and ethanol, it melted at 60⁰ and analysed for molecular formula $C_{15}H_{19}O_3$ and $M^+ 247$ (EIMS). It responded to positive hydroxamic acid test and other colour reactions.

HYDROXAMIC ACID TEST

A small quantity of compound was dissolved in acetone with few drops of alcoholic hydroxyl amine hydrochloride and 2-3 drops of saturated alc. KOH in a porcelin crucible. The mixture was then cooled, acidified with HCl and a drop of 1% FeCl₃ solution was added. Violet colour occur indicated; the presence of lactone ring in Sg.

COLOUR TESTS

(1) 2 ml. $H_2SO_4$ (50%) and 3-4 drops of aq. FeCl₃ were mixed with small quantity of compound on warming for some time on a boiling water bath, when red colour was obtained.

(2) Few drops of compound (Sg) dissolved in 50% methanol and were mixed, with alcoholic solution of m-dinitrobenzene and alc. KOH when a red colour was obtained.
(3) A small quantity of compound Sg when treated with 10% alc. solution of KOH, produced red colour.

(4) Small quantity of compound (Sg) when treated with dil HCl and resorcinol gave red orange colour.

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C_{15}H_{19}O_{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=73.30</td>
<td>C=73.20</td>
</tr>
<tr>
<td>H=7.32</td>
<td>H=7.55</td>
</tr>
</tbody>
</table>

Molecular weight 247
(By mass spectroscopy)

**ACETYLATION OF Sg TO Sg_{9}**

In 500 ml. R.B. flask fitted with an air condenser, 200 mg of Sg was mixed with 5 ml. pyridine, and 50 ml. acetic anhydride. The reaction mixture was refluxed on a water bath for 2 hrs, the mixture was then cooled and poured in cold water (100 ml) when a precipitate appeared. The precipitate was taken in ether (50 ml) and the ethereal layer was washed with water and sodium bicarbonate, then dried over anhydrous sodium sulphate. Ether was removed by evaporation and the residue was crystallised from acetone to get acetyl derivative (140 mg), molecular formula C_{17}H_{21}O_{4} m.p. 147-149°C M^+ = 289.
ELEMENTAL ANALYSIS

Found
C=76.70
H= 8.31

Calculated for $C_{17}H_{21}O_4$
C=76.75
H= 8.50

Molecular weight - 289
(By mass spectroscopy)

METHYLATION OF $Sg_9$ TO $Sg_{10}$

150 mg. of the compound $Sg$ was mixed with 5 ml. dimethyl sulphate, and anhydrous $K_2CO_3$ and 125 ml. acetone in a 500 ml R.B. flask, and refluxed for 24 hrs.

The reaction mixture was filtered and to it water was added and the acetone was distilled off, which yielded an oily residue which was chromatographed on silica gel and eluted with solvent ether. The resultant residue was crystallized from chloroform to yield colourless needleless ($Sg_9$) (170 mg) m.p. 131-133°, analysed for molecular formula $C_{16}H_{21}O_3$ and $M^+ 261$ (EIMS).

ELEMENTAL ANALYSIS

Found
C=78.50
H= 9.10

Calculated for $C_{16}H_{21}O_3$
C=78.55
H= 9.12

Molecular weight - 261
(By mass spectroscopy)
DEHYDRATION OF Sg TO Sg₁

Sg (215 mg) was heated with 75% conc. H₂SO₄ at 135-140°C. The solution was then allowed to stand at 0°C for 72 hrs. On cooling gave a residue (200 mg) which was crystallised twice from methanol to gave 195 mg. Sg₁ m.p. 58.6°C, analysed for molecular formula C₁₅H₁₈O₂ M⁺ - 230 (EIMS).

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₁₅H₁₈O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=78.10</td>
<td>C=78.15</td>
</tr>
<tr>
<td>H= 7.95</td>
<td>H= 7.90</td>
</tr>
</tbody>
</table>

Molecular weight - 230
(By mass spectroscopy)

CATALYTIC HYDROGENATION OF Sg TO Sg₅

(100 mg) Sg was dissolved in alcohol (0.50 ml) and hydrogenated under pressure using pd-black initially at room temperature and then 50°C, for 6 hrs. Alcohol was then removed and residue was diluted with water and extracted with ether. Removal of ether gave a liquid (75 mg) Sg₅ b.p. 130-132°C, analysed for molecular formula C₁₅H₂₃O₃.
ELEMENTAL ANALYSIS

Found  
C=76.70  
H= 9.85  

Calculated for C_{15}H_{23}O_{3}  
C=76.75  
H= 9.90  

NaBH\textsubscript{4} REDUCTION OF Sg\textsubscript{1} TO Sg\textsubscript{2}

175 mg. of the compound Sg\textsubscript{1} and sodium borohydride (50 mg) were taken in 50 ml. methanol and left for 2 hrs. at room temperature. 2 N H\textsubscript{2}SO\textsubscript{4} (1 ml.) was added in it and extract was washed with sodium carbonate, dried over sodium sulphate and evaporated to afford the crystalline product Sg\textsubscript{2} M.P. 109-112\degree, analysed for molecular formula C_{15}H_{22}O_{2} M\textsuperscript{+} 234 (EI MS).

ELEMENTAL ANALYSIS

Found  
C=77.50  
H= 9.56  

Calculated for C_{15}H_{22}O_{2}  
C=77.80  
H= 9.70  

Molecular weight - 234 
(By mass spectroscopy)

CHROMIC ACID OXIDATION OF Sg\textsubscript{2} TO Sg\textsubscript{6}

Sg\textsubscript{2} (40 mg) was dissolved in acetone (10 ml) and Jones reagent was added dropwise till a brown colour persisted. It was kept at room temperature for an hr. which and worked up to get a liquid on distillation afforded
Sg₃ (30 mg) b.p. 154-157⁰ analysed for molecular formula C₁₅H₂₀O₂.

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₁₅H₂₀O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=77.50</td>
<td>C=70.52</td>
</tr>
<tr>
<td>H= 8.70</td>
<td>H= 8.75</td>
</tr>
</tbody>
</table>

**HUANGIMINLON REDUCTION OF Sg₂ TO Sg₇**

The 150 mg Sg₂ was dissolved in freshly distilled diethylene glycol (100 ml.) in a flask fitted with a condenser, a thermometer, and N₂ gas was allowed to bubble. KOH (5 mg) and hydrazine hydrate (10 ml.) were introduced. The mixture was heated to 175⁰. After cooling it was diluted, with water and extracted with ether to gave a liquid (140 mg) which was chromatographed on si-gef.

**TABLE - 6**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluent collected</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 ml. each</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1-12</td>
<td>Pet ether (100 ml)</td>
<td>Sg₇ compound</td>
</tr>
<tr>
<td>2.</td>
<td>12-26</td>
<td>Pet ether, Benzene</td>
<td>Negligible</td>
</tr>
<tr>
<td>3.</td>
<td>26-36</td>
<td>Pet ether, Benzene (1:1)</td>
<td>Negligible</td>
</tr>
</tbody>
</table>
Fraction I (1-12) on distillation yielded Sg\(_7\) (25 mg) b.p. 122\(^\circ\) analysed for molecular formula C\(_{15}\)H\(_{24}\)O.

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C(<em>{15})H(</em>{24})O</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=81.55</td>
<td>C=81.70</td>
</tr>
<tr>
<td>H=10.80</td>
<td>H=10.90</td>
</tr>
</tbody>
</table>

**HUANGMINLON REDUCTION OF Sg\(_2\) TO Sg\(_8\)**

Mixture of Sg\(_2\) (50 mg), diethylene glycol (5 ml) KOH (1 gm) and hydrazine hydrate (5 ml) was heated at 110\(^\circ\) for 2.5 hrs. in an atmosphere of N\(_2\). After cooling it was diluted (50 mg) then chromatographed on silica gel and eluted with (1) Pet. ether : benzene (2:1), (2) Pet. ether : benzene (1:1). The fraction (1) was dried and crystallised from alcohol to gave Sg\(_8\) (47 mg.) m.p. 54-55\(^\circ\), analysed for molecular formula C\(_{15}\)H\(_{28}\)O \(M^+\) 224 (EIMS).

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C(<em>{15})H(</em>{28})O</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=81.10</td>
<td>C=80.90</td>
</tr>
<tr>
<td>H=11.90</td>
<td>H=11.80</td>
</tr>
</tbody>
</table>

Molecular weight - 224  
(By mass spectroscopy)
DEHYDRATION OF Sg₈ TO Sg₃

47 mg. of compound Sg₈ and KHSO₄ (50 mg) were taken in a flask fitted with reflux condenser and heated in an atmosphere of N₂ at 100° for 1.5 hrs. After cooling the product was dissolved in pet ether and chromatographed on si-gel and eluted with pet. ether to get Sg₃ (40 mg) b.p. 111-114° and analysed for molecular formula C₁₅H₂₆.

ELEMENTAL ANALYSIS

Found Calculated for C₁₅H₂₆
C=86.80 C=86.90
H=12.56 H=12.70

HYDROGENATION OF Sg₃ TO Sg₄

Sg₃ (40 mg) on hydrogenated in acetic acid using pt-catalyst at 50°. After hydrogenation, the residue was diluted with water and extracted with ether. Removal of ether gave a liquid (30 mg) Sg₄ b.p. 126-129° analysed for molecular formula C₁₅H₂₈.

ELEMENTAL ANALYSIS

Found Calculated for C₁₅H₂₈
C= H=12.95 12.95
H=13.11
REFERENCES


