EXPERIMENTAL
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GENERAL

Melting points were determined on a Boetius microheating table and are uncorrected. They are expressed in degree centigrade.

$^1$H-N.M.R. spectra were taken on Varian EM 390-90 MHz and Hitachi R-600 (FT) spectrometers, using tetramethyl silane as internal reference. The chemical shifts are quoted in parts per million (ppm) [s=singlet, d=doublet, t=triplet, q=quartet, dd=doublet of doublet, m=multiplet and bs=broad singlet]. The I.R. spectra were recorded on Perkin-Elmer - 597 spectrophotometer and the absorption frequencies are quoted in reciprocal centimeters.

Thin layer chromatography (t.l.c) was performed using glass plates coated with silica gel-G (incorporating CaSO$_4$ (13%) as binder), Benzene, chloroform and ethylacetate were used as the developing solvents. Spots were detected with iodine.

The solvents and reagents used for the synthesis were of reagent grade and were purified by standard methods. Petroleum ether (Pet. ether) used boils at 60-80°.
Anhydrous sodium sulphate was used to dry the solutions of organic extracts.

Purification of the crude products was carried out using chromatographic column packed with silicagel (60-120 mesh)/aluminium oxide active (basic or neutral) as the case may be.

* * *
EXPERIMENTAL

Extraction and fractionation of compounds from 'Limonia alata':-

Air dried bark material of the plant Limonia alata (250 g) was extracted exhaustively with petroleum ether (60-80\(^{\circ}\)) chloroform and in ethanol in a soxhlet apparatus. The extract was concentrated and chromatographed over silica gel column (60-120 mesh). Elution was commenced with and continued slowly with petroleum ether:benzene (5:1, 2:1 and 1:1) mixtures. The eluate was collected in 250 ml fractions and about 20 fractions were collected in each solvent system.

Isolation and characterization of the compounds:--

The first seven elutions of the above column with petroleum ether (1:1) as eluant furnished a product A after evaporation of the solvent (Yield 150 mg; m.p. 213\(^{\circ}\))

Recrystallization of the product from petrol-benzene furnished it as white needles and it was identified to be lupeol Lit.\(^{213}\) m.p. 215-16\(^{\circ}\); Mass : m/z 426.

Analysis: \[\text{C}_{30}\text{H}_{50}\text{O}\]

(426)

\[
\begin{align*}
\text{Found} & : \text{C} 84.50 \text{ H} 11.74 \\
\text{Calcd} & : \text{C} 84.44 \text{ H} 11.81
\end{align*}
\]

I.R (KBr): \[\nu_{\text{max}} = 3020, 2990, 2985, 1630, 1440, 1380 \text{ and } 1360 \text{ cm}^{-1}.\]
$^1$H N.M.R (CDCl$_3$) $\delta$ values

0.78, 0.83, 0.96, and 1.03 (4s 21H) ppm.
4.60 (2H, d C-30 = CH$_2$) ppm.
3.20 (1H, m 3β-OH) ppm.

The next 10 fractions of the above column with petrol:benezene (1:1) as eluant furnished a product after evaporation of the solvent. Yield: 250 mg, m.p. 132° (colourless prisms from petroleum ether Lit.212 m.p.133°.

Mass m/z 258.

Analysis:

C$_{15}$H$_{14}$O$_4$ Found C 69.75 H 5.42
(258) Calcd C 69.75 H 5.46

I.R(CCl$_4$) $\nu_{max}$=2900,1740,1610,1590,1390 and 1380 cm$^{-1}$.

$^1$H N.M.R (CDCl$_3$): $\delta$ values

1.47 (6H, s C$_2$, CH$_3$)$_2$] ppm.
3.86 (3H, s C$_5$ -OCH$_3$) ppm.
5.69 (1H, d, s$_3$ 4 = 9.5 Hz C$_3$, -H) ppm.
6.19 (1H, d, s$_3$, 4; = 9.5 Hz C$_3$-H) ppm.
6.55 (1H, d s$_3$, 4; = 9.5 Hz C$_4$,-H) ppm.
7.86 (1H, d, s$_3$,4 = 9.5Hz C$_4$-H) ppm.
and 6.58 (1H, s, C$_8$-H) ppm.
Further elution of the column with petrol-benzene (1:1) furnished xanthoxyletin into another product (trace) mixture m.p. 95-105. It was identified to be xanthyletin xanthoxyletin mixture. (C199 and C260)

I.R (CCl₄): $\nu_{\text{max}} = 2900, 1740, 1540, 1390, 1380$ and 1190 cm⁻¹.

Further elutions of the column with increasing polarity did not yield any isolable product.

**Acetylation of Lupeol (64a)**

A mixture of Lupeol (50 mg) and acetic anhydride (0.5 ml) and pyridine (0.5 ml) was allowed to stand at room temperature for 12 hrs. The reaction mixture was poured over crushed ice and extracted with chloroform (2 x 20 ml). Chloroform layer was dried and evaporated and put into a column of silicagel (60-120 mesh) and eluted with petroleum ether (60-80°). The compound obtained as colourless solid. (40 mg) which crystalized from the same solvent m.p. 215 (Lit. m.p 213)

I.R (KBr): $\nu_{\text{max}} = 3040, 1640, 880$ (Isopropenyl) 1730 and 1250 cm⁻¹.

H¹ N.M.R (CDCl₃) $\delta$ values

- 0.79, 0.94, 1.05 (3s, 9H, 3 Me) ppm.
- 0.85 (9H, s) ppm.
- 1.7 (3H, brs CH₃-C≡CH₂) ppm.
- 2.04 (3H, s, O-COCH₃) ppm.
Oxidation of Lupeol with chromium trioxide and pyridine:

Anhydrous chromium trioxide (30 mg) was added slowly into mixture of 10 ml of methylenechloride and 0.5 ml of pyridine at -10° with constant stirring, when the yellow (Py-CrO₃) complex separated, 50 mg of Lupeol in 5 ml of methylenechloride was added dropwise at 0°. The mixture was then filtered, the clear filtrate and the washing reduced to a small volume and was put into a column of silicagel. Elution with petrol:benzene (1:1) afforded the pure ketone namely Lupeonone (10 mg) with a m.p. 180° corresponded to the Literature value.213

Synthesis of Dihydroxanthoxyletin(65)

To a solution of xanthoxyletin (50 mg) in glacial-acetic acid (10 ml) was added 10% Pd-C (50 mg) and hydrogenated at 10 atm for 2 hr. in a Cooke's low pressure hydrogenator. Thereafter the catalyst was filtered off the solvent poured into water (100 ml) and washed with 5% sodium bicarbonate (100 ml) and then extracted with chloroform and dried (anhydrous sodium sulphate) and concentrated. The concentrated chloroform extract was put into a column of silicagel and while elution with benzene
afforded the compound the m.p. of which corresponded to the compound dihydroxanthoxyletin reported in the literature.

I.R (KBr): $\nu_{\text{max}} = 2900, 1740, 1610, 1590, 1440, 1380, 1390, 1190, 1150$ and $1060 \text{ cm}^{-1}$.

$^1$H N.M.R (CDCl$_3$): δ values

1.36 (s, 6H $-$C$^2'$ (CH$_3$)$_2$)
1.83 (t, 2H $C^3'$ -$\text{CH}_2$)
2.78 (t, 2H $C^4'$ -$\text{CH}_2$)
3.86 (s, 3H $C_4$ - OMe)
7.85 (d,1H,$C^4'$-CH)
6.15 (d,1H,$C_3$-CH)
6.52 (s,1H,$C_8$-H)

**Bromination of xanthoxyletin:**

To a compound of xanthoxyletin (50 mg) in chloroform was treated with a solution of Bromine in chloroform until the red colour persisted. The solvent and red colour bromine were removed under reduced pressure to yield an orange-coloured semi-solid product. This was dispersed in dry ether (10 ml) and activated zinc powder (500 mg) activated with copper sulphate was added carefully in small portions, so that the ether refluxed gently. When the reaction was completed the unchanced zinc powder was...
removed and the ether solution was also removed and the ether solution washed several times with water. The residue crystallized from ether to give 8-Bromoxanthoxyletin in pale yellow needles, m.p. 183-189 (12 mg).

I.R. (KBr). $\nu_{\text{max}}$ = 1720, 1605, 1585 and 1545 cm$^{-1}$.

**Analysis of the chloroform extract:**

The chloroform extract after concentration left a gummy residue (4 g) which was packed into column of silicagel (50 g). The elution was started with petrol and polarity was increased with petrol:benzene, (1:1, 1:2, 1:5) and then with benzene and benzene:ethyl acetate. In petrol:benzene (1:2) eluate xanthyletin - xanthoxyletin mixture was obtained in trace amounts (< 2 mg). On elution with benzene:ethyl acetate (10:1) the compound 'D' was obtained showing blue fluorescence in alcohol m.p. 221$^\circ$. The compound 'D' was identified to be a 7-hydroxycoumarin namely umbelliferone(C-118) with the synthetic sample.

The synthetic sample of umbelliferone was prepared by heating Resorcinol (0.01 mole) and Malic acid (0.01 mole) in concentrated sulphuric acid at 120$^\circ$ for 4 hrs. It was poured into about 200 gms of crushed ice and filtered and dried.
Extraction and isolation of the pyranocoumarin seselin from *Limonia alata*:

The fruits of the 'Limonia alata' were collected in the month of June and kept for air drying. The air dried fruits of *L. alata* (250 g) was powdered and exhaustively extracted with petroleum ether (60-80°C). The extract was concentrated and the residue obtained was chromatographed over silicagel. The elution was commenced with petrol and increased the polarity stepwise as in previous cases. Initial elutions with petrol gave a waxy residue showing mixture of many spots that couldn't be separated. On elution with petrol:benzene (1:1) the compound seselin (C-210) was obtained as colourless crystals. mp. 119, Lit. 218° 120°C.

Mass: 228.

I.R. (KBr): ν_max = 2910, 1730, 1620, 1590, 1390 and 1380 cm⁻¹.

^1_H N.M.R (CDCl₃): 6 values

1.51 (6H, s, C₂, -(CH₃)₂) ppm.
5.76 (1H, d, s₃,₄, = 9.7 Hz C₃-H) ppm.
6.25 (1H, d, s₃,₄, = 9.5 Hz C₃-H) ppm.
6.74 (1H, d, s₃,₄, = 9.5 Hz C₄-H) ppm.
6.91 (1H, d, s₅,₆, = 9Hz C₆-H) ppm.
7.25 (1H, d, s₅,₆, = 9Hz C₅-H) ppm.
7.64 (1H, d, s₃,₄, = 9.5Hz, C₄-H) ppm.
Chemical investigation of the plant *Evodia lunu-ankenda*

The various plant parts of *Evodia lunu-ankenda* like leaves, root bark and stem bark of the plant were collected separately and air-dried. The powdered *whole* bark of the plant (250 g) was put into a soxhlet apparatus and was extracted successively with petroleum ether 60-80°, chloroform and ethanol and each extract was concentrated to a small bulk. The concentrated petroleum ether extract (6 g) was put into a column of silicagel and elution was started with petrol and petrol:benzene (10:1, 5:1) and benzene mixtures.

On elution with petrol:benzene (10:1) mixture the compound *Alloevodionol methyl ether* (90) was isolated. m.p. 105. Yield: (150mg).

Mass: m/e 262.

Analysis:

\[
\text{C}_{15}\text{H}_{18}\text{O}_4 \quad \text{Found C 68.83 H 6.85} \\
(262.30) \quad \text{Calcd C 68.76 H 6.91}
\]

I.R (KBr): \( \nu_{\text{max}} = 2980, 1720, 1680, 1620, 1250, \text{and } 1050 \text{ cm}^{-1} \).
Further elution with still higher polar solvents did yield only a gummy material. Separation of chloroform extract by column chromatography didn't yield any crystallizable material in any of the solvent system.

**Extraction and fractionization of the alkaloids from Acidic extracts of stem bark of Evodia lunu-ankenda**

In the next experiment a further amount of air-dried, powdered bark of *Evodia lunu-ankenda* (2.5 kg) was exhaustively extracted with methylenechloride (3 lit). The extract was concentrated and the residue obtained was mixed with aqueous 10% HCl and kept aside for 3 days. It was then filtered and the filtrate neutralised with dilute ammonia. The neutralised solution was repeatedly extracted with methylenechloride (5 x 100 ml). The dried extract was concentrated and placed over a column of basic alumina.

\[\text{'H N.M.R (CDCl}_3\text{): } \delta \text{ values}\]

- 1.4 (6H, s C\(^2\)-(CH\(_3\)_2) ppm.
- 2.6 (3H s C\(^8\) -COCH\(_3\)) ppm.
- 3.43 (6H s C\(^5\) and C\(^7\)-OCH\(_3\)) ppm.
- 6.1 (1H, s C\(_6\)-H) ppm.
- 5.5 (1H, d C\(_4\)-H) ppm.
- 6.7 (1H, d C\(_3\)-H) ppm.
Elution of the column was commenced with petrol followed by petrol-benzene (5:1, 2:1, 1:1) and then with benzene. The eluate was collected in 150 ml fractions and about 20 fractions were collected in each solvent system. The fractions were monitored by TLC. (silicagelG, solvent benzene; Benzene:ethyl acetate 10:1).

Isolation of constituents:

a) The compound Dictamninine(A1) was obtained in the first six fractions of the petrol:benzene (1:1) eluate. m.p.132°. Yield: 75 mg. Lit. m.p. 133°; Picrate 162° (Lit. 164).

Mass: 199.

Analysis:

\[ \text{C}_{12}\text{H}_9\text{NO}_2 \]
\[ \text{Found C} \quad 72.38 \quad \text{H} \quad 4.52 \]
\[ \text{(199)} \quad \text{Calcd C} \quad 72.35 \quad \text{H} \quad 4.55 \]

I.R.(KBr): \[ \nu_{\text{max}} = 3020, 2990, 2980, 1620, 1580, 1440, 1400, 1360 \text{ and } 1080 \text{ cm}^{-1}. \]

\[ ^1\text{H N.M.R (CDCl}_3) : \] values.

4.38 (3H, s, C\textsubscript{4} -OCH\textsubscript{3}) ppm.
7.05 (1H, d, C\textsubscript{3}-H) ppm.
7.53 (1H, d, C\textsubscript{2}-H) ppm.
7.30-8.32 (4H, m, C\textsubscript{5},C\textsubscript{6},C\textsubscript{7} and C\textsubscript{8}-H) ppm.
b) The compound evolitrine (A₃) was obtained in the next 12 fractions of the petrol:benzene (1:1) eluant. m.p.112°.
Lit. 115; Yield: 125 mg; Picrate m.p.198 (Lit. 201°C).

Mass: 229.

Analysis:

\[ C_{13}H_{11}NO_3 \]  
Found C 68.20 H 4.78  
(229)  
Calcd C 68.11 H 4.84

I.R. (KBr): \( \nu_{\text{max}} = 3020, 2995, 2980, 1820 \) and \( 1580 \) cm\(^{-1}\).

\( ^1H \) N.M.R (CDCl₃): \nu values

- 3.95 (3H, s, C₇-OCH₃) ppm.
- 4.41 (3H, s, C₄-OCH₃) ppm.
- 7.01-7.56 (5H, m, C₂-H, C₆-H and C₅-H) ppm.
- 8.15 (1H, d, C₅-H) ppm.

C) The compound Kokusaginine (A₇) was obtained in the first 3 fractions of the benzene eluate. Yield 45 mg.
m.p. 168; Lit. 168. Picrate 216°C (Lit. 218°C.)

Mass: 259.

Analysis:

\[ C_{14}H_{13}NO_4 \]  
Found C 64.79 H 5.11  
(259)  
Calcd C 64.86 H 5.05
The compound N-Methyl-4-methoxy-2-quinolone was obtained from the 6th - 11th fractions of the benzene : Ethyl acetate (2:1) eluate. Yield 40 mg., m.p. 98°; Lit. 216 m.p. 100°.

Mass: 189

**Analysis:**

\[ \text{C}_{11}\text{H}_{11}\text{NO}_2 \]  

<table>
<thead>
<tr>
<th>Found</th>
<th>Calcd</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 69.78</td>
<td>H 5.92</td>
</tr>
<tr>
<td>(189)</td>
<td>C 69.83</td>
</tr>
</tbody>
</table>

**I.R. (KBr):**  

\[ \nu_{\text{max}} = 3025, 2990, 1640, 1620, 1580, 1460, 800 \text{ and } 700 \text{ cm}^{-1}. \]

**\(^1\text{H} \text{N.M.R. (CDC}_3\text{O)}:**  

\[ \delta \text{ values.} \]

3.7 (3H, s, NCH\(_3\)) ppm.
4.0 (3H, s, OCH\(_3\)) ppm.
6.04 (1H, s, C\(_3\)-H) ppm.
7.22 - 8.04 (4H, m, C\(_5\), C\(_6\), C\(_7\) and C\(_8\)-H) ppm.
e) The compound Marmesin (C-105) was obtained from the first 3 fractions of the benzene:ethylacetate (2:0:1) eluate. Yield 3 mg m.p. 189° Lit.217b m.p. 190°.

Mass: 246.

Analysis:

\[
\begin{align*}
\text{C}_{14}\text{H}_{14}^0_4 & \quad \text{Found C} \ 68.35 \ \text{H} \ 5.82 \\
(246) & \quad \text{Calcd C} \ 68.28 \ \text{H} \ 5.73
\end{align*}
\]

I,R.(KBr): \[
\nu_{\max} = 3025, 2990, 1720, 1620, 1580, 1460, 800 \text{ and } 700 \text{ cm}^{-1}.
\]

\(^1\text{H N.M.R (CDCl}\text{)}_3\): \(\delta\) values.

\[
\begin{align*}
1.24 \text{ and } 1.37 & \ (6\ H, 2s, 3H \ Each, -C(CH_3)_2) ppm \\
1.84 & \ (1H, \ br.s, -C(CH_3)_2) ppm \\
3.2 & \ (2H, d, C_3-CH_2-) ppm \\
4.7 & \ (1H, t, C_2, -H) ppm \\
6.2 & \ (1H, d, C_3-H) ppm \\
6.7 & \ (1H', s, C_5-H) ppm \\
7.6 & \ (1H, d, C_4-H) ppm \\
7.2 & \ (1H, s, C_8-H) ppm
\end{align*}
\]
Preparation of Alloevodionol Methyl ether (90).

Reaction of 7-hydroxy-5-methoxy-2,2-dimethylchroman (91) with Acetonitrile and hydrogen chloride: 217a

7-Hydroxy-5-methoxy-2,2-dimethylchroman (91) was prepared from 5,7-dihydroxy-2,2-dimethylchromanone by partial tosylation with p-toluene sulphonyl chloride followed by C5-methylation, detosylation and Clemmenson reduction. This (2.2 g) on reaction with acetonitrile (1.2 g) ZnCl2 (3.0 g) and dry HCl in ether (at 0°) gave a product which showed the presence of two compounds of TLC. Product 92 was eluted with pet. ether:ethyl acetate (98:2) in a silicagel column. This was closely followed by product 93 with same eluant.

92 : m.p. 77 (Lit. 222 78°C).
Yield (0.96 g).

93 : m.p. 66-67°.
Yield (80 mg).

8-Acetyl-7-hydroxy-5-methoxy-2,2-dimethyl chromene (Alloevodionol)

8-Acetyl-7-hydroxy-5-methoxy-2,2-dimethylchroman (200 mg) was dehydrogenated with DDQ (200 mg) in benzene gave the corresponding chromene (80 mg) which recrystallized from pet. ether.

m.p. 71-73°.
Methylation of alloevodionol (90)

The product Alloevodionol (0.1 g) was refluxed with dimethylsulphate (0.1 ml) in dry acetone (2 cm³) and K₂C₃O₃ (0.1 g) which on work-up corresponded to 'Alloevodionol methylether' isolated from the plant source. m.p. 105°. Yield: 80 mg.