CHAPTER - II

ISOLATION AND STRUCTURAL STUDY OF A NOVEL XANTHANOLIDE
14 HYDROXY XANTHUMIN
FROM THE STEMS OF XANTHIUM STRUMARIUM LINN*

* This work has been submitted for the publication to PHOTOCHEMISTRY, ENGLAND.
Xanthium strumarium Linn is commonly known as "Chhota Gokhru" and belongs to family Compositae. It is an unarmed herb which grows in throughout India and warmer parts of the world.

The plant has an acrid taste. It is laxative, anthelmintic, alexiteric, antipyretic, improves appetite and cures leucoderma. The whole plant is reported to be cytotoxic and very efficacious in long standing cases of malarial fever.

Earlier workers have already reported the presence of cytotoxic xanthanolides in it. During the last decade some significant reports have been published giving information that application of 50% ethanolic extract of plant for 10 days has exhibited 60% response in the cure of advanced breast cancer.

A consideration of above facts and in view of the claimed antitumour activity of sesquiterpene lactones and due to presence of unidentified compounds (as reported in the literature) in Xanthium strumarium, the authoress became very much interested and decided to undertake a comprehensive phytochemical study of Xanthium strumarium Linn.
ISOLATION OF THE XANTHANOLIDE:

Xanthium strumarium Linn (N.O. compositae) was procured from United Chemicals and Allied Product, Calcutta and authenticated by Botany Department of this University. A herbarium specimen (No. V-XXV) has been deposited at room no. 36 of the Chemistry Department.

Air dried and powdered STEMS of Xanthium strumarium Linn were extracted exhaustively with 90% ethanol. The extract was concentrated under reduced pressure to a viscous mass. It was segregated into petroleum ether (60-80°C), benzene, chloroform, ethyl acetate, acetone and methanol soluble fractions.

STUDY OF THE CHLOROFORM SOLUBLE FRACTION:

The chloroform soluble fraction on the removal gave of solvent a light brown coloured viscous mass which showed three spots on TLC examination using benzene: chloroform: ethyl acetate (65:25:10). The fraction was chromatographed on Si gel with solvents of increasing polarity. Eluent from benzene: chloroform (15:7,4v/v) were of same Rf value, hence combined. Removal of solvent yielded a colourless crystalline compound (0.05%), XP. Other two fractions from benzene: chloroform (15:10) and (1:1) on concentration gave residues in very small amount, for any substantive phytochemical investigation.
STUDY OF COMPOUND (XP):

This compound (XP) was soluble in methanol and acetone and crystallized from solvent ether. It analysed for $\text{C}_{17}\text{H}_{22}\text{O}_6$, m.p. $107-09^\circ$ and $M^+$ 322 (EIMS).

It gave all the characteristic colour reactions\textsuperscript{11} of xanthanolide and was positive to the hydroxamic acid test\textsuperscript{14}. So a basic structure to XP could be assigned as (1).

\[ \text{(1)} \]

IR SPECTRUM OF THE XANTHANOLIDE XP:

The characteristic peaks obtained in the IR spectrum of the compound and structural assignments made with the help of literature\textsuperscript{11,15-19} are given in Table-1.
TABLE - 1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Absorption (cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3460</td>
<td>OH groups(s).</td>
</tr>
<tr>
<td>2.</td>
<td>1760, 1750</td>
<td>(\alpha)-methylene-(Y)-lactone group</td>
</tr>
<tr>
<td>3.</td>
<td>1730</td>
<td>Acetoxy group</td>
</tr>
<tr>
<td>4.</td>
<td>1725</td>
<td>(\text{C:O of an aldihydic or ketonic group})</td>
</tr>
<tr>
<td>5.</td>
<td>1660</td>
<td>Ethylenic double group</td>
</tr>
<tr>
<td>6.</td>
<td>1060</td>
<td>(\text{C-O streching vibration of OH group})</td>
</tr>
<tr>
<td>7.</td>
<td>970</td>
<td>Cycloheptane ring</td>
</tr>
<tr>
<td>8.</td>
<td>890</td>
<td>(-\text{C=CH}_2) deformation</td>
</tr>
</tbody>
</table>

**PRESENCE OF HYDROXYL GROUP(S):**

IR spectrum of compound XP showed peak at \(v_{\text{KBr}}^\text{max}\) 3460 cm\(^{-1}\) indicating the presence of free hydroxyl group(s). On acetylation (Ac\(_2\)O/Py) the xanthanolide yielded an acetylated derivative XP\(_1\), m.p. 150-52\(^\circ\), molecular formula \(\text{C}_{19}\text{H}_{24}\text{O}_7\) and \(M^+ 364\) (EIMS).

IR examination of the acetoxyl derivative of XP indicated presence of peak at \(v_{\text{KBr}}^\text{max}\) 1715 cm\(^{-1}\) and absence of peak at 3460 cm\(^{-1}\) thereby concluding that all the hydroxyl groups must have undergone acetylation. Estimation of the acetoxyl group (1.20%) by Wiesenberger
method\textsuperscript{20} as described by Belcher and Godbert\textsuperscript{21} suggested the presence of one hydroxyl group in XP.

\[
\text{Xanthanolide } \xrightarrow{\text{Ac}_2\text{O/Py}} \text{ Xanthanolide mono acetate}
\]

\[
\begin{align*}
\text{C}_{17}\text{H}_{22}\text{O}_6 & \quad \text{C}_{19}\text{H}_{24}\text{O}_7 \\
(\text{XP}) & \quad (\text{XP}_1)
\end{align*}
\]

Presence of one hydroxyl group was further supported by methylation when the xanthanolide XP gave a methylated derivative XP\textsubscript{2}, m.p. 135-37\textdegree, molecular formula \text{C}_{18}\text{H}_{24}\text{O}_6 and \text{M}^+ 336 (EIMS).

\[
\text{DMS+K}_2\text{CO}_3 \text{ in} \xrightarrow{\text{Xanthanolide}} \text{Methyl ether of xanthanolide}
\]

\[
\begin{align*}
\text{C}_{17}\text{H}_{22}\text{O}_6 & \quad \text{acetone} \quad \text{C}_{18}\text{H}_{24}\text{O}_6 \\
(\text{XP}) & \quad (\text{XP}_2)
\end{align*}
\]

In the IR spectrum of XP\textsubscript{2}, appearance of peak at 2865 cm\textsuperscript{-1} and disappearance of hydroxyl peak at 3460 cm\textsuperscript{-1} indicated complete methylation. Estimation of the methoxy groups (4.40\%) by Ziesel’s method\textsuperscript{22} confirmed the presence of one methoxy group in XP.

**NATURE OF THE OH GROUP :**

On reduction with conc. HI and red phosphorous\textsuperscript{23}, XP gave a compound XP\textsubscript{3}, m.p. 120-22\textdegree, molecular formula \text{C}_{17}\text{H}_{22}\text{O}_5, \text{M}^+ 262 (EIMS) which was identified as xanthumin
and \(^1\text{H} \text{NMR}\) (by superimposable IR/spectral analysis, Fig. No. 2,3) thereby indicating the conversion of \(-\text{CH}_2\text{OH}\) group in XP to methyl group in XP\(_3\) as given below:

\[
\begin{align*}
\text{Xanthanolide} & \rightarrow \text{HI/Red P} \\
\text{OAc} \\
\text{C}_{17}\text{H}_{22}\text{O}_6 & \quad \text{C}_{17}\text{H}_{22}\text{O}_5 \\
(\text{XP}) & \quad (\text{XP}_3)
\end{align*}
\]

In the \(^1\text{H} \text{NMR}\) spectrum of XP, doublet of two proton intensity appeared at \(\delta 4.10\) and confirmed the presence of primary hydroxyl group in it (Fig. 4).

**POSITION OF OH GROUP:**

The conversion of XP into the known compound Xanthumin on reduction with HI and red phosphorous as described above established the fact that the primary hydroxyl group must be at C-10.

The examination of \(^1\text{H} \text{NMR}\) spectrum of XP indicated the presence of a triplet at \(\delta 5.39\) for the proton at C-2 and supported the fact that C-3 was
$^1$H NMR SPECTRUM OF THE XANTHANOLIDE (X$\beta_3$)
unsubstituted. Another signal as multiplet at $\delta$ 3.28 showed $\text{CH}_7$ proton and confirmed that C-6 position was unsubstituted, thereby leaving the only possibility for -CH$_2$OH group at C-9 or C-10. Signal in the $^1$H NMR at $\delta$ 1.95 as dd established the presence of two protons at C-9.

The above deliberations finally established that the only possibility for the presence of -CH$_2$OH group is at C-10. These facts finally concluded that the partial structure to XP may be assigned as (2).

![Chemical Structure](image)

**PRESENCE OF ACETOXYL GROUP:**

Absorption in IR spectrum at $\nu_{\text{max}}^{\text{KBr}}$ 1730 cm$^{-1}$ indicated the presence of acetoxyl group in XP (Fig.1).
POSITION OF ACETOXYL GROUP:

The xanthanolide (XP) on treatment with alcoholic solution of sodium acetate{superscript 19} gave a viscous des-acetylated product XP{subscript 4}, molecular formula C{subscript 15}H{subscript 18}O{subscript 4} and M{superscript +} 262, which showed a peak in IR spectrum at 1670 cm{superscript -1} for the presence of α, β unsaturated carboxyl group and indicating desacetylation of XP, concluding in turn the presence of acetoxyl group at C-2 in XP.

\[
\text{Xanthanolide} \xrightarrow{\text{Acetate}} \text{Des-acetylated Product}
\]

C{subscript 15}H{subscript 22}O{subscript 6} \rightarrow C{subscript 15}H{subscript 18}O{subscript 4}

(XP) \quad (XP{subscript 4})

This fact was further supported by the {superscript 1}H NMR spectrum of XP which showed a triplet of one proton intensity at δ 5.39 for the methine proton of C-2 because had C-2 been unsubstituted than a triplet of two proton intensity would have been observed instead of one. Thus a partial structure to XP may be assigned as (3).
PRESENCE OF ACETYL GROUP:

The IR spectrum showed a peak at $\nu_{\text{max}}^{\text{KBr}} 1725 \text{ cm}^{-1}$ for the presence of acetyl group in XP. The presence of acetyl group was further supported by the formation of 2:4 dinitrophenylhydrazone XP$_5$, m.p. 211-12$^\circ$, molecular formula $\text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_9$, $M^+$ 502 (EIMS) from 2:4 dinitrophenylhydrazone. 11

\[
\begin{align*}
\text{Xanthanolide} & \quad \xrightarrow{2:4 \text{ DNPH}} \quad 2:4 \text{ dinitrophenylhydrazone of XP} \\
\text{C}_{17}\text{H}_{22}\text{O}_6 & \quad \text{C}_{23}\text{H}_{26}\text{N}_4\text{O}_9 \\
(\text{XP}) & \quad (\text{XP}_5)
\end{align*}
\]

POSITION OF ACETYL GROUP:

XP on treatment with sodium hypoiodite gave iodoform as one of the product alongwith a compound XP$_6$, m.p. 262-63$^\circ$, molecular formula $\text{C}_{14}\text{H}_{16}\text{O}_3$ and $M^+$ 264 (EIMS). The UV absorption spectrum of this compound gave an absorption maxima $\lambda_{\text{max}}$ 250 nm which is characteristic of $\omega$-Y dienoic acid, thereby fixing acetyl group at C-3 in XP.

\[
\begin{align*}
\text{Xanthanolide} & \quad \xrightarrow{\text{NaIO}_4} \quad \text{An acid derivative} \\
\text{C}_{17}\text{H}_{22}\text{O}_6 & \quad \text{C}_{14}\text{H}_{16}\text{O}_3 \\
(\text{XP}) & \quad (\text{XP}_6)
\end{align*}
\]
Thus a partial structure to XP may be given as (4).

\[ \text{(4)} \]

**Presence of Double Bonds:**

A peak in the IR spectrum of the xanthanolide (XP) at $\nu_{\text{max}}^\text{KBr} 1645 \text{ cm}^{-1}$ showed the presence of unsaturation in it.

On catalytic hydrogenation with Pd/C, XP yielded a tetrahydro derivative $\text{XP}_7$, m.p. 170-71°C, molecular formula $\text{C}_{17}\text{H}_{26}\text{O}_6$, $M^+ 326$ (EIMS), thus indicating the presence of two double bonds in it.

\[
\text{Xanthanolide} \xrightarrow{\text{Pd/C}} \text{Tetrahydro derivative} \\
\text{C}_{17}\text{H}_{22}\text{O}_6 \quad \text{C}_{17}\text{H}_{26}\text{O}_6 \\
(\text{XP}) \quad (\text{XP}_7)
\]
NATURE AND POSITION OF DOUBLE BONDS:

Peaks in the IR spectrum of XP at $\nu_{\text{max}}^{\text{KBr}}$ 1654 cm$^{-1}$ (for exocyclic methylene group) and 890 cm$^{-1}$ (for ethylenic double bond) exhibited the presence of two types of double bonds (which may be either exocyclic or endocyclic).

XP on reduction with sodium borohydride gave a viscous compound, molecular formula $C_{17}H_{26}O_6$, which was found to be dihydroxy derivative (by spectral studies) of XP and so giving inference that one double bond escaped reduction giving the clue that it must be in the ring (so endocyclic) and the reducible double bond must be exocyclic.

POSITION OF EXOCYCLIC METHYLENE GROUP:

Peaks in IR spectrum at $\nu_{\text{max}}^{\text{KBr}}$ 1760 cm$^{-1}$ and 1750 cm$^{-1}$ showed the presence of exocyclic methylene group in the lactone ring.$^{13}$

The position of exocyclic methylene group in XP was established at C-11 due to a doublet of two proton intensity at $\delta$ 5.51 and $\delta$ 6.24 for C-13.

The position of exocyclic methylene group at C-11 was further supported by the $^1H$ NMR spectrum of dihydroxy derivative XP$_8$, molecular formula $C_{17}H_{26}O_6$,
M+ 326 (EIMS) obtained by the reduction of XP with sodium borohydride in which doublet of three proton intensity appeared at C-13 along with a multiplet of one proton intensity which must be of C-11 proton formed during the reduction of XP to XP8.

\[
\text{Xanthanolide} \xrightarrow{\text{NaBH}_4} \text{Dihydrohydroxy derivative}
\]

\[
\text{C}_{17}^\text{red} \text{H}_{22}^\text{red} \text{O}_6
\]

\[
\text{C}_{17}^\text{red} \text{H}_{26}^\text{red} \text{O}_6
\]

(XP) (XP8)

Thereby further establishing a partial structure of XP as (5).

\[
\text{(5)}
\]

**POSITION OF THE ENDOCYCLIC ETHYLENE DOUBLE BOND:**

XP on reduction with NaBH4 yielded a compound XP8 (dihydrohydroxy derivative) which still showed unsaturation on IR spectral studies thereby confirming the presence of endocyclic double bond in XP.
XP on reduction with HI and red phosphorous gave a known xanthanolide-xanthumin, which was identified by superimposable spectral studies (as described on page 27 of the thesis) thereby concluding that the endocyclic double bond must be at C_1-C_5. This position was further supported by $^1H$ NMR spectrum, which displayed a dd at δ 5.82 of one proton intensity for C-5.

Based on the above facts further partial structure to XP may be assigned as (6).

![Chemical Structure](chart)

**PRESENTATION OF LACTONE RING:**

Peaks in IR spectrum at $\nu_{\text{max}}^{\text{KBr}}$ 1760 cm$^{-1}$ and 1750 cm$^{-1}$ indicated the presence of $\alpha$ methylene-$\gamma$-lactone ring$^{11}$ in XP.

**POSITION OF LACTONE RING:**

There are following possibilities for the fusion of the lactone ring in the xanthanolide XP.
The lactone ring may be fused either at C₆-C₇ or at C₇-C₈. In the case of fusion at C₆-C₇; ¹H NMR will display dd for C₆ and C₇, m for C₈ and C₉ separately, but in the case of fusion at C₇-C₈ the ¹H NMR will display dd for C₆ and C₉, and m for C₇ and C₈.

A critical examination of ¹H NMR spectrum of the xanthanolide XP showed that it's ¹H NMR spectrum is similar to the later case thereby confirming the position of the lactone ring at C₇-C₈ in XP. ¹H NMR spectrum of XP displayed signal of one proton intensity (at C-8) which appeared downfield at δ 4.65 and further proved the cis fusion of the lactone ring.

The above deliberations finally established the structure of the xanthanolide as; 14-hydroxy xanthumin (7).

The above structure (7) satisfactorily explains all the reactions of Xanthanolide XP as described in the Scheme-I.
The identification of the xanthanolide XP was further confirmed by $^1$H NMR, $^{13}$C NMR and mass spectral studies.

$^1$H NMR SPECTRUM OF THE XANTHANOLIDE (XP):

The chemical shifts in $^1$H NMR (Fig. 2) of XP were noted and the structural assignments made with the help of available literature$^{26-28}$ are given in Table-2.

**TABLE - 2**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (δ)</th>
<th>Pattern</th>
<th>J value (Hz)</th>
<th>No.of proton</th>
<th>Assignment</th>
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<tr>
<td>01.</td>
<td>5.39</td>
<td>t</td>
<td>-</td>
<td>1H</td>
<td>C$_2$-H</td>
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<tr>
<td>02.</td>
<td>2.78</td>
<td>d</td>
<td>4.0</td>
<td>2H</td>
<td>C$_3$-H$_2$</td>
</tr>
<tr>
<td>03.</td>
<td>5.82</td>
<td>dd</td>
<td>8,8</td>
<td>1H</td>
<td>C$_5$-H</td>
</tr>
<tr>
<td>04.</td>
<td>2.35</td>
<td>dd</td>
<td>4,4</td>
<td>2H</td>
<td>C$_6$-H$_2$</td>
</tr>
<tr>
<td>05.</td>
<td>3.28</td>
<td>m</td>
<td>-</td>
<td>1H</td>
<td>C$_7$-H</td>
</tr>
<tr>
<td>06.</td>
<td>4.65</td>
<td>m</td>
<td>-</td>
<td>1H</td>
<td>C$_8$-H</td>
</tr>
<tr>
<td>07.</td>
<td>1.95</td>
<td>dd</td>
<td>7,8</td>
<td>2H</td>
<td>C$_9$-H$_2$</td>
</tr>
<tr>
<td>08.</td>
<td>2.64</td>
<td>t</td>
<td>-</td>
<td>1H</td>
<td>C$_{10}$-H</td>
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<tr>
<td>09.</td>
<td>5.51, 6.24</td>
<td>d,d</td>
<td>3,3</td>
<td>2H</td>
<td>C$_{13}$-H$_2$</td>
</tr>
<tr>
<td>10.</td>
<td>4.10</td>
<td>d</td>
<td>2.5,2.5</td>
<td>2H</td>
<td>C$_{10}$-CH$_2$OH</td>
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<tr>
<td>11.</td>
<td>2.82</td>
<td>s</td>
<td>-</td>
<td>3H</td>
<td>C$_{15}$-H$_3$</td>
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<tr>
<td>12.</td>
<td>2.03</td>
<td>s</td>
<td>-</td>
<td>3H</td>
<td>C$_2$-OAc</td>
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</tbody>
</table>
$^1$H NMR of spectrum of the xanthanolide (XP)

FIG. - 4
MASS SPECTRUM\textsuperscript{29} OF THE XANTHANOLIDE:

The significant fragmentation pattern obtained in the electron impact mass spectrum of the xanthanolide XP is given below and confirmed the above structure 7 of the xanthanolide. The important fragments in EIMS: \text{M}^{+} 322 and m/e 262, 247, 219, 165, 193, 139. (Scheme - 2)

\textbf{13}C NMR OF THE XANTHANOLIDE XP:

The \textbf{13}C NMR spectrum of the xanthanolide XP showed important signals which are recorded in the Table-3 and further supported its identity as 14-hydroxy xanthumin (7).

\begin{table}[h]
\centering
\begin{tabular}{ccc}
Carbon No. & Pattern & Value (ppm) \\
1 & 2 & 3 \\
01 & s & 142.3 \\
02 & t & 28.9 \\
03 & t & 42.0 \\
04 & s & 206.8 \\
\end{tabular}
\caption{Table - 3}
\end{table}
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<thead>
<tr>
<th></th>
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<th>3</th>
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<tr>
<td>07</td>
<td>d</td>
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<tr>
<td>08</td>
<td>d</td>
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<td>09</td>
<td>t</td>
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<td>11</td>
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<td>12</td>
<td>s</td>
<td>169.4</td>
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<td>13</td>
<td>t</td>
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<tr>
<td>14</td>
<td>d</td>
<td>66.3</td>
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<tr>
<td>15</td>
<td>q</td>
<td>30.6</td>
</tr>
<tr>
<td>CH₃COO</td>
<td>q</td>
<td>20.9</td>
</tr>
<tr>
<td>CH₃COO</td>
<td>s</td>
<td>168.5</td>
</tr>
</tbody>
</table>
SCHEME-2
EXPERIMENTAL

The plant *Xanthium strumarium* Linn was supplied by M/s United Chemicals and Allied Product, Calcutta and authenticated by Botany Department of this university.

Air dried and powdered stems (4 Kg.) were extracted exhaustively with 90% ethanol (5 lit) in 10 lit R.B. flask, which was fitted with a water condenser and the extraction was continued for several days. The extract concentrated under reduced pressure to a brown coloured viscous mass (145 g). It was successively extracted with petroleum ether (800 ml), benzene (450ml), chloroform (750 ml), ethylacetate (400 ml), acetone (700 ml) and methanol (650 ml).

**STUDY OF THE CHLOROFORM SOLUBLE PART:**

The concentrated extract of petroleum ether soluble fraction gave a known xanthanolide - Xanthumin. Benzene and ethyl acetate fractions afforded small amount of residues and so these were not worked up further. Acetone soluble part yielded another xanthanolide namely Xanthinosin and methanol soluble fraction showed negative response to the test of xanthanolide hence discarded.

The chloroform soluble fraction was concentrated under reduced pressure to a dark brown coloured viscous
mass (3.20 g). It showed three spots when examined by thin layer chromatography on Si gel G plates using benzene:chloroform:ethyl acetate (65 : 20 : 10). The chloroform soluble fraction was therefore column chromato
tographed on si gel G with solvents of increasing polarity.

**COLUMN CHROMATOGRAPHY**:

Length of the column                     150 cm
Diameter of the column                    5.0 cm
Weight of the crude extract              3.2 g
Weight of the Si gel (60-120 mesh)        140 g

**TABLE - 4**

<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>1000 ml each</td>
<td></td>
</tr>
<tr>
<td>01.</td>
<td>1-25</td>
<td>( \text{C}_6\text{H}_6 : \text{C}_6\text{H}_5\text{CHCl}_3 ) (15:7,4)</td>
<td>Compound XP</td>
</tr>
<tr>
<td>02.</td>
<td>26-35</td>
<td>-do-</td>
<td>Negligible</td>
</tr>
<tr>
<td>03.</td>
<td>36-50</td>
<td>-do-</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

**STUDY OF THE FRACTION (1-25)**:

Fractions 1-25 were of same \( R_f \) value and so these were mixed and solvent removed to get a colourless crystalline compound (2.00 \( \chi \)), \( R_f 0.65 \) TLC Si gel G
\( \text{C}_6\text{H}_6 : \text{C}_6\text{H}_6; \text{CHCl}_3, 15:7:4 \).

**STUDY OF THE COMPOUND (XP):**

The compound was soluble in methanol and acetone. It melted at 107-09\(^\circ\), analysed for \( \text{C}_{17}\text{H}_{22}\text{O}_6 \) and \( M^+ 322 \) (EIMS), yield (0.050%). It responded to positive hydroxamic acid test and other colour reactions.

**HYDROXAMIC ACID TEST:**

A small quantity of the compound was dissolved in acetone and taken in a porcelain crucible with 2-3 drops of alcoholic hydroxyl amine hydrochloride solution and few drops of saturated alc. KOH and heated until the reaction started. Thereafter the mixture was cooled, acidified with HCl and a drop of 1% FeCl\(_3\) solution was added. Violet colour appeared indicating the presence of lactone ring in XP.

**COLOUR TESTS:**

1. A small quantity of the compound XP was treated with 10% alc. solution of KOH; rose red colour was obtained.

2. Few drops of compound (dissolved in 50% MeOH) were mixed with ethanolic solution of meta dinitrobenzene and alc. KOH; red colour was produced.

3. Few crystals of XP, gave orange red colour when treated with dil. HCl and resorcinol.
4. A small portion of compound was mixed with 2 ml \( \text{H}_2\text{SO}_4 \) (50%) and 3-4 drops of aq. \( \text{FeCl}_3 \). Red colour was obtained on warming for few minutes on water bath.

**ELEMENTAL ANALYSIS**

Found

<table>
<thead>
<tr>
<th>Element</th>
<th>Value</th>
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<tr>
<td>H</td>
<td>6.80</td>
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</tbody>
</table>

Calculated for \( \text{C}_{17}\text{H}_{22}\text{O}_6 \)

<table>
<thead>
<tr>
<th>Element</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>63.35</td>
</tr>
<tr>
<td>H</td>
<td>6.82</td>
</tr>
</tbody>
</table>

Molecular weight 322

(By mass spectroscopy)

**ACETYLATION OF THE COMPOUND XP TO \( \text{XP}_1 \)**

Compound XP (200 mg) was mixed with 3 ml pyridine and 25 ml acetic anhydride in a 500 ml R.B. flask, fitted with an air condenser. The reaction mixture was refluxed on a water bath for 3 hrs. After cooling, the mixture was poured in cold water (100 ml) to give a precipitate which was extracted with solvent ether (50 ml), the ethereal layer was washed with water and \( \text{NaHCO}_3 \) solution. It was dried over anhydrous sodium sulphate and ether was removed by evaporation. The residue was crystallized from acetone to get the acetyl derivative (150 mg) which analysed for \( \text{C}_{19}\text{H}_{24}\text{O}_7 \), m.p. 150-52°, \( M^+ \) 364 (EIMS).
ELEMENETAL ANALYSIS:

Found                       Calculated for $C_{19}H_{24}O_7$
C = 62.60                   C = 62.63
H = 6.57                    H = 6.59

Molecular weight 364
(By mass spectroscopy)

METHYLATION OF THE XANTHANOLIDE XP TO XP₂:

200 mg of the xanthanolide was mixed with 2 ml dimethyl sulphate, anhydrous $K_2CO_3$ and 150 ml acetone in a 500 ml R.B. flask and refluxed for 18 hrs. Reaction mixture was filtered to remove the impurities. Water was added in the filtrate and the acetone was distilled off yielding an oily residue which was chromatographed over a small column of Si gel and eluted with solvent ether. The resultant residue was crystallized from chloroform to yield colourless needles (160 mg), m.p. 135-37° and analysed for $C_{18}H_{24}O_6$, $M^+$ 336 (EIMS).

ELEMENETAL ANALYSIS:

Found                       Calculated for $C_{18}H_{24}O_6$
C = 64.26                   C = 64.28
H = 1.74                    H = 7.14

Molecular weight 336
(By mass spectroscopy)
REDUCTION OF THE XANTHANOLIDE XP TO XP₃:

200 mg of XP was taken in 500 ml R.B. flask, 50 ml conc. HI and 25 mg red phosphorous were added. The mixture was refluxed on sand bath for 4 hrs and cooled; a crystalline compound was obtained (130 mg), m.p. 120-22°C molecular formula C₁₇H₂₂O₅, M⁺ 306 (EIMS).

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₁₇H₂₂O₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 66.62</td>
<td>C = 66.65</td>
</tr>
<tr>
<td>H = 7.21</td>
<td>H = 7.24</td>
</tr>
</tbody>
</table>

Molecular weight 306
(By mass spectroscopy)

DES-ACETYLATION OF THE XANTHANOLIDE XP TO XP₄:

A solution of XP (200 mg) in acetone and 2.0 g sodium acetate in 30 ml of ethanolic HCl was refluxed for 1 hr. The solution was made acidic with 3N HCl (10 ml) and water was added to dilute it. On cooling, the des-acetylated derivative was obtained as a viscous oily substance (180 mg), molecular formula C₁₅H₁₈O₄, M⁺ 262 (EIMS).
ELEMENTAL ANALYSIS:

Found                     Calculated for C\textsubscript{15}H\textsubscript{18}O\textsubscript{4}
C = 68.69                C = 68.70
H = 6.84                 H = 6.87
Molecular weight 262
(By mass spectroscopy)

2:4 DINITROPHENYLHYDRAZONE OF THE XANTHANOLIDE XP:

150 mg of the xanthanolide XP in 10 ml of 95% ethyl alcohol was mixed with a hot solution of 2:4 dinitrophenylhydrazene (100 mg) in 20 ml of ethanol. After the addition of few drops of conc. HCl and boiling a yellow precipitate was obtained. The solution was cooled and the derivative was collected (100 mg), m.p. 175-76°, molecular formula C\textsubscript{23}H\textsubscript{26}N\textsubscript{4}O\textsubscript{9}, M\textsuperscript{+} 502 (EIMS).

ELEMENTAL ANALYSIS:

Found                     Calculated for C\textsubscript{23}H\textsubscript{24}N\textsubscript{4}O\textsubscript{9}
C = 53.04                C = 53.07
H = 5.36                 H = 5.38
Molecular weight 502
(By mass spectroscopy)

OXIDATION OF XP TO XP\textsubscript{6}:

600 mg iodine was dissolved in KI solution (50 ml) and aq. sodium hypochlorite solution was added until the colour of iodine was discharged. 350 mg of
the xanthanolide XP was added while stirring. After 15 minutes the solution was acidified and then made basic. Iodoform was precipitated which was collected after filtration. The reaction completed after 8 hrs. Acidification yielded white crystals (90 mg), m.p. 262-63°C which analysed for \( \text{C}_{14}\text{H}_{16}\text{O}_3 \), \( M^+ 264 \) (EIMS).

**ELEMENTAL ANALYSIS:**

Found Calculated for \( \text{C}_{14}\text{H}_{16}\text{O}_3 \)

\[
\begin{array}{ll}
C &= 63.61 & C &= 63.63 \\
H &= 6.05 & H &= 6.06 \\
\end{array}
\]

Molecular weight 264
(By mass spectroscopy)

**CATALYTIC HYDROGENATION OF THE XANTHANOLIDE XP TO XP:**

100 mg of XP was taken in 30 ml MeOH and reduced with hydrogen in pressure of 5% Pd/C, which yielded a tetrahydro derivative (50 mg), m.p. 170-71°C, molecular formula \( \text{C}_{17}\text{H}_{26}\text{O}_6 \), \( M^+ 326 \) (EIMS).

**ELEMENTAL ANALYSIS:**

Found Calculated for \( \text{C}_{17}\text{H}_{26}\text{O}_6 \)

\[
\begin{array}{ll}
C &= 62.57 & C &= 62.57 \\
H &= 7.94 & H &= 7.97 \\
\end{array}
\]

Molecular weight 326
(By mass spectroscopy)
\[ \text{NaBH}_4 \text{ REDUCTION OF THE XANTHANOLIDE XP TO XP}_8 : \]

400 mg XP was dissolved in acetone and mixed with 80 mg NaBH\(_4\) in 15 ml MeOH. The whole mixture was left for 2 hrs. at room temperature. 2N H\(_2\)SO\(_4\) (6 ml) was added and thereafter extracted with ether. The ethereal extract was washed with Na\(_2\)CO\(_3\), dried over anhydrous sodium sulphate and evaporated to get the product as a viscous oil (250 mg), molecular formula \(\text{C}_{17}\text{H}_{26}\text{O}_6\)\(^+\) 326 (EIMS).

**ELEMENTAL ANALYSIS:**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for (\text{C}<em>{17}\text{H}</em>{26}\text{O}_6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 62.52</td>
<td>C = 62.57</td>
</tr>
<tr>
<td>H = 7.94</td>
<td>H = 7.97</td>
</tr>
</tbody>
</table>

Molecular weight 326
(By mass spectroscopy)
REFERENCES


