CHAPTER 2

CHEMICAL INVESTIGATION OF IRIS GERMANICA

(IRIS KASHMIRIANA BAKER) AND IRIS KUMAONENSIS WALL

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EXPERIMENTAL SECTION

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INTRODUCTION

*Iris* is a genus of rhizomatous or bulbous herbs distributed in the north temperate region of the world. It constitutes about 150 species and contains many plants of ornamental value. The plants are perennials and are mostly spring and early summer bloomers. The genus is characterised by a simple or branched erect stem bearing one or more flowers at its top; the leaves are mostly radical and basal, linear to sword shaped, flat and many-nerved lengthwise; the segments of flowers are generally united into a long or short tube (the perianth tube) the three outer segments are hanging or reflexed and narrowed towards the base, the inner three segments are usually erect and often arched. Style is three branched; the branches are coloured, petal like, expanded, spreading outwardly and covering the three stamens.

About a dozen species of *Iris* occur in India and a few are cultivated for ornamental purposes. In the Kashmir valley the genus is represented by a number of species out of which *Iris germanica, I.kumaonensis, I.ensata, I.spuria, I.aurea, I.florentina* and *I. kashmiriana* are a few worth mentioning. All the species are wild and occupy large strips of land. Some species especially *I.germanica* and *I.florentina* are cultivated for their rhizomes which constitute the orris of commerce.

*Iris kashmiriana* is a local variety reported by Baker 98 years ago. Due to extensive hybridisation with other varieties especially *I.germanica* this species has lost its identity. Since this plant resembles both *I.germanica* and *I.kashmiriana* (as described by Baker) in morphology, it was thought worthwhile to retain both the
names and write the species as Iris germanica (Iris kashmiriana Baker)

Rhizomes of Iris have been used in perfumery from Greek and Roman times. An important source of the drug in the middle ages was Florence. The ancient arms of that city being a white iris on a red shield. The peeled, dried and aged rhizomes of Iris germanica, I. florentina and I. pallida are called as orris. The characteristic violet-like odour of orris is due to the presence of an essential oil, orris oil, which contains irones. Removal of fatty acids from orris oil gives absolute resinoids, a most valuable and expensive perfume used in high class soaps, cosmetics, dentrifices and as a fixative. Orris oil is also used for flavouring soft drinks, candies and gelatine desserts.

Roots and rhizomes of Iris species have been used in indigenous system of medicine as alterative, aperient, diuretic, cathartic, gallbladder diseases, liver complaints, dropsy, purification of blood, venereal infections, fever, ringworm, bilious obstructions and a variety of heart diseases.

Extracts of iris rhizomes are employed in meat curing pickle solutions to prevent food poisoning. Iris powder is used as an ingredient in formulating creams, lotions, shampoos and dentrifice compounds.

IRIS GERMANICA (IRIS KASHMIRIANA Baker)

It is a perennial herb occurring in the valley of Kashmir at an altitude of 5,400 to 8,500 ft. It covers large areas of land especially on graveyards and blooms in May. Plant with rhizomes; stem erect up to 2.5 ft. high; leaves radical, basal, few on the stem, flat,
sword-shaped and bearing many nerves lengthwise; flowers: pure white, sweet scented, arising from the base of a stem leaf, many on the stem, one flower following another, sepals of flowers united into a short perianth tube, three outer sepals large, reflexed and narrowed towards the base, three inner sepals smaller, erect and arched towards the inner side, spathe valves scarious at the tips only; stamens inserted at the base of the outer sepals; anthers linear, basifixed; ovary three gonous, style branched into three petal like milk white stigmas two third as long as the perianth, arching over the stamens, two fid and with a large dorsal yellow crust.

MEDICINAL USES:— Rhizomes used as alterative, aperient, diuretic, cathartic, used in gallbladder diseases and as powder or in poultice applied to sores and pimples. Crude alcoholic extract of this plant has been shown to possess hypotensive and anti-inflammatory action. An aqueous solution of *Iris germanica* has been shown to decrease smooth muscle activity *in vivo* and has a musculotropic spasmylytic effect on the duodenum and oddis sphincter *in vivo* and *in vitro*. It has also been shown to stimulate respiration, shows a central anti-serotonin hypotension accompanied by a negative-inotropic effect. It has no toxicity and psychotropic activity in mice.

No work has so far been done on the chemical investigation of the title species.

The herbarium has been deposited with Regional Research Laboratory, Jammu, under Collection No. 11742.
IRIS KUMAONENSIS Wall

It is a dwarf perennial herb occurring in the western Himalayas, from Kashmir to Kumaon, at an altitude of 8,000 - 12,000 ft. Root stock thick and creeping; stems crowded and upto one foot high; leaves usually longer than the stem, linear; flowers bright lilac with a long perianth-tube, spathe one-fid and 2-3 in. long, enveloped by the uppermost leaf, petals erect, blade oblong, capsule 1-2 in. long, ellipsoid.

MEDICINAL USES: Roots and leaves of this plant are reported to be given in fever.

The herbarium has been deposited with Regional Research Laboratory, Jammu, under Collection No. 12603.

REVIEW OF LITERATURE

A variety of compounds of different classes has been identified, isolated and characterised from various Iris species. These include flavonoids, steroids, amino acids, fatty acids, polysaccharides, irones, uncharacterised alkaloids etc.

Flavonoids form the major group of compounds isolated from Iris species. All the four members namely: anthocyanins, xanthones, flavones and isoflavones are represented. The main pigment is delphinidin-3,5-diglycoside and 3-(p-coumaroylrutinoside)-5-glucoside. A partly characterised malvidin derivative has been reported from I. ensata, I. chrysographes and I. delavayi. Delphanin in pseudobase form occurs in some white petalled varieties of the garden iris.
Mangiferin is the main xanthone isolated from Iris species. Recently a new xanthone irisxanthone has been reported from I. florentine. Flavones and flavone glycosides occur widely in Iris genus plants. These include embinin, orientin, isoorientin, homo-orientin, saponarin, swertisin, O-xylosylswertisin, swertiajaponin, flavoayarnenin, kaempferol, quercitin, dihydroquercitin-3',7-dimethyl-ether, lucenin, vicenin, vitexin etc.

Isoflavones are well known in Iris and seem to be of erratic distribution in the genus. Irigenin was the first isoflavone reported from iris in 19th century. This was followed by tectorigenin and tectoridin from I. tectorum, irisolone, irisolidone and iridin from I. nepalensis. Other isoflavonoids isolated are irifloside, irisflorentin, iristectorigenin B, iristectorin A, iristectorin B, 5,2'-dimethoxy-6,7-methylenedioxyisoflavone, 5,2',3'-trimethoxy-6,7-methylenedioxyisoflavone, 5,7,2'-trihydroxy-6,4'-dimethoxyisoflavone, 5,7,3'-trihydroxy-6,2'-dimethoxyisoflavone etc.

Steroids are not a permanent feature in Iris. Only a few namely: β-sitosterol and its glucoside, stigmasterol, campesterol and octacosanol have been isolated so far.

A number of amino acids, all the three classes acidic, neutral, and basic are present in the genus. These are cysteine, cystine, ornithine, lysine, histidine, asparagine, aspartic acid, serine, aminoacetic acid, glutamic acid, alanine, proline, β-alanine, tyrosine, methionine, valine, norvaline, phenylalanine, leucine, norleucine, γ-L-glutamyl-β-aminoisobutyric acid, m-carboxy-2-phenylglycine, β-aminoisobutyric acid, γ-L-glutamyl-β-alanine, γ-L-glutamyl-L-valine and L-hydroxyproline.
Oil isolated from *Iris* is rich in fatty acids; caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid etc., have been detected. Essential oil of *Iris* is a mixture of $\alpha$, $\beta$ and $\gamma$ irones. Other odouriferous substances present are acetovanillone and tectoruside.

*Iris* species are rich sources of ascorbic acid. Dihydrothiamine has also been reported from *I. tectorum* and *I. pseudacorus*. *I. drepanophylla* contains a high percentage of alkaloids but these have not been characterised so far. Gibberellin like substances and ethylene are also reported from the genus. The list of chemical compounds isolated from various *Iris* species is given in the following table:

<table>
<thead>
<tr>
<th><em>Iris</em> species</th>
<th>compounds isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. germanica</em></td>
<td>Homotectoridin, tectoridin$^8$ 5,2'-dimethoxy-6,7-methylenedioxyisoflavone, 5,2,3'-trimethoxy-6,7-methylenedioxyisoflavone, 5,7,2'-trihydroxy-6,4'-dimethoxyisoflavone, 5,7,3'-trihydroxy-6,2'-dimethoxyisoflavone, acetovanillone, irigenin, irisolone, iridolate, tectorigenin, dihydroquercitin-3',7-dimethyl ether; delphinidin glycosides$^{10}$ mangiferin$^{11}$ starch$^{12}$ caprylic, capric, lauric, myristic, palmitic, oleic and linoleic acids$^{13}$ embinin$^{14}$ ascorbic acid$^{15}$ L-hydroxyproline$^{16}$ irones$^{17}$</td>
</tr>
<tr>
<td><em>I. florentina</em></td>
<td>Iriflogenin, irifloside, irisolone, irisflorentin, iristectorigenin B, irigenin, iriflophenone, and iridin$^{18}$ mangiferin, irisxanthone$^{19}$ $\beta$-sitosterol</td>
</tr>
<tr>
<td>Iris species</td>
<td>compounds isolated</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>I. japonica</td>
<td>Embelinin and svertisin</td>
</tr>
<tr>
<td>I. tingitana</td>
<td>β-alanine; m-carboxy-L-phenylglycine; vitexin, iso-orientin, svertiajaponin, svertisin, O-xylosylsvertisin and delphinidin-3-(p-coumaroylrutinoside)-5-glucoside; β-aminoisobutyric-acid.</td>
</tr>
<tr>
<td>I. tectorum</td>
<td>Iristectorin A and androsin; iristectorin B, tectoruside; dihydrothiamine; embelinin.</td>
</tr>
<tr>
<td>I. pallida</td>
<td>Irones; ascorbic acid; starch</td>
</tr>
<tr>
<td>I. elegantissima</td>
<td>Cystine, cysteine, ornithine, lysine, histidine, asparagine, aspartic acid, serine, glycine, glutamic acid, alanine, proline, β-alanine, tyrosine, methionine, valine, norvaline, phenylalanine, leucine and norleucine.</td>
</tr>
<tr>
<td>I. nepalensis</td>
<td>Irigenin; irisolone; irisolidone</td>
</tr>
<tr>
<td>I. hollandica</td>
<td>β-sitosterol, stigmasterol, campesterol and octacosanol.</td>
</tr>
<tr>
<td>I. pumila</td>
<td>Starch; ascorbic acid</td>
</tr>
<tr>
<td>Iris species</td>
<td>compounds isolated</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------------------------------------------------------------------</td>
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<tr>
<td><em>I. terax</em> &amp;</td>
<td>Homo-orientin, saponaretin, kaempferol and</td>
</tr>
<tr>
<td><em>I. chrysophylla</em></td>
<td>quercitin glycosides, saponaretin glycosides, orientin, vitexin, lucenin, and vicenin glycosides</td>
</tr>
<tr>
<td><em>I. pseudacorus</em></td>
<td>Shikimic acid, maleic acid and quinic acid; dihydrothiamine.</td>
</tr>
<tr>
<td><em>I. nertchinska</em></td>
<td>Flavoayarnenin</td>
</tr>
<tr>
<td><em>I. alubiflora</em></td>
<td></td>
</tr>
<tr>
<td><em>I. drepanophylla</em></td>
<td>Uncharacterised alkaloids</td>
</tr>
<tr>
<td><em>I. kumaonensis</em></td>
<td>Iridin</td>
</tr>
<tr>
<td><em>I. aphylloa</em> ,</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td><em>I. sambucina</em> ,</td>
<td></td>
</tr>
<tr>
<td><em>I. variegata</em></td>
<td></td>
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<tr>
<td>and <em>I. heigo</em></td>
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</table>

In addition to above compounds ethylene, gibberellin like substances, \( \gamma-L\)-glutamyl-L-alanine and \( \gamma-L\)-glutamyl-L-valine, \( \gamma-L\)-glutamyl-\( \beta\)-alanine, m-carboxyphenyl-L-alanine, \( \gamma-L\)-glutamyl-\( \beta\)-aminoisobutyric acid etc., have also been isolated from various *Iris* species.

**ISOFLAVONES**

Isoflavones form a group of structurally related natural products occurring in plants. They are characterised by a particular type of ring system structurally related to the parent compound 3-phenyl-
The number of isoflavones found to date in nature is relatively small compared with the widespread occurrence of flavones and coumarins. They have attracted the attention of organic chemists because some of them possess estrogenic activity. The estrogenic activity of the subterranean clover (Trifolium subterraneum), a plant of great importance for sheep breeding in the drier areas of Western Australia, has been attributed to the presence of two isoflavones, genistein and formononetin in this plant. The so-called "spring flush" in diary cows is also probably due to the presence of isoflavones in the spring grass.

Isoflavones are also reported to have antifungal, anti-spasmodic, insecticidal and piscicidal activity. Some of the isoflavones are potent fish poisons.

**BIOSYNTHESIS OF ISOFLAVONES**

The biosynthesis of isoflavones can be subdivided into two definite steps: (i) the formation of a C\textsubscript{15} unit; (ii) modification of this unit to form a range of different isoflavones encountered in nature.

Observations based on feeding labelled acetate, phenylalanine, cinnamic acid and shikimic acid to tobacco, buck wheat and red cabbage, have indicated that the C\textsubscript{15} skeleton of flavonoids is derived from two separate pathways — acetate and shikimic acid.

As in the synthesis of aromatic rings in fungi the A-ring of
flavonoids arises by head-to-tail condensation of two molecules of malonyl CoA (co-enzyme A) and one molecule of acetyl CoA. The B-ring and the central C₃ unit is derived from a C₆-C₃ precursor which may be cinnamic acid itself. The C₆-C₃ precursor is itself derived via shikimic acid pathway presumably by enzymatic one step deamination of phenylalanine.

Monohydroxylation of ring B can occur at C₆-C₃ stage but other hydroxyls appear to be introduced at C₁₅ stage.

\[
\text{Shikimic acid} \rightarrow HOOCH₂CH₂\Phi \quad \text{phenylalanine} \\
\text{NH₂} \quad \text{→} \quad -\text{NH₃}
\]

\[
2 \text{HOOCH₂CH₂CO-CoA} + \text{CH₃CO-CoA} \rightarrow \text{Flavanone etc.} \quad \text{C₁₅-intermediate}
\]

Grisebach has proposed that chalcone should be the first stable intermediate in the biosynthesis. According to his scheme cinnamic acid molecule (probably the CoA ester) condenses with three malonyl CoA units resulting in the formation of a β-polyketo acid. Owing probably to the surface structure of the enzyme this acid would then undergo intramolecular ring closure whereby a chalcone is formed.
Hypothetical formation of a chalcone from p-coumaryl-CoA and malonyl-CoA.

The nature of the $C_6-C_3-C_6$ precursor is not yet known. Either it enjoys a transient existance or is in rapid equilibrium with an activated, perhaps phosphorylated chalcone or flavanone. Which of the above $C_{15}$ units is the actual intermediate in the biosynthesis of isoflavones has yet to be decided.

After the formation of $C_{15}$ intermediate, the next step in the biosynthesis is the introduction of $C_3$-OH group followed by the closure of heterocyclic ring. Support for the plausibility of this mechanism is given as: by feeding labelled phenylalanine at each position in the $C_3$ side-chain to clover seedlings it has been observed
that isoflavone formononetin gets labelled according to the scheme:

\[
\text{HOOC-CH-CH}_2\text{-NH}_2 \quad \rightarrow \quad \text{HO-\text{OCH}_3}
\]

and also label from chalcones fed to the legumes has been found in the subsequently isolated isoflavones.

Glycosylation and methylation presumably occur towards the end of the synthesis because these processes affect some flavonoids and not others. Formation of methylenedioxy group may involve an oxidative cyclisation of the methoxyls as suggested by Barton in connection with the biogenetic origin of the methylenedioxy group in alkaloids, etc.

In spite of all the above observations there is still a lingering doubt about what appears to be a proven biosynthetic route. Modification of pathway leading to C\text{15} intermediate cannot be completely ruled out at the present time.
Defatted rhizomes of Iris germanica (Iris kashmiriana Baker) were extracted exhaustively with methanol. Methanol extract was vacuum dried and re-extracted with hot CHCl₃ and hot EtOAc.

The CHCl₃ extract on concentration deposited a pale brown crystalline solid, Igk-1. The mother liquor was filtered, concentrated and separated on a column of silica gel (using CHCl₃-EtOAc as eluents) into fractions A, B, C, D and E.

Fraction B was rechromatographed over silica gel (using CHCl₃-EtOAc as eluent) to give Igk-2.

Fractions C and D were pooled and subjected to rechromatography over silica gel using a linear gradient of light petrol against EtOAc as eluents. Various sections of the column were followed by TLC and suitably pooled to give the following compounds:

Igk-3
Igk-4

The above EtOAc extract was chromatographed over silica gel using CHCl₃ and CHCl₃-acetone as eluents. Various fractions of the column were suitably pooled on the basis of TLC results to give Igk-5 as a brown solid.
Igk-3

**Solubility:**
Igk-3 is soluble in hot benzene, chloroform, ether, alcohol, 5% aq. NaOH solution and cold conc. H$_2$SO$_4$. It is insoluble in light petrol, water, 5% aq. NaHCO$_3$, 5% aq. HCl and 85% aq. phosphoric acid solutions. The compound, therefore, falls in "A$_2$-N$_2$", weakly acidic to neutral, class of compounds according to Shriner, Fuson and Curtin.$^{52}$

**Tests for Elements and Functional groups (chemical methods):**

1. Detection of elements:– It showed the absence of nitrogen, sulphur and halogens in the Lassaigne's test.


3. Phenolic hydroxyl group:– The compound gave a green colour on treatment with 1% alcoholic FeCl$_3$ and a very faint grey colour with 1% aq. FeCl$_3$ solutions – one or more phenolic -OH groups present.

4. Methylenedioxy group:–
   (a) Labat test$^{53}$:– Dissolved 2 mg. of Igk-3 in a little amount of alcohol. Added to it 0.2 ml. of 10% H$_2$SO$_4$ and then 2 ml. of conc. H$_2$SO$_4$ with shaking. Then added 0.1 ml. of 5% gallic acid in alcohol to the mixture and warmed on water bath for about 30 min. Presence of a green colour against a blank test indicated the presence of methylenedioxy group.

   (b) Gaebel test:– Took 1 g. of phloroglucinol and dissolved
it in 75 ml. water and 30 ml. conc. $\text{H}_2\text{SO}_4$ by warming. Allowed the mixture to stand for a few hours and then filtered. Took a few drops of this phloroglucinol - $\text{H}_2\text{SO}_4$ reagent and added to 4 mg. of Igg\textsuperscript{3} in alcohol. Warmed, a red colour against the blank test, showed the presence of a methylenedioxy group in Igg\textsuperscript{3}.

5. Methoxyl groups\textsuperscript{54} took about 2 mg. solution of Igg\textsuperscript{3} in chloroform in a tube (apparatus described in Feigl\textsuperscript{54}) and added two drops of a 10% solution of benzoyl peroxide in benzene to it. Evaporated the solvent. Charged the knob of the stopper with chromotropic acid - conc. $\text{H}_2\text{SO}_4$ mixture (mixed by centrifuging) and inserted it into the tube in such a way that the knob just remained above the compound. Heated the tube at 120 - 130\textdegree. The hanging drop did not turn violet. This indicated the absence of methoxyl group in Igg\textsuperscript{3}.

6. Magnesium and conc. HCl test for flavonoids took 15 mg. of Igg\textsuperscript{3} with conc. HCl (1.5 ml.) and a small piece of magnesium. Heated the mixture on a steam bath. A yellow coloured solution appeared. The test did not lead to any positive conclusion.

The information gathered so far indicates that Igg\textsuperscript{3} contains the following functionalities:

(i) One or more phenolic hydroxyl groups.

(ii) A methylenedioxy group.

**Element analysis**

Found: C, 64.1; H, 3.41.

Calculated for $\text{C}_{16}\text{H}_{10}\text{O}_6: C$, 64.42; H, 3.35%.

The molecular formula suggests that the compound contains total sites of unsaturation plus rings = 12.
Spectral data:-

UV Spectrum:-- The UV spectrum of Igk-3 shows $\lambda_{max}$ in MeOH at 269 and 332 nm (inflexion). This type of UV is characteristic for iso-flavones. The low intensity peak or Band I is associated with absorption due to B-ring phenyl system and intense peak at 269 nm or Band II involves the A-ring benzoyl system.

Addition of AlCl$_3$ showed a bathochromic shift of 13 nm, which did not change after the addition of HCl, of Band II. This shows the presence of a 5-OH group in the molecule. The UV spectrum was unaffected by the addition of fused NaOAc to the system.

Infrared spectrum (Fig. 1):-- IR spectrum of Igk-3 shows principal peaks at 3200 - 3600 (broad band), 1680, 1627, 1600, 1570, 1520, 1470, 1325, 1270, 1180, 1100, 1055, 926, 827 and 772 cm$^{-1}$.

The 1680 cm$^{-1}$ band arises due to $>C=O$ stretching vibration of pyrone ring carbonyl. The broad band at 3200 - 3600 cm$^{-1}$ with two peak pattern is due to $O-H$ stretching vibration of $-OH$ groups and band at 926 cm$^{-1}$ shows the presence of methylenedioxy group.

Igk-3 diacetate I(b)

Igk-3 diacetate was prepared by refluxing a mixture of Igk-3, acetic anhydride and pyridine for one hour.

Element analysis:--

Found: C, 62.3; H, 3.29.

Calculated for $C_{20}H_{14}O_8$: C, 62.82; H, 3.66 %.

The molecular formula suggests that the total number of rings
plus sites of unsaturation present in Igk-3 acetate = 14.

**Spectral data :-**

IR spectrum (Fig.2) :- IR shows principal peaks at 1782, 1748, 1654, 1630, 1510, 1477, 1372, 1260, 1225, 1210, 1192, 1100, 1085, 1052, 928 and 783 cm\(^{-1}\). Disappearance of peaks at 3200 – 3600 cm\(^{-1}\) region shows that all the \(-\text{OH}\) groups have got acetylated and the acetates appear at 1782 and 1748 cm\(^{-1}\) Bands at 1654 and 928 cm\(^{-1}\) are due to pyrone carbonyl and methylenedioxy groups respectively.

NMR spectra (Fig.3) :- The NMR spectrum of Igk-3 diacetate is recorded in Table 1 and accounts for the presence of 14 protons in the molecule. The signal at \(\delta\) 7.87s indicates the C-2-H of an isoflavone. Since this proton is beta to the C-4 keto function and attached to \(-\text{C-C-}\) system, therefore, in NMR it occurs in the region down field from where most aromatic proton signals appear.

The typical four-peak pattern of two distorted doublets suggests an aromatic AA'BB' type of system. This is only possible in ring B when 4' position will be oxygenated. Thus 4' position contains an -OAc group and therefore, an -OH group in the unacetlated compound. The doublet at \(\delta\) 7.55 allocates two protons to C-2', C-6' positions. The C-3', C-5' protons, which are shielded by C-4 oxygen substituent appear up field at \(\delta\) 7.17d. The single sharp peak at \(\delta\) 6.15s is characteristic for a methylenedioxy group which can either be placed at 6,7 or 7,8 positions. The single peak at \(\delta\) 6.84 (a typical down field shift in 5-acetoxy flavonoids\(^{55-57}\)) suggests a proton at C-8 because the NMR signal of C-6 proton in isoflavones appears at slightly
up field than C-8 proton. So the methylenedioxy group is at 6,7 positions. The two singlets at δ 2.41 and 2.29 are due to two acetate proton signals.

The NMR suggests the following structure I(b) for Igk-3 acetate and, therefore, I(a) for Igk-3 with various groups arranged on a 3-phenyl-4-chromone nucleus.

\[
\text{I(a) } R = R' = H \\
\text{(b) } R = R' = \text{COCH}_3 \\
\text{(c) } R = R' = \text{CH}_3
\]

### Table 1

<table>
<thead>
<tr>
<th>δ</th>
<th>No. of Protons</th>
<th>Multiplicity</th>
<th>Proton Assignment</th>
<th>See I(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.87</td>
<td>1</td>
<td>s</td>
<td>C - 2(H)</td>
<td></td>
</tr>
<tr>
<td>6.84</td>
<td>1</td>
<td>s</td>
<td>C - 8(H)</td>
<td></td>
</tr>
<tr>
<td>6.15</td>
<td>2</td>
<td>s</td>
<td>O - CH\textsubscript{2} - O</td>
<td></td>
</tr>
<tr>
<td>7.55</td>
<td>2</td>
<td>d(J\textsubscript{AB} = 9Hz)</td>
<td>C - 2', 6' (2H)</td>
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</tr>
<tr>
<td>7.17</td>
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<td>d(J\textsubscript{AB'} = 9Hz)</td>
<td>C - 3', 5' (2H)</td>
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<tr>
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<td>3</td>
<td>s</td>
<td>C - 5 (0.C0.CH\textsubscript{3})</td>
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<tr>
<td>2.29</td>
<td>3</td>
<td>s</td>
<td>C - 4' (0.C0.CH\textsubscript{3})</td>
<td></td>
</tr>
</tbody>
</table>

\( s = \text{singlet}, \ d = \text{doublet} \)

Mass spectrum (Fig. 4) : The molecular ion (M\textsuperscript{+}) peak in the MS appeared at m/e 382. This supports the structure I(b) and its micro-analytical data. Peaks at m/e 340(M\textsuperscript{+} - 42) and 298 (M\textsuperscript{+} - 2 x 42) show the presence of two acetyl\textsuperscript{-} groups in the molecule and, therefore, two -OH groups in I(a). The molecule undergoes Diels - Alder retro -
addition to give peaks at m/e 180 and 118.

\[
\begin{align*}
I(b) & \rightarrow ^{+} \quad m/e \ 342 \\
\text{m/e 382} & \rightarrow ^{+} \quad I(a) \\
& \rightarrow \quad \text{m/e 298}
\end{align*}
\]

Igk-3 dimethyl ether (C)

Igk-3 dimethyl ether was prepared by refluxing a mixture of Igk-3, Me₂SO₄, K₂CO₃ and acetone for 18 hr.

Element analysis :-

Found; C, 66.53; H, 4.09.

Calculated for C₁₈H₁₄O₆ : C, 66.25; H, 4.32 %.

The molecule contains total 12 number of rings and sites of unsaturation.

Spectral data :-

UV spectrum :- UV shows \( \lambda_{\text{max}}^{\text{EtOH}} \) at 266 and 325 nm (shoulder).

The absorption maximum was unaffected by the addition of AlCl₃ or NaOAc.

IR spectrum (Fig.5) :- IR shows >C=O at 1648 cm\(^{-1}\) and \(-O-\text{CH}_2-\text{O-}\) at 922 cm\(^{-1}\).

Partial synthesis of Igk-3 :-

The structure I(a) for Igk-3 was finally confirmed by its partial synthesis from irisolone. Irisolone was treated with anhydrous AlCl₃ in ether for 3 hr. The solvent was removed and the residue refluxed with HCl - AcOH (1:1) for 15 min. The product after usual
work up was found to be identical with Igk-3 in all respects.

CONCLUSION

Chemical, spectral data and partial synthesis indicate that Igk-3 has got structure I(a) - 5,4'-dihydroxy-6,7-methylenedioxy-isoflavone. It is a new isoflavone which has been named here as irilone.
**Fig. 1** - IR (KBr) spectrum of Igk-3

**Fig. 2** - IR (KBr) spectrum of Igk-3 diacetate.

**Fig. 3** - NMR (100 MHz, CDCl₃) spectrum of Igk-3 acetate.
**Fig. 4** - MS of Igk-3 acetate.

**Fig. 5** - IR (nujol) spectrum of Igk-3 methyl ether.
Solubility:

Igk-5 is soluble in ether, ethyl acetate, alcohol, 5% aq. NaOH and cold conc. \( H_2SO_4 \) solutions. It is insoluble in cold water, 5% aq. NaHCO\(_3\), 5% aq. HCl and 85% aq. phosphoric acid solutions. The compound, therefore, falls in "A\(_2\)-N\(_2\)", weakly acidic to neutral class of compounds according to Shriner, Fusan and Curtin.\(^{52}\)

Tests for elements and functional groups (chemical methods):

1. Detection of elements: It showed the absence of nitrogen, sulphur and halogens in the Lassaigne's test.

2. Free -COOH group: The compound gave no effervescence on treatment with aq. NaHCO\(_3\) solution. This shows the absence of -COOH in the compound.

3. Phenolic hydroxyl group: The compound gave a blackish-green colour on treatment with alcoholic 1% FeCl\(_3\) solution and a light brown colour with 1% aq. FeCl\(_3\) solution — This shows the presence of phenolic -OH group or groups in the molecule.

4. Free carbonyl group: To 5 mg. of 2,4-dinitrophenylhydrazine added 2.5 ml. of alcohol and 0.5 ml. of conc. \( H_2SO_4 \). Warmed the solution. Added a solution of 5 mg. of Igk-5 in 2 ml. alcohol to it and heated just to boiling. A red precipitate was formed. This shows the presence of free carbonyl group in the molecule.

5. Ether linkage: -

(a) Methylenedioxy group: Igk-5 did not give any colour reactions in Labat and Gaebel tests for methylenedioxy group.
(b) Methoxyl group :- The compound showed the absence of methoxyl groups in the chromotropic acid test (see Igk-3 for details of this test).

6. Magnesium and conc. HCl test for flavonoids :- Treated 15 mg. of Igk-5 with conc. HCl (1.5 ml.) and a small piece of magnesium. Heated the mixture on a steam bath. A yellow coloured solution appeared. The test did not lead to any conclusion.

The information gathered so far indicates that Igk-5 contains the following functional groups :-

(i) It contains one or more phenolic hydroxyl groups.

(ii) It contains a free carbonyl group.

**Element analysis :-**

Found : C, 63.38; H, 4.1.

Calculated for $\text{C}_{13}\text{H}_{10}\text{O}_5$: C, 63.41; H, 4.06 %.

The molecular formula of the compound suggests that it contains total 9 sites of unsaturation plus rings.

**Spectral data :-**

UV spectrum :- UV shows $\lambda_{\text{MeOH}}^{\text{max}}$ at 311 nm (broad intense band) and 225 nm (shoulder).

IR spectrum (Fig. 6) :- IR shows principal peaks at 3515, 3420, 3268, 1645, 1600, 1510, 1315, 1263, 1160, 1061, 1000, 923 and 795 cm$^{-1}$. Bands at 3515, 3420 and 3268 cm$^{-1}$ show free and chelated $-\text{OH}$ groups in the molecule. Peaks at 1645 and 1600 cm$^{-1}$ indicate carbonyl and aromatic functions in the molecule.
Tetra-acetate of Igk-5 was prepared by refluxing a mixture of Igk-5 with Ac₂O and pyridine for 2 hr.

Element analysis:—

Found: C, 60.73; H, 4.46.

Calculated for C₂₁H₁₈O₉: C, 60.86; H, 4.34%.

The molecular formula shows that the compound contains total 13 sites of unsaturation and rings.

Spectral data:—

IR spectrum (Fig. 7):— IR spectrum shows principal bands at 1775, 1680, 1600, 1375, 1272, 1195, 1125, 1055, 1023, 933, 912 and 780 cm⁻¹. IR shows that all the phenolic groups in the compound have got acetylated. The peak at 1680 cm⁻¹ and broad bands at 1775 and 1272 cm⁻¹ are due to carbonyl and acetyl carbonyls respectively.

NMR spectra (Fig. 8):— The NMR spectrum of Igk-5 tetra-acetate is recorded in table 2 and accounts for the presence of 18 protons in the molecule.

The two singlets at δ 2.28 and δ 1.89, each showing six protons, indicate the presence of four acetyl groups in the molecule and, therefore, 4 -OH groups in Igk-5. Out of four acetyl groups two each are similar. The four peak pattern of two distorted doublets suggests an aromatic AA' BB' type of system in the molecule. This is only possible when one of the aromatic rings contains a substituent at para position. The singlet of two protons at δ 7.0 shows two equivalent aromatic protons in the molecule. The NMR of Igk-5 tetra-acetate suggests the following structure II(b) for the acetate and
therefore, II(a) for Igk-5. The various groups are arranged on a benzophenone nucleus.

\[
\begin{align*}
\text{II(a) } & R = H \\
\text{(b) } & R = \text{CO.CH}_3 \\
\text{(c) } & R = \text{CH}_3
\end{align*}
\]

<table>
<thead>
<tr>
<th>(\delta)</th>
<th>No. of protons</th>
<th>multiplicity</th>
<th>proton assignment</th>
</tr>
</thead>
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<td>s</td>
<td>C - 3, 5 (2H)</td>
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<tr>
<td>7.835</td>
<td>2</td>
<td>d((J_{AB} = 9\text{Hz}))</td>
<td>C - 2', 6' (2H)</td>
</tr>
<tr>
<td>7.175</td>
<td>2</td>
<td>d((J_{AB'} = 9\text{Hz}))</td>
<td>C - 3', 5' (2H)</td>
</tr>
<tr>
<td>2.28</td>
<td>6</td>
<td>s</td>
<td>C - 4, 4' (0.C0.CH(_3))</td>
</tr>
<tr>
<td>1.89</td>
<td>6</td>
<td>s</td>
<td>C - 2, 6 (0.C0.CH(_3))</td>
</tr>
</tbody>
</table>

\(s = \text{singlet, } d = \text{doublet}\)

Mass spectrum (Fig.9) :- MS shows \(M^+\) at 414 and other major peaks at m/e 372, 330, 288, 246, 245, 153, 152 and 121. The MS supports the structure II(b) for Igk-5 tetra-acetate and, therefore, II(a) for Igk-5. The fragmentation can be explained as:

\[
\begin{align*}
\text{II(b) } & \longrightarrow \text{II(a)} \longrightarrow \\
& \text{m/e 414} \quad \text{m/e 372} \quad \text{m/e 330} \quad \text{m/e 288} \quad \text{m/e 246} \quad \text{m/e 153} \quad \text{m/e 121}
\end{align*}
\]
Igk-5 tetramethyl ether II(c)

Tetramethyl ether of Igk-5 was prepared by treating it with Me₂SO₄, K₂CO₃ and acetone followed by refluxing for 25 hr.

Element analysis:–

Found : C, 67.43; H, 6.0.
Calculated for C₁₇H₁₈O₅: C, 67.55; H, 5.96 %.

Spectral data:–

UV spectrum:– UV shows λ max at 285 nm (intense band) and 225 nm (shoulder).

IR spectrum (Fig.10):– IR shows principal peaks at 1653, 1600, 1278, 1252, 1223, 1154, 1117, 1025, 946, 910, 841 and 805 cm⁻¹. IR indicates the presence of >C=O (1653 cm⁻¹), aromatic (1600 cm⁻¹) and methoxyl (1025 cm⁻¹) functions in the molecule.

Synthesis of Igk-5 tetramethyl ether:–

2,4,6-trimethoxybenzene on heating with p-methoxybenzoic acid, fused ZnCl₂ and POCl₃ at 85° for 3 hr. gave a solid which on chromatographic fractionation over silica gel gave a shining crystalline compound of 2,4,6,4’-tetramethoxybenzophenone which was found to be identical with Igk-5 tetramethyl ether in all respects.

Chemical, spectral data and synthesis of Igk-5 methyl ether indicate that Igk-5 has got structure II(a) – 2,4,6,4’-tetrahydroxybenzophenone. It is known in literature as a synthetic compound but isolated for the first time from a natural source. (The compound has also been isolated recently from Iris florentina.)
Fig. 6 - IR (nujol) spectrum of Igk-5

Fig. 7 - IR (KBr) spectrum of Igk-5 tetra-acetate.

Fig. 8 - NMR (100 MHz, CDCl₃) spectrum of Igk-5 tetra-acetate.
Fig. 9 - MS of Igk-5 tetramethyl ether.

Fig. 10 - IR (nujol) of Igk-5 tetramethyl ether.
It behaves like Igk-3 in solubility and thus falls in the group "A_2^- - N_2^-" of compounds according to Shriner et al.

**Tests for elements and functional groups (chemical methods):**

1. Detection of elements: It showed the absence of N, S and halogens in the Lassaigne's test.

2. Groups found absent: The following functional groups tested in a way similar to Igk-3 were found absent.
   (i) Free -COOH group.

3. Groups found present: The compound indicated the presence of the following types of groups:
   (i) Phenolic hydroxyl group: The compound gave a faint green colour on treatment with alcoholic 1% FeCl_3 solution and no colour with aq. 1% FeCl_3 solution. This shows the presence of phenolic -OH group or groups in Igk-1.
   (ii) Methylenedioxy group:
      (a) Labat test - The compound gave a green colour in the Labat test.
      (b) Gaebel test - Igk-1 gave a red colour in this test.
   (iii) Methoxyl group: The compound imparted a violet colour to the hanging drop of a mixture of chromotropic acid - conc. H_2SO_4 in the test.

4. Magnesium and conc. HCl test for flavonoids: The compound gave a bright yellow colour when heated with a piece of magnesium and conc. HCl.
Element analysis :-

Found : C, 65.49; H, 3.96.

Calculated for \( \text{C}_{17}\text{H}_{12}\text{O}_{6} \): C, 65.38; H, 3.87 %.

The molecular formula shows the presence of 12 rings plus sites of unsaturation in the molecule.

Spectral data :-

UV spectrum :- UV shows \( \lambda_{\text{max}} \) at 266 and 325 nm (shoulder).

Addition of anhydrous \( \text{AlCl}_3 \) and \( \text{NaOAc} \) did not show any shift in the UV. UV shows the presence of an isoflavone skeleton and absence of chelated 5, and 7-OH groups in the molecule.

IR spectrum (Fig.11) :- IR spectrum shows principal peaks at 3270, 1645, 1610, 1517, 1472, 1441, 1262, 1107, 1080, 1060, 927, 838 and 775 cm\(^{-1}\). Broad band at 3270 cm\(^{-1}\) and other bands at 1645, and 927 cm\(^{-1}\) support the presence of -OH, >C=O and methylene-dioxy group in the molecule.

NMR spectra (Fig.12) :- The NMR is recorded in table 3 alongwith proton assignments. NMR (DMSO) accounts for the presence of 12 protons in the molecule. The singlet at \( \delta 8.14 \) is due to C-2 proton of isoflavones. The two doublets at \( \delta 7.29 \) and 6.75 suggest the presence of an aromatic AA'BB' system in the molecule. The sharp singlet of two protons at \( \delta 6.15 \) is characteristic for methylene-dioxy group and three proton singlet at \( \delta 3.88 \) indicates -OCHO\(_3\) group in the molecule. The singlets at \( \delta 6.96 \) and 9.45 allocates protons to C-8 and C-4 -OH, respectively.

\( \text{D}_2\text{O} \) exchange of the molecule makes the peak at \( \delta 9.45 \) to disappear. This confirms one -OH group.

The UV, IR and NMR suggest the following structure III(a) for
the molecule of Igk-1 with various groups arranged on a 3-phenyl-4-chromone nucleus.

\[ III(a) \hspace{1cm} R = H \]
\[ (b) \hspace{1cm} R = CO.CH_3 \]
\[ (c) \hspace{1cm} R = CH_3 \]

Table 3

<table>
<thead>
<tr>
<th>δ</th>
<th>No. of protons</th>
<th>multiplicity</th>
<th>proton assignment</th>
</tr>
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<tbody>
<tr>
<td>9.45</td>
<td>1</td>
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<td>C - 4'(OH)</td>
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<td>8.14</td>
<td>1</td>
<td>s</td>
<td>C - 2 (H)</td>
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<td>2</td>
<td>d(J_{AB} = 9Hz)</td>
<td>C - 2', 6'(2H)</td>
</tr>
<tr>
<td>6.755</td>
<td>2</td>
<td>d(J_{AB} = 9Hz)</td>
<td>C - 3', 5'(2H)</td>
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<tr>
<td>6.96</td>
<td>1</td>
<td>s</td>
<td>C - 8 (H)</td>
</tr>
<tr>
<td>6.15</td>
<td>2</td>
<td>s</td>
<td>C - 6, 7 (O-CH_2-O)</td>
</tr>
<tr>
<td>3.88</td>
<td>3</td>
<td>s</td>
<td>C - 5 (OCH_3)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet

Igk-1 acetate III(b)

Element analysis :-

Found : C, 64.57; H, 4.1.

Calculated for C_{19}H_{14}O_{7}: C, 64.40; H, 3.98%.

Spectral data :-

IR spectrum (Fig.13) :- IR shows principal bands at 1753, 1644, 1616, 1476, 1440, 1261, 1224, 1206, 1107, 1082, 1062, 938, 870 and 786 cm\(^{-1}\). Absence of band between 2500 - 3600 cm\(^{-1}\) and appearance
of bands at 1753 cm\(^{-1}\) shows that -OH has got acetylated.

NMR spectra (Fig.14): The NMR spectrum shows peaks at \(\delta\) : 7.87s, C-2 proton; 7.53d (\(J_{AB}= 9\)Hz), C-2',6' protons; 7.11d (\(J_{AB}= 9\)Hz), C-3',5' protons; 6.6s, C-8 proton; 6.02s, C-6,7 (O-CH\(_2\)-O); 4.07s, C-5 -OMe and 2.29s, C-4' -COCH\(_3\).

Mass spectrum (Fig.15): MS shows M\(^+\) at 354 and other major fragments at m/e 312 (M\(^+\)-CH\(_2\)CO), 311 (M\(^+\)-CH\(_2\)CO-H), 266 (M\(^+\)-CH\(_2\)CO - CO - H\(_2\)O), 194, 166, 164, 118, 76, 53, 43 and 27.

Retro-Diels-Alder reaction accounts for the peaks at m/e 118 and 194.

\[
\begin{align*}
\text{III(b)} & \xrightarrow{\text{m/e 354}} \text{CH\(_3\)CO} \quad \text{III(a)} & \xrightarrow{\text{m/e 312}} & \text{m/e 194} & \text{m/e 118}
\end{align*}
\]

Igk-1 methyl ether III(c)

Igk-1 methyl ether was identical in m.p., TLC, element analysis, UV and IR with Igk-3 dimethyl ether.

**CONCLUSION**

Chemical and spectral data indicate that Igk-1 has got structure III(a) - 4'-hydroxy-5-methoxy-6,7-methylenedioxyiso-flavone (irisolone).\(^{58}\) This is the second report of the isolation of this isoflavone from a natural source and first report from the present species.
Fig. 11 - IR (KBr) spectrum of Igk-1

Fig. 12 - NMR (100 MHz, DMSO) spectrum of Igk-1

Fig. 13 - IR (KBr) spectrum of Igk-1 acetate.
Fig. 14 - NMR (100 MHz, CDCl₃) spectrum of Igk-1 acetate.

Fig. 15 - MS of Igk-1 acetate.
Solubility: 
It behaved like Igk-3 in solubility and thus falls in the class "A_2 - N_2" of compounds according to Shriner et al.

Tests for elements and functional groups (chemical methods):-
1. Detection of elements: - It showed the absence of N, S and halogens in the Lassaigne's test.
2. Groups found absent: - The following groups, tested in a way similar to Igk-3, were found absent.
   (i) Free -COOH group.
   (ii) Methylene dioxy group.
3. Groups found present: -
   (i) Phenolic hydroxyl group: - The compound gave a green colour on treatment with 1% alcoholic FeCl_3 and a light brown colour with aq. 1% FeCl_3 solutions.
   (ii) Methoxyl group: - The compound was tested for methoxyl groups in a way similar to Igk-3. A violet colour was imparted to the hanging drop of chromotropic acid - conc. H_2SO_4 mixture. This indicated the presence of methoxyl group or groups in the molecule.
4. Magnesium and conc. HCl test for flavonoids: - The compound gave a bright yellow colour with conc. HCl and magnesium.

Element analysis: -

Found: C, 64.72; H, 4.51.

Calculated for C_{17}H_{14}O_6: C, 64.96; H, 4.45%.

The molecular formula shows that the compound contains 11 sites of unsaturation and rings.
Spectral data :-

UV spectrum :- UV spectrum shows $\lambda_{\text{max}}^{\text{MeOH}}$ at 268 and 330 nm (shoulder). Addition of anhydrous $\text{AlCl}_3$ to the solution shifted the $\lambda_{\text{max}}$ to 278 nm with no change after the addition of $\text{HCl}$, and the addition of $\text{NaOAc}$ showed a bathochromic shift of 8 nm of this band. The UV spectrum suggests that the molecule has got an isoflavone skeleton and contains a chelated 5-OH group and a 7-OH group.

IR spectrum (Fig.16) :- IR shows principal peaks at 3400, 1655, 1633, 1580, 1463, 1368, 1296, 1232, 1160, 1072, 830 and 815 cm\(^{-1}\). IR supports the presence of a $>\text{C}=\text{O}$ (1655 cm\(^{-1}\)) and a chelated $-\text{OH}$ group in the molecule.

NMR spectra (Fig.17) :- The NMR is recorded in table 4 along with proton assignments. It accounts for the presence of 14 protons in the molecule. A singlet at $\delta$ 7.96 is due to C-2 proton of an isoflavone. The two doublets at $\delta$ 7.38 and 6.93 show an AA' BB' type of system in ring B which is, therefore, substituted at 4' position. Two singlets at $\delta$ 3.9 and 4.08 suggest two $-\text{OCH}_3$ groups. One $-\text{OCH}_3$ can be placed at C-4' position and the other at either C-6 or C-8 position. Out of these two positions former appears to be more appropriate because on biogenetic grounds C-6 position is more favourable than C-8 position for substitution. The two proton signal at $\delta$ 6.61 allocates one proton to C-8 position and one proton of $-\text{OH}$ group to C-7 position. The signal due to C-5 OH which appears above $\delta$ 10 has not been recorded.

$\text{D}_2\text{O}$ exchange of the molecule reduces the two proton signal at $\delta$ 6.61 to one proton signal. Other signals remain unaffected.

The NMR, UV and IR suggest the following structure IV(a) for Igk-2 with various groups arranged on a 3-phenyl-4-chromone nucleus.
\[ \text{IV(a) } R = H \]
\[ \text{(b) } R = \text{CO.CH}_3 \]

Table 4

<table>
<thead>
<tr>
<th>δ</th>
<th>No. of protons</th>
<th>multiplicity</th>
<th>proton assignment</th>
</tr>
</thead>
<tbody>
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<td>s</td>
<td>C - 2 (H)</td>
</tr>
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<td>d((J_{AB} = 9)Hz)</td>
<td>C - 3', 5' (2H)</td>
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<tr>
<td>6.61</td>
<td>2</td>
<td>s</td>
<td>C - 8, C - 7 (H, -OH)</td>
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<td>3.9</td>
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<td>s</td>
<td>C - 4' and</td>
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<tr>
<td>4.08</td>
<td>3</td>
<td>s</td>
<td>C - 6 -OCH\textsubscript{3} protons</td>
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</tbody>
</table>

s = singlet, d = doublet

Mass spectrum (Fig. 18) :- MS shows \(M^+\) at 314 and other major fragments at m/e 315 (P + 1), 299 (M\(^+\)-15), 296 (M\(^+\)-18), 271 and 69. MS supports the formula IV(a).

\textbf{Igk-2 diacstate IV(b)}

Element analysis :-

Found : C, 63.52; H, 4.37.

Calculated for \(C_{21}H_{18}O_8\) : C, 63.31; H, 4.52 %.

Spectral data :-

IR spectrum (Fig. 19) :- IR shows bands at 1773, 1648, 1623, 1515, 1481, 1435, 1373, 1297, 1247, 1185, 1062, 1028, 904, 855 and 750 cm\(^{-1}\).

IR supports that both -OH groups have got acetylated.

NMR spectra (Fig. 20) :- The NMR accounts for the presence of 18
protons in the molecule. It shows peaks with proton assignments at $6: 7.82s$, C-2 proton; $7.38d$ ($J_{AB} = 9$Hz), C-2', 6' protons; $6.93d$ ($J_{AB}' = 9$Hz), C-3', 5' protons; $7.15s$, C-8 proton (a typical down field shift in 5-acetoxyflavonoids); $3.81s$ and $3.85s$, two $-OCH_3$ proton signals; $2.34s$ and $2.42s$, two acetate proton signals.

CONCLUSION

Chemical and spectral data indicate that Igk-2 has got structure IV(a) - 5,7-dihydroxy-6,4'-dimethoxyisoflavone (irisolidone). It is a rare isoflavone and has been isolated for the second time from Iris plants, but for the first time from the present species.
Fig. 16 - IR (KBr) spectrum of Igk-2

Fig. 17 - NMR (100 MHz, CDCl₃) spectrum of Igk-2

Fig. 18 - MS of Igk-2
Fig. 19 - IR (KBr) spectrum of Igk-2 diacetate.

Fig. 20 - NMR (60 MHz, CDCl₃) spectrum of Igk-2 diacetate.
Solubility :-

It behaved like Igk-3 in solubility and, therefore, falls in "A_2 - N_2", weakly acidic to neutral class of compounds.

Tests for elements and functional groups (chemical methods) :-

1. Detection of elements :- It showed the absence of N, S and halogens in the Lassaigne's test.

2. Groups found absent :- The following groups tested in a way similar to Igk-3 were found absent.
   (i) Free -COOH group.
   (ii) Methylenedioxy group.

3. Groups found present :- The compound indicated the presence of the following types of groups :
   (i) Phenolic hydroxyl groups :- It gave a blackish-green colour with alcoholic 1% FeCl_3 and violet-brown colour with aq. 1% FeCl_3 solutions.
   (ii) Methoxyl groups :- The compound imparted a violet colour to the hanging drop of chromotropic-conc. H_2SO_4 acid mixture in the spot test.

4. Magnesium and conc. HCl test for flavonoids :- The compound gave a bright yellow colour when treated with magnesium and conc. HCl.

Element analysis :-

Found : C, 60.72; H, 4.21.

Calculated for C_{18}H_{16}O : C, 60.0; H, 4.44 %.

The molecular formula shows the presence of 11 sites of unsaturation plus rings in the molecule.
Spectral data :-

UV spectrum:- UV shows $\lambda_{\text{max}}^{\text{MeOH}}$ at 268 and 336 nm (shoulder).
Addition of anhydrous AlCl$_3$ shows a bathochromic shift of 7 nm in the 268 nm band and addition of anhydrous NaOAc shifts this band to 273 nm. The UV spectrum shows that the molecule has got an isoflavone nucleus and contains one acidic 7-OH and a chelated 5-OH group.

IR spectrum (Fig.21) :- IR shows principal peaks at 3430, 1655, 1620, 1578, 1290, 1193, 1150, 1105, 1048, 975, 835, 800 and 697 cm$^{-1}$. The bands at 3430 and 1655 cm$^{-1}$ support the presence of $-\text{OH}$ and $\geq\text{C}=\text{O}$ in the molecule. The IR was superimposable on the IR of an authentic sample of irigenin.

Igk-4 diacetate V(b)

Element analysis :-

Found : C, 60.03; H, 4.64.
Calculated for $C_{22}H_{20}O_{10}$ : C, 59.46; H, 4.50 %.

IR spectrum (Fig.22) :-

IR shows principal bands at 1764, 1645, 1623, 1585, 1510, 1312, 1286, 1183, 1083, 1008, 976, 811 and 685 cm$^{-1}$. The bands at 1764 and 1645 cm$^{-1}$ are due to acetyl and carbonyl groups in the molecule, respectively.

Igk-4 triacetate V(c)

Element analysis :-

Found : C, 60.0; H, 4.32.
Calculated for $C_{24}H_{22}O_{11}$ : C, 59.25; H, 4.52 %.
IR spectrum (Fig. 23):-

IR shows principal peaks at 1775 (acetate carbonyls), 1650 (>C=O), 1620, 1500, 1426, 1300, 1190, 1080, 1050, 997, 900, 854 and 680 cm$^{-1}$

Igk-4 trimethyl ether V(d)

Element analysis:-

Found: C, 62.71; H, 5.32.

Calculated for C$_{21}$H$_{22}$O$_8$: C, 62.68; H, 5.54%.

Spectral data: -

UV spectrum: - UV shows $\lambda_{\text{max}}$ at 263 and 304 nm (inflection).

IR spectrum (Fig. 24): - IR shows principal peaks at 1640 (>C=O), 1600, 1580, 1407, 1284, 1120, 1025, 803 and 662 cm$^{-1}$

The chemical and spectral data suggest that Igk-4 has got structure V(a) with various functional groups arranged on an isoflavone nucleus.

\[ V(a) \quad R = R' = H \]
\[ (b) \quad R' = \text{CO.CH}_3, \quad R = H \]
\[ (c) \quad R = R' = \text{CO.CH}_3 \]
\[ (d) \quad R = R' = \text{CH}_3 \]

CONCLUSION

Chemical, spectral data and superimposable IR on irigenin indicate that Igk-4 has got structure V(a) - 5,7,3'-trihydroxy-6,4',5'-trimethoxyisoflavone. It is a well known natural isoflavone and has been obtained for the first time from the title species.
Fig. 21 - IR (nujol) spectrum of Igk-4.

Fig. 22 - IR (nujol) spectrum of Igk-4 diacetate.
Fig. 23 - IR (nujol) spectrum of Igk-4 trimethyl ether.

Fig. 24 - IR (nujol) spectrum of Igk-4 triacetate.
STRUCTURE ELUCIDATION OF
COMPOUNDS OF IRIS KUMAONENSIS WALL

The defatted plant material of *Iris kumaonensis* Wall was extracted with hot MeOH. The extract on concentration deposited a greenish-yellow amorphous powder. This powder when subjected to chromatography over silica gel, using CHCl₃ and CHCl₃-MeOH as eluents, provided cuts A, B and C.

Cut A was again subjected to chromatography over silica gel using a linear gradient of benzene against EtOAc as eluents. Various fractions of the column were suitably pooled to give Ik-1 as a TLC pure compound.

Cut C, which was found to be TLC pure, on concentration deposited silky needles of Ik-2.
Ik-1 behaved like Igk-4 in solubility, Lassaigne's test, phenolic hydroxyl and methoxyl group tests, element analysis and spectral data. It formed a diacetate, triacetate and a trimethyl ether. These derivatives were found to be identical with the corresponding derivatives of Igk-4 in all respects. Ik-1 is, therefore, identical with Igk-4.

CONCLUSION

The chemical and structural data and identical properties with Igk-4 suggest that Ik-1 is Igk-4 and has, therefore, got structure - 5,7,3'-trihydroxy-6,4',5'-trimethoxy-isoflavone (irigenin\textsuperscript{61}). This compound has been isolated for the first time from the title species.
Solubility :–

The compound behaved like Igk-3 in solubility. It, therefore, falls in " \( H^+ \) - \( N_2 \) " weakly acidic to neutral, class of compounds according to Shriner et al.52

Tests for elements and functional groups (chemical methods) :–

1. Detection of elements :- It showed the absence of \( N \), \( S \) and halogens in the Lassaigne's test.

2. Groups found absent :- The following groups, tested in a way similar to Igk-3, were found absent:
   (i) Free \(-\text{COOH} \) group.
   (ii) Methylenedioxy group.

3. Groups found present :- The following groups, tested in a way similar to Igk-3 and Igk-2, were found present:
   (i) Phenolic hydroxyl groups.
   (ii) Methoxyl groups.

4. Hydrolysis :- Refluxed 40 mg. of Igk-2 with aq. \( 2\% \ H_2\text{SO}_4 \) for 6 hr. Extracted this solution with \( \text{CHCl}_3 \). The aq. acidic solution was neutralized and tested for Molisch's test. A violet ring appeared at the common surface of the liquids. This shows that the solution and, therefore, Igk-2 contains a sugar group or groups.

   Took another portion of the aq. acidic solution and neutralized it with aq. \( \text{NaOH} \). Then added Fehling's solution to it and warmed – a red precipitate indicated that the sugar was reducing.

5. Magnesium and HCl test for flavonoids :- The compound gave a
yellow colour when heated with a piece of magnesium and conc. HCl.

**Element analysis:**

**Found:** C, 55.3; H, 5.6.

**Calculated for C\textsubscript{24}H\textsubscript{26}O\textsubscript{13}:** C, 55.1; H, 5.0 %.

The molecular formula shows that the compound contains 12 sites of unsaturation (double bonds plus rings).

**Spectral data:**

**UV spectrum:** UV shows λ\textsubscript{max}\textsuperscript{NaOH} at 268 and 332 nm (inflection). Addition of anhydrous AlCl\textsubscript{3} to the solution shifted the band at 268 nm to 277 nm. Addition of NaOAc did not show any shift in this band.

**IR spectrum (Fig.25):** IR shows principal bands at 3040 - 3570 cm\(^{-1}\) (-OH groups), 1657 cm\(^{-1}\) (>C=0), 1590, 1506, 1295, 1200, 1146, 1085, 1040, 987, 815 and 710 cm\(^{-1}\).

**Hydrolysis of Ik-2**

Acid hydrolysis of Ik-2 gave a sugar and an isoflavone which was found to be identical in all respects with Ik-1 and Igk-4. The sugar was chromatographed on a Whatman No.1 paper using glucose as the reference compound. The sugar and the glucose spots appeared at the same Rf. This shows that Ik-2 is a glucoside of Ik-1.

The chemical and spectral data, and acid hydrolysis of Ik-2 show that the compound has got structure VI with various groups arranged on an isoflavone nucleus.
CONCLUSIONS

The chemical and spectral data indicate that Ik-2 has got structure VI: 7-glucosyloxy-5,3'-dihydroxy-6,4',5'-trimethoxy-isoflavone (iridin). It is a well known natural isoflavone.
Fig. 25 - IR (mujol) spectrum of Ik-2
EXPERIMENTAL SECTION

Melting points were taken in open capillaries and are uncorrected. UV spectra were recorded on a Beckman DB spectrophotometer. Infra-red spectra were recorded on Hilger & Watts infrascan, Perkin-Elmer, Unicam SP 200 and Carl Zeiss Specord 71R spectrophotometers. The NMR spectra were recorded at 100 MHz on JEOL MH 100, Varian HA 100 and at 60 MHz on Varian A60D NMR spectrometers using tetramethylsilane as the internal standard. Mass spectra were obtained on LKB 9000 and Hitachi-Perkin-Elmer mass spectrometers using a direct inlet system and operated with an ionization energy of 70 eV. TLC was carried out on silica gel G layers (0.3 mm.). The plates were activated at 100 - 105°C for 35 min. and then stored in a desiccator. 10% aq. H$_2$SO$_4$ (containing 7 g. ceric sulphate per 100 ml.) spray, followed by heating at 130°C, was used for visualization of TLC spots. The analytical samples were dried in vacuo at 35°C over P$_2$O$_5$ for 24 hr. and then stored in a desiccator.

ISOLATION OF COMPOUNDS OF
IRIS GERMANICA (I. KASHMIRIANA BAKER)

Rhizomes of I. germanica (I. kashmiriana Baker), harvested in late August, were air dried and finely pulverized. The powder was defatted with light petrol (60 - 80°C) and then exhaustively extracted (60 hr.) with MeOH in a soxhlet extractor. Removal of solvent left a dark brown gummy mass which was re-extracted with hot chloroform (three 1L portions). After the removal of CHCl$_3$ soluble portion the gummy mass was extracted with hot ethylacetate (two 600 ml. portions).
**Isolation of Igk-1.**

The above combined chloroform extracts were filtered and concentrated to about 200 ml. in vacuo and the resulting solution was allowed to stand for 50 hr., a pale yellow crystalline dust (1.3 g.) got deposited at the bottom of the flask. The solid was filtered and dissolved by refluxing with excess of ether. The ether solution was diluted with a small amount of light petrol (40 - 60°) and this solution on concentration furnished colourless crystalline needles of Igk-1 (0.99 g.), m.p. 250 - 52°, Rf: 0.3 (EtOAc – light petrol, 1:1), C_{17}H_{12}O_6 (Found: C, 65.49; H, 3.96. C_{17}H_{12}O_6 requires: C, 65.38; H, 3.87 %). UV, IR, NMR and MS described in the text.

**Igk-1 acetate**: Igk-1 (200 mg.), acetic anhydride (1.5 ml.) and pyridine (1.5 ml.) were heated to reflux for 1 hr. The reaction mixture was cooled and poured into ice-cold water with thorough stirring. The mixture was extracted with chloroform and the extract dried over anhydrous sodium sulphate. Removal of chloroform left a solid which on crystallization from ethylacetate – light petrol gave colourless crystalline plates of Igk-1 acetate (180 mg.), m.p. 162 - 63°, Rf: 0.4 (EtOAc – light petrol, 1:1), C_{19}H_{14}O_7

(Found: C, 64.57; H, 4.1. C_{19}H_{14}O_7 requires: C, 64.40; H, 3.98 %).

**Igk-1 methyl ether**: Igk-1 (200 mg.), dimethyl sulphate (0.3 ml.) anhydrous potassium carbonate (0.5g) and dry acetone (5 ml.) were refluxed on water bath for 6 hr. The reaction mixture was then filtered and the inorganic residue washed several times with hot acetone. The filtrate and the acetone washings were concentrated and diluted with water. A white crystalline precipitate appeared. The precipitate was filtered, washed with water and dried. The dried product on crystallization from ethyl acetate gave colourless shin-
ing plates of Igk-1 methyl ether (220 mg.), m.p. 154°, m.p. of anhydrous product 183 - 84°, Rf: 0.47 (EtOAc - light petrol, 1:1), C_{18}H_{14}O_6 requires: C, 66.25; H, 4.32 %).

**Isolation of Igk-2.**

The chloroform filtrate obtained after filtering Igk-1 was vacuum dried to give a brown gummy mass (25.8 g.). 5 g. of this mass were dissolved in a small quantity of methanol and mixed with silica gel (7 g.). The mixture was loaded on a column of silica gel (100 g/100 - 200 mesh; 42 cm. X 3.2 cm.). The column was eluted (solvent rise 14 cm.) as follows and various cuts monitored by TLC (for CHCl_3 fractions, CHCl_3 and CHCl_3 - EtOAc, 25:2; for other fractions, EtOAc - light petrol, 1:1):

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Solvent</th>
<th>Volume</th>
<th>Quantity</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CHCl_3</td>
<td>100ml x 14</td>
<td>0.43g</td>
<td>least polar fraction Igk-2 &amp; 3</td>
</tr>
<tr>
<td>B</td>
<td>CHCl_3</td>
<td>100ml x 12</td>
<td>0.35g</td>
<td>Igk-2 &amp; 3</td>
</tr>
<tr>
<td>C</td>
<td>CHCl_3</td>
<td>100ml x 4</td>
<td>0.12g</td>
<td>Igk-3 &amp; 4</td>
</tr>
<tr>
<td></td>
<td>CHCl_3 : EtOAc (9:1)</td>
<td>100ml x 10</td>
<td>0.35g</td>
<td>Igk-3, 4 &amp; 1</td>
</tr>
<tr>
<td>D</td>
<td>CHCl_3 : EtOAc (8:2)</td>
<td>100ml x 40</td>
<td>0.7g</td>
<td>Igk-4 &amp; 1</td>
</tr>
<tr>
<td>E</td>
<td>EtOAc</td>
<td>100ml x 20</td>
<td>1.93g</td>
<td>Igk-1 and a complex mixture of unidentified compounds.</td>
</tr>
</tbody>
</table>

Fraction B (0.25g) was mixed with 2g of silica gel and the mixture chromatographed on silica gel column (20g/100 - 200 mesh; 14 cm x 2cm, solvent rise 14 cm).

Fractions 1 - 50 CHCl_3 : EtOAc (96:4) 10ml x 50

Fractions 9, 10, 12, 13, 14, 15 and 16 were pooled and on
concentration gave a pale yellow silky solid which was crystallized from ethylacetate - light petrol to give pale yellow silky needles of Igk-2 (70mg), m.p. 188 - 188.5°, Rf: 0.4 (chloroform - EtOAc, 25:2), C_{17}H_{14}O (Found: C, 64.72; H, 4.51. C_{17}H_{14}O requires: C, 64.96; H, 4.45 %).

Igk-2 diacetate: Igk-2 (50mg), acetic anhydride (1ml) and pyridine (1ml) were refluxed for 1 hr. After cooling the mixture was poured into ice-cold water and shaken thoroughly. It was then extracted with benzene. Benzene extract was washed several times with water and vacuum dried. The dried product was crystallized from benzene - light petrol to give Igk-2 diacetate (35mg), m.p. 212 - 13°, Rf: 0.66 (EtOAc - light petrol, 1:1), C_{21}H_{18}O_{8} (Found: C, 63.52; H, 4.37. C_{21}H_{18}O_{8} requires: C, 63.31; H, 4.52 %).

Igk-2 dimethyl ether: Igk-2 (30mg), dimethyl sulphate (0.2ml), anhydrous potassium carbonate (0.3g) and dry acetone (5ml.) were refluxed for 18 hr. The reaction product was worked up in the same manner as described for Igk-1 methyl ether. The dried product was crystallized from CHCl₃ - light petrol to give colourless crystals of Igk-2 dimethyl ether (32mg), m.p. 180 - 81° (Lit. 181°), C_{19}H_{18}O (Found: C, 66.46; H, 5.42. C_{19}H_{18}O requires: C, 66.66; H, 5.23 %).

Isolation of Igk-3.

Fractions C and D (2.2g) were adsorbed over a small quantity of silica gel. This gel was charged over a silica gel column (80g/100 - 200 mesh, 32cm x 2.6cm) using light petrol - EtOAc (solvent rise 18cm) as eluents.

Fractions 1 - 13 light petrol : EtOAc (80:20) 50ml x 13
Fractions 14 - 23 light petrol : EtOAc (70:30) 100ml x 10
Fractions 24 - 30 light petrol : EtOAc (60:40) 100ml x 7

Fractions 15 - 21 (80mg) and 22 - 25 (610mg) were combined on the basis of TLC results (light petrol : EtOAc, 2:3).

Fractions 15 - 21 were rechromatographed over a column of silica gel (10g/100 - 200 mesh, 7cm x 2cm, solvent rise 21.5cm) using light petrol - EtOAc (1:1) as eluent. A total of 20 10ml fractions were collected. Fractions 4 - 13, which were found to be TLC pure, were pooled, dried and crystallized from EtOAc - light petrol to give pale yellow crystals of Igk-3 (50mg), m.p. 231 - 32°, Rf: 0.49 (EtOAc : light petrol, 1:1), C_{16}H_{10}O_6 (Found: C, 64.1; H, 3.41. Calculated for C_{16}H_{10}O_6: C, 64.42; H, 3.35 %).

Igk-3 diacetate :-- Igk-3 (50mg), acetic anhydride (1ml) and pyridine (1ml) were refluxed for 1 hr. After cooling the solvent was removed in vacuo and the product crystallized from benzene - light petrol to give Igk-3 diacetate (56mg) as colourless silky crystals, m.p. 206 - 8°, Rf: 0.54 (EtOAc : light petrol, 1:1), C_{20}H_{14}O_8 (Found: C, 62.3; H, 3.29. C_{20}H_{14}O_8 requires: C, 62.82; H, 3.66 %).

Igk-3 dimethyl ether :-- Igk-3 (50mg), Me_2SO_4 (0.2ml), anhydrous K_2CO_3 (0.5g) and dry acetone (7ml) were refluxed for 18 hr. After usual processing the product was crystallized from benzene - light petrol to give colourless shining crystals of Igk-3 dimethyl ether (53mg), m.p. 184°, Rf: 0.47 (EtOAc : light petrol, 1:1), C_{18}H_{14}O_6 (Found: C, 66.53; H, 4.09. C_{18}H_{14}O_6 requires: C, 66.25; H, 4.32 %).

Partial synthesis of Igk-3 :-- Igk-1 (100mg), anhydrous AlCl_3 (1.0g), and dry ether (10ml) were refluxed for 3 hr. Removal of ether gave a yellow solid which was refluxed with a mixture of conc. HCl (2.5ml)
and G. acetic acid (2.5ml) for 15 min. The reaction mixture was
diluted with water and extracted with ether. Ether extract showed
two spots on TLC (EtOAc : light petrol, 1:1). It was subjected to
preparative layer chromatography on silica gel G (25g) using
EtOAc - light petrol (1:1). The two bands which appeared at Rf:
0.49 and Rf: 0.3 gave Igk-3 and Igk-1 respectively. The former
was crystallized from EtOAc - light petrol as prisms (32mg), m.p.
231 - 32°, mixed m.p. (with natural Igk-3) 231 - 32°.

Isolation of Igk-4.

Combined fractions 22 - 25 (see isolation of Igk-3) were
concentrated and allowed to stand. After a few days greenish-
yellow crystalline solid got deposited at the bottom of the flask.
It was filtered and recrystallised from CHCl₃ - light petrol to
give greenish-yellow crystals of Igk-4 (490mg), m.p. 185°, Rf: 0.45
(EtOAc : light petrol, 1:1), C₁₈H₁₆O₂ (Found: C, 60.42; H, 4.21.
C₁₈H₁₆O₂ requires: C, 60.0; H, 4.44 %).

Igk-4 triacetate :- Igk-4 (100mg), acetic anhydride (3ml) and
conc. H₂SO₄ (0.1ml) were heated on a water bath for 10 min. The
reaction mixture was cooled and poured into ice-cold water. After
usual processing the product was crystallized from EtOAc - light
petrol to give a colourless crystalline solid (85mg), m.p. 127 -
28°, Rf: 0.45 (EtOAc : light petrol, 1:1), C₂₄H₂₂O₁₁ (Found: C,
60.0; H, 4.32. C₂₄H₂₂O₁₁ requires: C, 59.25; H, 4.52 %).

Igk-4 diacetate :- Igk-4 (100mg), acetic anhydride (2ml) and
pyridine (2ml) were kept over night. After usual processing the
product was crystallized from ethyl acetate - light petrol to give
crystalline dirty white crystals of Igk-4 diacetate (81mg), m.p.
Isolation of Igk-4 trimethyl ether:

To Igk-4 (100mg) in dry acetone, anhydrous K$_2$CO$_3$ (2g) and dimethyl sulphate (0.3ml) were added. The mixture was refluxed for 18 hr. After usual processing the product was crystallized from EtOAc - light petrol to give colourless shining crystalline rosettes of Igk-4 trimethyl ether (98mg), m.p. 163°, Rf: 0.24 (EtOAc - light petrol, 1:1), $C_{21}H_{22}O_8$ (Found: C, 62.71; H, 5.32. $C_{21}H_{22}O_8$ requires C, 62.68; H, 5.54 %).

Isolation of Igk-5.

The combined ethyl acetate extracts (see extraction procedure) were vacuum dried to a brown powder. 1g of this brown powder was dissolved in a small quantity of methanol and the methanol solution adsorbed over silica gel (2.5g). This gel was charged over a column of silica gel (100g/100 - 200 mesh, 37cm x 3cm) in CHCl$_3$ and the column eluted (solvent height 23cm) as follows:

<table>
<thead>
<tr>
<th>Fractions</th>
<th>1 - 12</th>
<th>CHCl$_3$</th>
<th>50ml x 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractions</td>
<td>13 - 27</td>
<td>CHCl$_3$ : acetone (94:6)</td>
<td>50ml x 15</td>
</tr>
<tr>
<td>Fractions</td>
<td>28 - 41</td>
<td>CHCl$_3$ : acetone (90:10)</td>
<td>50ml x 14</td>
</tr>
<tr>
<td>Fractions</td>
<td>42 - 61</td>
<td>CHCl$_3$ : acetone (88:12)</td>
<td>50ml x 20</td>
</tr>
<tr>
<td>Fractions</td>
<td>62 - 70</td>
<td>CHCl$_3$ : acetone (85:15)</td>
<td>50ml x 7</td>
</tr>
</tbody>
</table>

Fractions 52 - 67, which were found to be TLC pure (ether : light petrol, 3:0.5), were pooled and concentrated to about 50ml. The solution was allowed to evaporate slowly. After two days light brown crystalline needles of Igk-5 (160mg) got deposited in the flask, m.p. 208 - 210°, Rf: 0.75 (EtOAc : light petrol, 2:1).
Igk-5 tetra-acetate:--- Igk-5 (50mg), acetic anhydride (2ml) and pyridine (1.5ml) were refluxed for 2 hr. After cooling the product was poured in ice-cold water and extracted with benzene. The benzene extract was washed several times with water, vacuum dried and dissolved again in benzene (50ml). This benzene extract was filtered through 3g of dry silica gel (100 - 200 mesh) and the filtrate concentrated to dryness. The dry mass crystallized from a mixture of benzene - light petrol into colourless crystalline needle bearing rosettes of Igk-5 tetra-acetate (39mg), m.p. 112°, Rf: 0.64 (EtOAc : light petrol, 1:1), C\textsubscript{21}H\textsubscript{18}O\textsubscript{9} (Found: C, 60.73; H, 4.46. C\textsubscript{21}H\textsubscript{18}O\textsubscript{9} requires: C, 60.86; H, 4.34 %).

Igk-5 tetramethyl ether:--- Igk-5 (50mg), anhydrous K\textsubscript{2}CO\textsubscript{3} (1g) and dimethyl sulphate (0.5ml) were refluxed with 15ml of dry acetone for 25 hr. After usual processing the compound was crystallized from CHCl\textsubscript{3} - light petrol to give colourless crystals of Igk-5 tetramethyl ether (50mg), m.p. 146.5°(Lit. 146°), Rf: 0.58 (EtOAc : light petrol, 1:1), C\textsubscript{17}H\textsubscript{18}O\textsubscript{5} (Found: C, 67.43; H, 6.0. C\textsubscript{17}H\textsubscript{18}O\textsubscript{5} requires : C, 67.55; H, 5.96 %).

Synthesis of Igk-5 tetramethyl ether:--- 2,4,6-trimethoxybenzene (0.4g), anisic acid (0.36g), freshly fused ZnCl\textsubscript{2} (0.5g) and POCl\textsubscript{3} (7.5ml) were heated at 85° for 3 hr. The mixture was cooled and poured on crushed ice. After stirring thoroughly a purple coloured solution appeared. This solution was extracted with benzene. Benzene extract was concentrated and adsorbed over a small quantity of silica gel. This gel was charged over a column of silica gel (30g/100 - 200 mesh, 22cm x 2cm). Column was eluted with CHCl\textsubscript{3} (solvent height 8cm).
A total of 25 fractions of 25ml each were taken. Fractions 4 - 20, which were found to be TLC pure (EtOAc : light petrol, 3:2), were concentrated and allowed to crystallize. A very light pink crystalline solid appeared. This solid was recrystallized from CHCl₃ - light petrol to give colourless crystalline rosettes of Igk-5 tetramethyl ether (490mg), m.p. 146°, m.m.p. 146°, Rf: 0.58 (EtOAc : light petrol, 1:1), C₁₇H₁₈O₅ (Found: C, 67.51; H, 6.1. C₁₇H₁₈O₅ requires: C, 67.55; H, 5.96 %).

ISOLATION OF COMPOUNDS
OF IRIS KUMAONENSIS WALL

The plant material (whole plant) of I. kumaonensis Wall was collected in late August, air dried under shade and finely powdered. The powder (1kg) was defatted exhaustively with light petrol (60 - 80°) for 20 hr. and then dried free of petrol. It was then soxhleted with methanol for 56 hr. The methanol extract was filtered, concentrated to about 700ml and its volume was then raised to 1L. by the addition of 85% aq. EtOH. After three days a bulky greenish-yellow amorphous powder (55g) got deposited at the bottom of the flask. It was filtered, washed with alcohol and dried. TLC (CHCl₃ : MeOH, 2:0.5) of the powder revealed that it was a mixture of compounds.

1.5g of the above powder was dissolved in a little quantity of MeOH and the solution adsorbed over a small amount of silica gel. This gel was charged over a column of silica gel (100g/100 - 200 mesh, 36cm x 3cm) in chloroform. Elution (solvent height 14cm) was started as follows:
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Range</th>
<th>Solvent</th>
<th>Volume</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1-30</td>
<td>CHCl₃</td>
<td>30 x 100ml</td>
<td>80mg</td>
</tr>
<tr>
<td>B</td>
<td>31-60</td>
<td>CHCl₃:MeOH (95:5)</td>
<td>30 x 100ml</td>
<td>150mg</td>
</tr>
<tr>
<td>C</td>
<td>61-105</td>
<td>CHCl₃:MeOH (90:10)</td>
<td>45 x 100ml</td>
<td>1.05g</td>
</tr>
</tbody>
</table>

**Isolation of Ik-1.**

Fraction A was concentrated and adsorbed over a small quantity of silica gel. This gel was charged over a silica gel column (30g/100 - 200 mesh, 20cm x 2cm) in benzene and eluted (solvent height 10cm) as follows:

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Range</th>
<th>Solvent</th>
<th>Volume</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>1-6</td>
<td>EtOAc:benzene (25:75)</td>
<td>25ml x 6</td>
<td></td>
</tr>
<tr>
<td>7-16</td>
<td>7-16</td>
<td>EtOAc:benzene (30:70)</td>
<td>25ml x 10</td>
<td></td>
</tr>
<tr>
<td>17-30</td>
<td>17-30</td>
<td>EtOAc:benzene (40:60)</td>
<td>25ml x 14</td>
<td></td>
</tr>
<tr>
<td>31-45</td>
<td>31-45</td>
<td>EtOAc:benzene (50:50)</td>
<td>50ml x 15</td>
<td></td>
</tr>
</tbody>
</table>

Fractions 26 - 40, which were found to be TLC pure (EtOAc: benzene, 1:1), were pooled and concentrated to dryness. Crystallization from CHCl₃ - light petrol gave greenish yellow silky crystals of Ik-1 (35mg), m.p. 185°, Rf: 0.45 (EtOAc : light petrol, 1:1), C₁₈H₁₆O₈ (Found: C, 60.22; H, 4.5. C₁₈H₁₆O₈ requires: C, 60.0; H, 4.44 %).

Ik-1 diacetate, Ik-1 triacetate, and Ik-1 trimethyl ether were prepared in a way similar to the corresponding derivatives of Igk-4.

**Isolation of Ik-2.**

Fraction C (1.05g), which was found to be TLC pure (CHCl₃: MeOH, 2:0.5), was concentrated and vacuum dried to give a yellowish-palegreen powder. It was recrystallized from MeOH to give silky needles of Ik-2 (0.92g), m.p. 207 - 208°, Rf: 0.38 (CHCl₃: MeOH,
Chemical formula of Ik-2 is $\text{C}_{24}\text{H}_{26}\text{O}_{13}$ (Found: C, 55.3; H, 5.6. requires: C, 55.1; H, 5.0 %).

**Acid hydrolysis of Ik-2 to Ik-1 and glucose:**

Ik-2 (300mg) in 50ml of 2% acidic (HCl) alcohol was refluxed for 6 hr. (The reaction was monitored on TLC). The solution was cooled, diluted with water and vacuum distilled to remove alcohol. A greenish-yellow precipitate appeared in the aqueous solution. The precipitate was extracted with CHCl$_3$ and the remaining aqueous solution neutralized with sodium bicarbonate.

The above aq. solution, which gave positive Molisch's and Fehling solution tests was chromatographed on Whatman No.1 filter paper using glucose as the reference compound. The chromatogram was sprayed with a solution of aniline phthalate in butanol saturated with water and then heated at 130° for 10 min. The sugar and glucose spots (brown spots) appeared at the same Rf: 0.21 (n-butanol : AcOH : H$_2$O, 4:1:5).

The above CHCl$_3$ extract was washed with water, dried and concentrated. A pale greenish-yellow crystalline solid, m.p. 185°, Rf: 0.45 (EtOAc : light petrol, 1:1) got crystallized. The compound was identical in all respects with Ik-1 and Igk-4.
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