

PHYTOCHEMICAL INVESTIGATION OF SOME INDIGENOUS ANTI-ASTHMATIC PLANTS

By

Prachi Saxena

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PHYTOCHEMICAL INVESTIGATION OF SOME INDIGENOUS ANTI-ASTHMATIC PLANTS

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Prachi Saxena

(This work was submitted by Prachi Saxena as a Ph.D. Thesis for the partial fulfillment of Ph.D. degree in Department Of Chemistry, Dr. H.S. Gour Vishwavidyalaya, Formerly University of Saugor, M.P., INDIA in the year 2005)

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PHYTOCHEMICAL INVESTIGATION OF SOME INDIGENOUS ANTI-ASTHMATIC PLANTS

A THESIS

submitted for the degree of

DOCTOR OF PHILOSOPHY

Submitted By

Prachi Saxena

(M.Sc.)



DEPARTMENT OF CHEMISTRY

*Dr. H.S. Gour Vishwavidyalaya
(Formerly University of Saugor)*

Sagar (M.P.) INDIA

2005

Declaration

I declare that the thesis entitled “PHYTOCHEMICAL INVESTIGATION OF SOME INDIGENOUS ANTI-ASTHMATIC PLANTS” is my own work, conducted under the supervision of PROF. V.K. SAXENA, Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.), approved by research Degree committee. I have put in more than 200 days of attendance with supervisor at the centre.

I further declare that to the best of my knowledge. The Thesis does not contain any part of any work, which has been submitted for the award of any degree either in this University or in any other University/Deemed University without proper citation.

Signature of the Supervisor

(PROF. V.K. SAXENA)

Signature of Candidate

(PRACHI SAXENA)

(PROF. S.C. GARG)

*Head
Department of Chemistry
Dr. H.S. Gour University, Sagar*

Acknowledgement

"No man is an island". This saying is true in present context. As without the blessings, help and assistance of many people this thesis would not have been initiated and accomplished. Standing on the last step of my journey, looking back I recognized my omniscient, omnipotent majesty who has hold me through all my sufferings and trials and escort me to successfully completion of my thesis work,

Lord is my Shepherd; I shall want nothing.

He makes me lie down in green pastures, and leads me beside the waters of peace;

He renews life within me, and for His name's sake guides me in the right path.

Even though I walk through a valley dark as death,

I fear no evil, for thou art with me,

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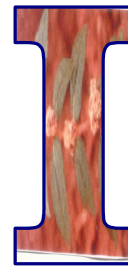
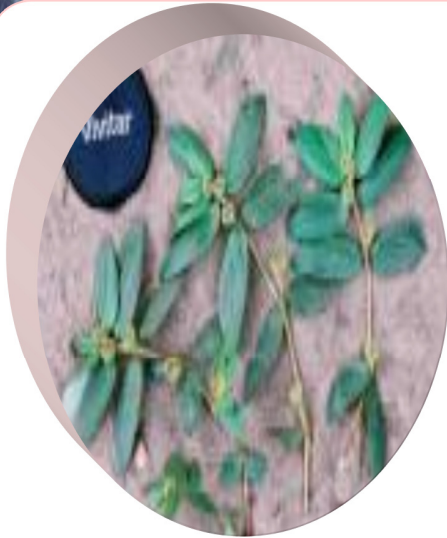
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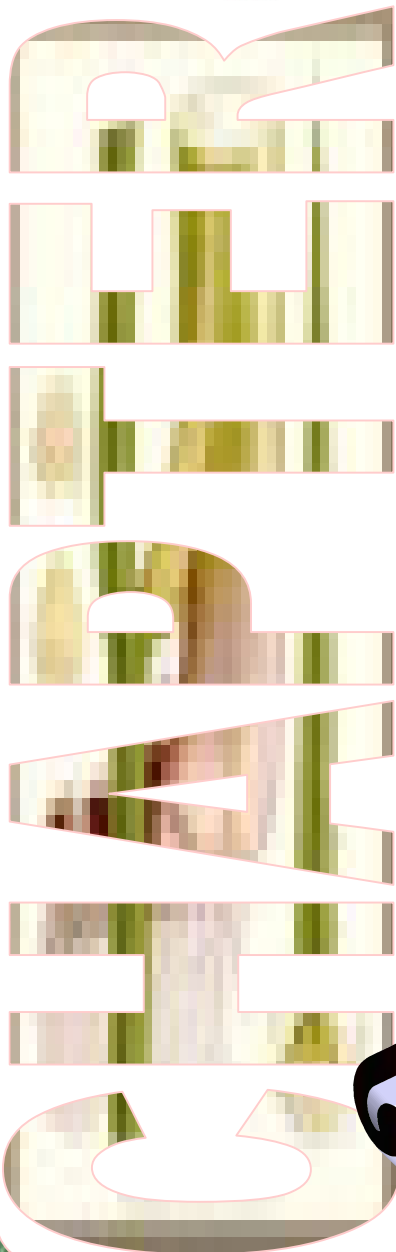
Prachi Saxena

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Our character is basically a composite of our habits. Because they are consistent, often unconscious patterns, they constantly, daily, express our



Introduction

Asthma affects more than 5% of the global population including children. It is chronic respiratory disease. It is a frightening condition and some times worrisome and inconvenient. It is predicted to be pre-eminent occupational lung disease, which is allergic in origin. It is also known as **bronchial asthma**.¹⁻³

Asthma known as reactive airway disease, is defined as a chronic lung condition, which associated with inflammation (swelling) of the airways and chest tightness.⁴

Asthma is chronic inflammation disorder of airways, which affects millions of people worldwide, and it is characterised by⁵

- 1) Inflammation of the lining of the airways, which lead to swelling, and narrowing of airway lining.
- 2) Increasedin snsitivity of the airways, which make the asthma worse.
- 3) Airway obstruction occurs with asthma, which is caused by tightening of muscles that surrounds the airways.
- 4) Airway obstruction is also called bronchospasm, which further causes the narrowing of the inflamed airways.

The causes of asthma is unknown, however its symptoms can develop at any stage and strikes the individual without any discrimination.⁶⁻⁸The symptoms are more severe and become worse if it is not diagnosed and treated. The first step in diagnosing asthma is a good evaluation even when the symptoms are mild.

A variety of inflammatory mediators and “triggers” may initiate and activate several target cell, in the airway, resulting in bronchoconstriction, micro-vascular leakage, oedema, mucus hypersecretion and stimulation of pleural reflexes thus worsen an asthma attack.

Asthma is known as a “Complex” heritable disease. There are a number of genes that contribute towards a person's susceptibility to a disease but in case of asthma, chromosomes 5, 6, 11, 12 and 14 have all been implicated.^{9, 10}

Asthma is associated with lung disorder in which spasms and inflammation of the bronchial passages restrict the flow of air in and out of the lungs. It causes bronchospasm, swelling of the mucus membranes and increased bronchial mucus secretion leading to respiratory disease.¹¹⁻¹⁶

The lungs form integrated mechanisms by means of which the constancy of the internal environment is maintained.

The lungs are considered as dual organ whose function is:-

- (1) To supply the tissues with oxygen and to remove carbon-dioxide.
(Respiration)
- (2) To maintain the acid-base balance.¹⁷

The Fig. A showed the **structure of lungs**.

The bronchial asthma¹⁸⁻²⁰ is a clinical syndrome characterized by increase in responsiveness of the tracheo-bronchial tree to a variety of stimuli.

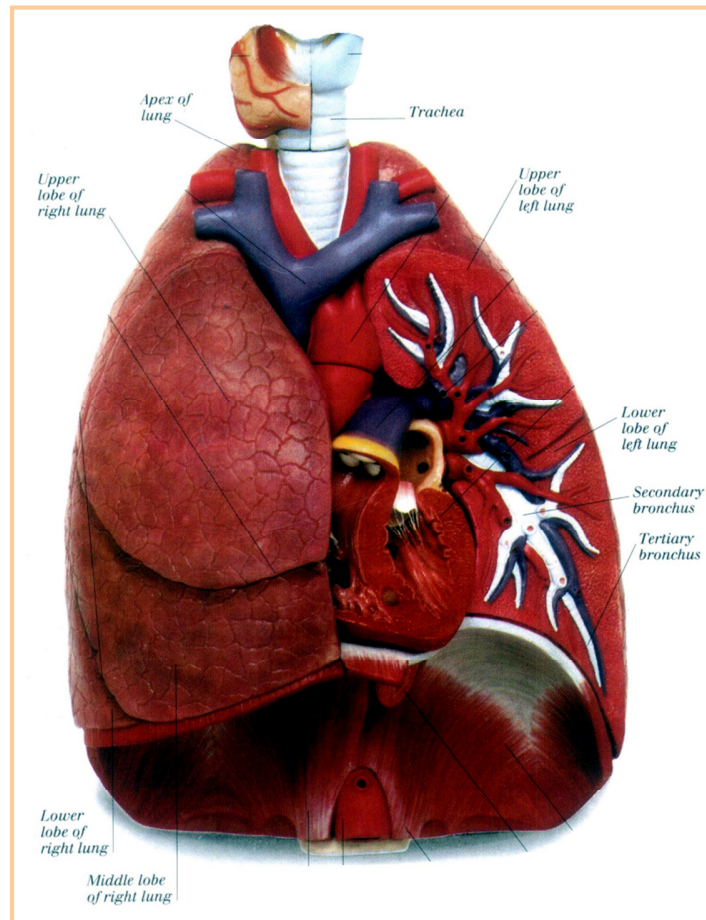


Fig. A: Structure of lungs

The asthmatic suffers from recurring attack of paroxysmal dyspnoea, his lungs become voluminous and emphysematous, filling the entire pleural cavities.

The air-conducting tubes in the lungs carry the air from the upper airway and trachea before reaching the air sacs (alveoli) that bring the air in intimate close contact with tiny blood vessels, where exchange of oxygen and carbon-dioxide can occur between air and blood. The circulation then transfers oxygen rich blood to the tissues where it diffuses by simple concentration gradients into the tissues. In the mean time, waste carbon

dioxide diffuses back into the blood in these tiny blood vessels (capillaries) to be returned for removal by the lungs. Because of irritated membranes in these little air tubes (bronchi) and bronchioles, reflexes make smooth muscles surrounding them contract, thereby narrowing the airways. Other reflexes stimulate normal glands in the bronchi to secrete extra mucus thus narrowing the lumen of the bronchioles so asthma is known as **crippling disease**. In a typical attack, the breathing is slowed and prolonged. All the chest muscles are called into action in an attempt to force the air from the lungs. The patient becomes more uncomfortable as the attack proceeds and his anxiety increases. He coughs frequently and eventually, the constricted bronchial tubes relax. He coughs up the secretions, and its attack is over.

The Fig. B shows the normal bronchial tube and constricted bronchial tube due to asthmatic attack.

A MECHANISM²⁷ IN ASTHMATIC ATTACK

The term allergy²¹ was first defined in the year 1906 by 'Von Pirquel'.

When an individual develops an unusual reply to substances, then 'an individual is said to allergic and the allergens are antigenic substances which are capable of sensitizing the body in hypersensitive individuals.

When a body first comes in contact with allergen (antigen), then this is known as primary exposure, at this time no symptoms of allergy can be diagnosed because no antibody has been formed.

Like way asthma is a type of allergy, which begins when an allergen called antigens for e.g. animal dander's, dust-mites, drugs, pollens etc are inhaled.

The inhaled allergen (antigens) binds with an antibody called **reagin**, which is an allergen bind receptor. This antibody (reagin) is known as **IgE**. It is a type of gamma globulin. These IgE molecules bound to the mast cell in lungs via Fe receptors. IgE molecules dwell on the surface of a mast cell of blood or organ. When an allergen crosslinked with two molecules of IgE antibody, then basophiles and mast cell get activated and walls of the mast cell get rupture.^{22, 23}

During the antigen-antibody-reaction, a large number of inflammatory mediators get releases. Immunological stimulation of basophils and mast cell has resulted in synthesis of several proinflammatory mediators.

Inflammatory mediators released by mast cells such as histamine, leukotrienes and proinflammatory mediators cytokinins such as interleukin (IL-4) and (IL-13).^{24, 25}

The substances histamines and leukotrienes cause the smooth muscles cells of bronchi to contract, which make the lumen of bronchi narrow. This represents the early phase.

Further, these substances attract an accumulation of inflammatory cells, especially eosinophils and lymphocytes that results in production of mucus. This recognized as the late phase.

Histamine²⁶ and leukotriene come from mast cell in an acute reaction and in chronic diseases; these mediators come along with IL-4 and IL-13 from basophils. Repeated attack of these cells damages the lining of bronchi.

So Fig. C shows the activity of affected Bronchial tube.

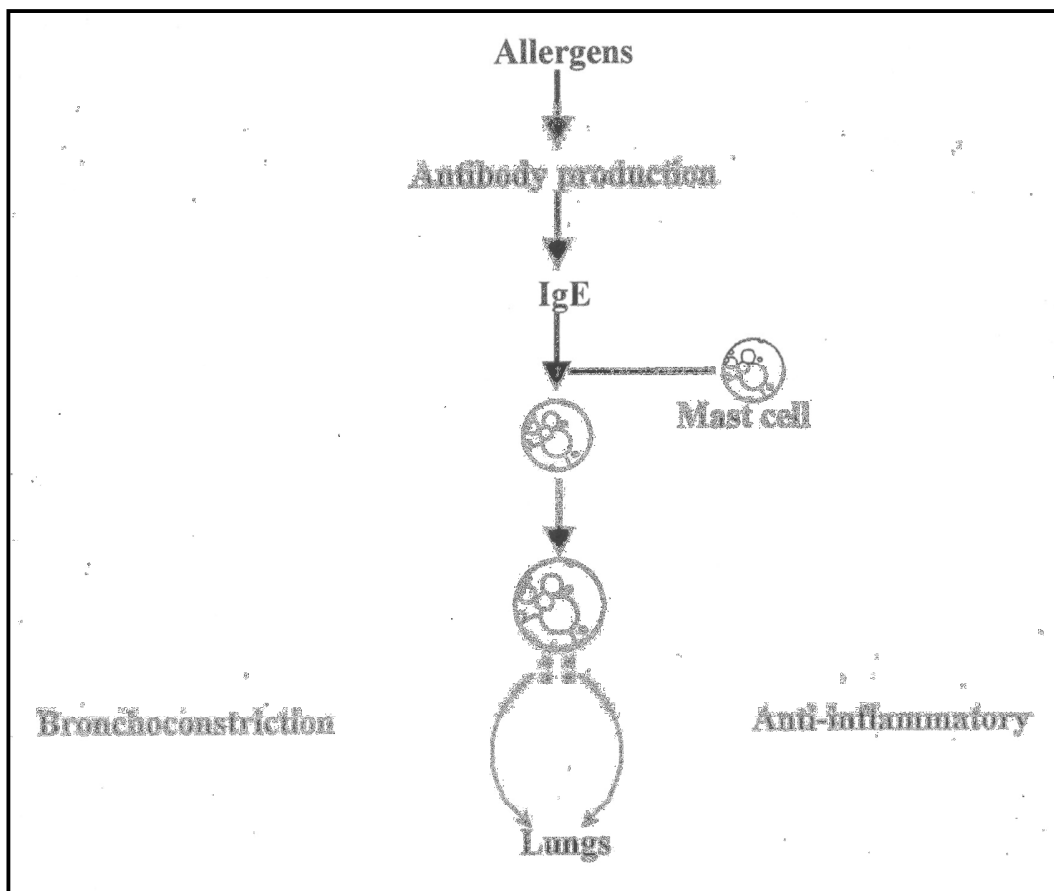


Fig. C: Activity of affected Bronchial tube

So while keeping in view, the major causes for the airway obstruction are: -

- (1) Bronchoconstriction due to histamine, leukotriene LTC₄ etc.
- (2) Mucosal odema due to prostaglandins, bradykinin, PAF (Platelet Activating Factor), leukotrienes; LTD₄, LTC₄ and LTE₄).

- (3) Mucus secretion formed generally by submucosal gland, inflammatory exudates consisting of eosinophils and neutrophils, other mediators like histamine and prostaglandins.
- (4) Migration of inflammatory cells like neutrophils and eosinophils to the site of inflammation.

A variety of “Triggers Factors” may initiate or worsen an asthmatic attack, including allergens, exercise and exposure to irritants such as smoke (tobacco) and psychological stimuli.

So, to meet the symptoms of asthma and to provide relaxant in bronchi, an ideal anti-asthmatic drug should possess bronchodilating, expectorant, anti-inflammatory, anti-allergic and anti-histaminic properties, and in addition to this properties drugs should also be able to perform some action like²⁸⁻³¹

- (1) It should dilate the bronchial muscles permanently and irreversibly.
- (2) To avoid the formation of mucus.
- (3) To eliminate the inflammation in lungs.
- (4) To avoid the further attack of allergen.
- (5) To reduce the early allergic responses by decreasing the level of circulating IgE.
- (6) To reduce the inflammatory response by decreasing IL-4 and IL-13 levels in the airways.

TYPES OF ASTHMA³²

There are various types of asthma: -

(1) Cardiac Asthma:

Cardiac asthma is a condition arising from a sudden failure of the left side of the heart, but this is not an allergic condition. Shortness of breath is a striking feature.

(2) Bronchial Asthma:

Bronchial asthma is the term we usually mean for asthma. This is a common condition. It is an allergic disorder due to an altered reaction within the lining of bronchial tubes or windpipes. It is a respiratory tract infection (viral or bacterial) cause inflammation of the mucus lining of the bronchial.

Etiology: The hypersensitivity is due to a variety of agents, particularly pollens, dusts, foods, drugs and bacterial and other causes also.

Lesions: The smaller and medium sized bronchi are occluded with thick, tough and tenacious mucus, which results in characterized hyalinized thickening.

Features: Bronchial asthmatics troubled with marked shortness of breath and a wheezing type of respiration. This peculiar type of breathing is brought on by spasm in the smaller bronchial tubes, as well as swelling in the mucous membranes.

(3) Acute Asthma:

Acute inflammation of the bronchi affects either the large bronchi and trachea or the small bronchioles. . Bronchioles affection associated with pneumonia and tracheal affection which occurs in a pure form known as tracheobronchitis. Generally, acute bronchitis passed from person to person.

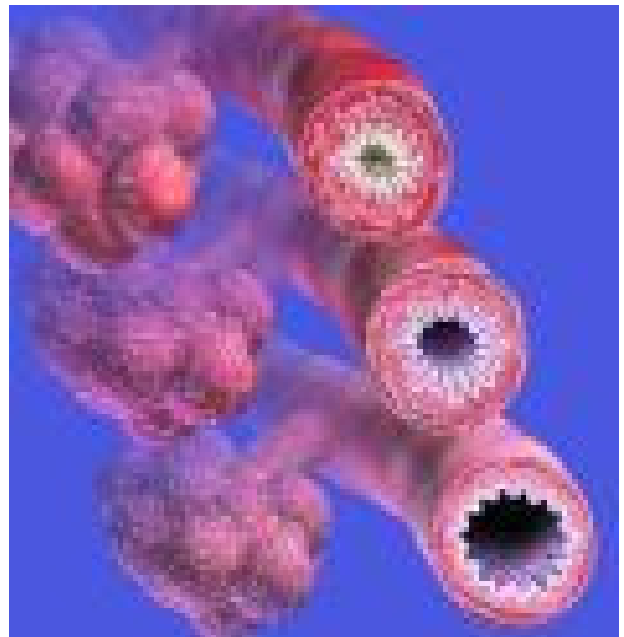
Etiology: The acute bronchitis is affected by bacteria, dust, poisonous gases and steam.

Lesions: The mucus membrane of the trachea and large bronchi is red, swollen and covered with tenacious exudates, which may be mucoid or purulent. The lumen of the bronchus is filled with pus.

Features: Acute asthmatics feel tightness of his lungs. He may feel that his chest will burst unless he can expel the air that is trapped within.

Symptoms:

- (1) Cough that produces mucus or pus
- (2) Burning sensation in the chest
- (3) Sore throat and fever
- (4) Fatigue
- (5) Blue tinted lips
- (6) Wheezing
- (7) Weight gain



(4) Chronic Asthma:

Chronic bronchitis is the result of repeated attack of acute bronchitis. The condition appears to begin with a slight dilatation of the bronchi (bronchiectasis), which leads to structural and functional disorder of the mucosa.

Etiology: The main causes of chronic bronchitis are cigarette smoking and prolonged exposure to air pollution other irritants such as dust and grain.

Lesions: The mucus membranes may be swollen and hypertrophic, and bathed with mucus or pus.

Features:

- Chronic cough that produces excessive amounts of mucus or pus.
- Wheezing, shortness of breath.

APPROACH OF THERAPIES

There are various physical as well as herbal therapies, which are put forward to control an asthmatic attack.

(1) Breathing exercises and yoga are extremely helpful for asthmatics, because they:-

- Strengthen respiratory muscles.
- Eliminate inefficient use of accessory muscles of respiration such as abdominal or neck muscles.
- Can reduce hyperventilation by increasing expiration.

- Can reduce the sensation of breathlessness by increasing the functioning of the diaphragm.
 - Can enhance clearing of airways.
- (2) Hydrotherapy is a physical Medicine technique in which steam inhalation with a few drops of eucalyptus oil in the water can be use.
- (3) For an acute attack, drink some hot water with the juice of clove of garlic.
- (4) Specific therapeutic foods for controlling asthma are:
- Juicy pungent foods such as garlic, onions, leeks, turnips, grapes, apricots Kernels, almonds, walnuts, carrots, pumpkin, figs, lychee, honey & sesame seeds which reduces mucus and enhance moisture.
 - Foods, which reduce inflammation as well as reducing mucus are cauliflower, garlic, onions, turnips, cherries, raw nuts, grains and green vegetables.
 - Foods high in flavonoids and carotenoids such as dark green leafy vegetables and deep yellow and orange vegetables.
- (4) Specific vitamins and mineral supplementation to be consider with asthma are:
- Vit B₁₂ 1000mg injected intramuscularly.
 - Vit B₆, vit E, vit C.
- (5) Lycopene, an antioxidant related to beta carotene and found in tomatoes, help to reduce the symptoms of asthma caused by exercising.

- (6) Magnesium levels are frequently low in asthmatics. Current evidence suggested that high dietary magnesium intake associated with better lung function and prevent spasms of the bronchial passages & reduces bronchial reactivity.
- (7) Vitamin C per day reduces the tendency of the bronchial passages to go into spasm.
- (8) The Eating principles for reducing the number of asthmatic attack are a diet, which is:
 - Moderately low fat
 - Low sugar
 - High complex carbohydrates, such as whole grains and starchy vegetables.
- (9) Foods to strictly avoid are the mucus forming ones, such as:
 - Cow milk and other dairy products,
 - White bread, sugar and sweets,
 - Tomatoes, Salty foods, cold foods,
 - Water-melon, bananas,
 - Shellfish, meat,
 - Ice-cream,
 - Refined foods and processed foods.

MEDICATION THERAPY³³

The following are the drugs generally prescribed for people suffering with asthma. Medications fall into two major groups:-

(1) Long-term control Medications (2) Quick-Relief Medications

- (1) Long-term control Medications are used daily to maintain control of asthma symptoms.
- (2) Quick-Relief Medications are used to treat asthma symptoms or an asthma episode.

The following anti-asthmatic drugs are elaborated in table-1.1.

INHALED THERAPY

The inhaler is the preferred routes for routine management of deliver medication directly to the airways, which is useful for lungs disease. It allows maximum bronchodilation to occur and fewer side effects.^{49, 50}

Aerosol therapy depends on the delivery of drugs and which further depends on the particle size of the drugs

- 1) Particles larger than 10um get s deposited in mouth.
- 2) Particles smaller than 0.5um are inhaled to alveoli and exhaled subsequently.
- 3) Particles with a diameter of 1 to 5 um allow deposition of drugs in the small airways.⁵¹

Aerosol devices^{52,53} for inhaled medication fall in two categories:-

- Metered-dose inhaler
- Dry powder inhaler
- Nebulizer (for infants and young children).

DRAWBACK OF SYNTHETIC DRUGS⁵⁴

- (1) These drugs may causes feeling of restlessness, apprehension and anxiety, which limit the use of β_2 -agents.
- (2) They result in various side effects, including skin, cataracts, decreased glucose tolerance, increased blood pressure & acne.
- (3) Higher doses lead to increase in heart rate, cardiac arrhythmias and CNS effects.
- (4) They decrease steady state clearance of Theophylline.
- (5) The adverse effect includes bronchospasm, cough or wheezing, joint swelling, pain, headache, rash and nausea.
- (6) They increase prothrombin times.
- (7) Rapid intravenous administration of therapeutic doses results in palpitation, hypotension, and pericardial pain.

There are various types of drugs like - Inhaled steroids, Leukotriene modifiers, Cromolyn sodium and Nedocromil, β_2 -adrenergic agonists, Theophylline etc., which control the asthma but none of them can cure the diseases. Therefore, the drugs with the established facts to contingent the treatment of asthma can be achieved by natural drug therapies.

PLANTS AS POTENTIAL ANTI-ASTHMATIC AGENTS

Phytochemistry is an impetus branch of science, which involves the role of medicine. The practices of traditional medicine are based on hundred of years of belief and observations which predate the development and spread the modern medical system. The traditional medicines remain an integral part of the formal health system and exist at an equal footing with modern medicine. The method of practicing of traditional medicine, some of them appear to be numerous and dissimilar but they all represent variations of three basic activities: faith healing, hygienic measures and drug therapy. Traditional medicine plays an important role in health care.

A deep exploration⁵⁵⁻⁵⁷ of the compounds regarding the anti asthmatic properties, isolated from the Indian plants has disclosed the fact that the some compounds isolated from the plants which possess an anti asthmatic properties are used to assassinate the disease. The potential importance of the plants is due to the presence of some specified chemical substance in the parts of the plants.

N-isobutyl 3, 4-methylenedioxy benezamide isolated from *Asarumsagittarioides* possess, antihistamine activity.⁵⁸ **Baicalein** is the flavone isolated from *Scutellaria baicalensis* has been reported to posses an antiallergic property. **Tylophorine** is an alkaloid isolated from *Tylophora asthmatica* play a decisive role in the anti-asthmatic activity.⁵⁹

Thiosulfinates dithietanes and α -sulfinyl-disulfides (cepaenes) has been isolated from *Allium cepa*. The main antiasthmatic activity is due to the thiosulfinates.⁷⁵

A combination of the two alkaloids: **vasicine** and **vasicinone** isolated from the plant “*Adhatoda vasica*” showed bronchodilatory activity.^{60,61}

Nobeletin, tangeretin and **3, 4, 6, 7, 8, 3, 4-heptamethoxy flavone** have been isolated from the plant *Citrus aurantium* were found to be good inhibitors.⁶²

Flavonoid, coumarin and sesquiterpene have been isolated from the plant *Magnolia salicifolia* and *centipeda minima* possess anti allergic properties.

Essential fatty acids⁶³ such as GLA in fish oil and flaxseed oil are often used to inhibit inflammatory responses (asthma).

Various species of *Annona* have been used as anti-inflammatory, as well as the roots of *Annona squamosa*⁶⁴ reported to possess antispasmodic, antihistaminic, and bronchodilator effect.

Dry fruits of Indian medicinal plant “*Piper longum*”⁶⁵ are often used for prophylaxis of asthma and in children; it significantly decreases the frequency and severity attack of bronchial asthma.

The Indian medicinal plant “*Abies pindow*”⁶⁶ is reported to possess anti-asthmatic and bronchodilating properties.

The bark of the deciduous tree “*Ailanthus*”⁶⁷ is used as an antispasmodic for treatment of asthma and bronchitis.

The leaves of **Tulsi** have very interesting medicinal values. The juice of its leaves have strong expectorant properties and are used in cough, bronchitis and other respiratory diseases.⁶⁸

India is rich in wealth of Indian medicinal plants. They have contributed their aspect in the development of ancient medicine in India, as *Tephrosia purpurea* has been used as traditional medicine for bronchial asthma. A saponin isolated from *Clenodendron serratum* and *Gardinia latifolia* has been reported to possess bronchodilator properties after performing activity on guinea pigs. *Ocimum sanctum* as well as *Terminalia belarica* also resolute for their antiasthmatic properties.⁶⁹

A natural product isolated from *Khellin*⁷⁰ is disodium cromoglycate which has been reported to possess alleviate colic pains.

Pepper cubebs also known as Tailed pepper⁷¹ is employed for the treatment of asthma as well as an expectorant and stimulant to the bronchial mucus membrane.

The leaves and roots of *Adhatoda vasica*, *Solanum xanthocarpum*, and bark of *Albizzia lebbeck*, roots of *Glycyrrhiza glabra*, and roots of *Picrorhiza kurroa* are reported to possess curative properties in the asthma treatment.⁷²

Lobelia inflata is a traditional herb⁷³, which has been reported to possess anti-asthmatic properties.

Numbers of natural anti-asthmatic drugs were isolated from many plant species in nature are given in table-1.2.

The prudential investigation has marked that the following two plants exhibit significant medicinal properties therefore it is obligatory to investigate these phytochemically.

The descriptions of both the plants are given below:-

(1) ***Acorus calamus* Linn.⁸⁸⁻⁹¹**

Acorus calamus (Linn.) belongs to natural order Araceae.

It is known as Bach in Hindi and also known by analogous name as Sweet flag, Calamus root, Calamus, Sweet edge, Sweet Calomel, Rat root, Pine root, Beewort, Muskrat root.

Acorus calamus grows in northern latitudes around the globe. It is hydrophillic and is usually found in wetlands and around ponds and in ditches and sweeps. This plant can be often found growing close to the sites of Indian villages, camping areas and trails.

This plant is a grass-like rhizome, forming iris-like perennial that can grow to 2 meters high. It has long creeping roots that spread just below the soil. The roots of this plant spread horizontally and can extend upto 2 meters long. The leaves of this plant are thick and erect. The flowers of this plant are 3-8 cm long, they are cylindrical in shape, and green-brownish in colour and covered in a multitude of rounded spike. The fruits of this plant are small and berry-like.

An alcoholic extract of this plant has sedative and analgesic effects and also moderate depression in blood pressure and respiration. Its rhizome is used in emetic, stomach, dysentery, colic, remittent fever and as a nerve tonic in bronchitis and snakebite. It has also been used to improve memory retention and recall.

The rhizome of this plant is a potent tranquilizer with neuroleptic and anxiety properties. It possesses anti-inflammatory, antiepileptic, antihypertensive, sedative, analgesic, antiulcer and antiherpes properties. **(Photograph-1)**

(2) *Euphorbia hirta* Linn.⁹²⁻⁹⁸

This plant belongs to natural order Euphorbiaceae. It is known as Dudhi in Hindi. It is known by analogous names as Pill-bearing spurge, Snakeweed, Cat's-hair, Queensland asthma-weed, Flowery-headed spurge.

Euphorbia hirta is an annual herb with a slender hairy stem and lanceolate opposite – toothed leaves, which is oblique oblong – ovate shape. The flowers are small and yellow in color and occur in dense cluster in leaf axil, head of flower are cymose, minute, numerous, crowded and collected on stalk. The fruit of this plant are an acute – angled, hairy pod, inclosing the reddish 4-angled seeds. This plant produces milky latex, which irritate the mucus membranes.

This plant has a relaxing action on the muscles of the lungs. It also has a prominent effect in asthma treatment and bronchitis. The whole plant is used for the treatment of diseases of children especially in worms, bowels complain and cough. Its juice is used in dysentery and colic and also in warm infection. The decoction of leaves is used in bronchial asthma, infections and chronic coughs. The latex of this plant is used as an application for warts. The ripe fruit of this plant is given to stop vomiting, where as its extracts are spasmolytic, antihistaminic and anti-inflammatory. The roots of this plant are an anti-emetic. **(Photograph-2)**

The compounds isolated from these following plants are elaborate in a given table 1.3 :-

PROBLEM TAKEN AND WORK DONE

Asthma affects more than 5% of the world population. It is a chronic inflammatory disorder of the airways, characterised by coughing, shortness of breath and chest tightness. The physiology symptoms of asthma are narrowing of airways caused by oedema.

Asthma prevalence and mortality have increased dramatically over the past decades and occupational asthma is predicted to be the pre-eminent occupational lung disease in the next decade.

From very old time throughout the world man has tried to alleviate human suffering from many diseases by making proper use of the plant drugs and of course the more useful ones have been unclouded in modern pharmacopoeia of the different countries, but some of the useful plant drugs are yet to be studied properly by research workers.

Numbers of herbal drugs have been used in the treatment of asthma and proven therapy in the treatment of this disease have been tried to give combined beneficial effects. The pharmacological actions of active principles isolated from various medicinal plants have been studied in our world.¹¹⁴

Presently various synthetic drugs and physical medicine have been discovered and are being used on a large scale but the significant of the natural drugs have not been relegated.¹¹⁵

A numbers of alternative therapies are useful in treatment of this diseases and which still in use as white horhound (*Marrabium valgare*) is a gentle stimulating expectorant, sundew (*Drosera rotundifolia*) help in cough up mucus and relax spasm.

Finding from animals and human studies confirmed that DTP or tetanus vaccines induce allergic responses.

Several anti-asthmatic compounds which were isolated from plant have possess the property to cure this disease as β -solarmine from *Solanum xanthocarpum*, Tylophorine from *Tylorphora indica*, Picorrhizin from roots of *Picorrhiza* genus has been reported to posses anti-asthmatic properties and picracin are potent inhibitor of mitogen-induced proliferation of T-lymphocytes, Friedelin from *Abies pindrow*, by Earlier workers.

Though many effective drugs to treat acute symptoms of asthma are available, these are not completely safe for long-term use, hence the search has been on traditional medicinal plant to treat asthma. Most of the allopathic medicines are reported to have side effects. Therefore there is aneed to search for effective and safe drugs from medicinal plants for asthma.

So in view of disclosing the secrets of the therapeutic importance of the two medicinal plants, author has accept defy to combat this disease.

Therefore, the phytochemical investigation of the extracts from the following plant (1) *Acorus calamus* Linn. (2) *Euphorbia hirta* (Linn.) were performed with view to isolate, purify and identify the constituents and the findings of the author are summarised below:-

- (I) Isolation and study of the flavonoidal glycoside PS-I: Galangin-3-O- β -D-glucopyranosyl-7-O- α -L-rhamnopyranoside from the roots of *Acorus calamus* Linn.

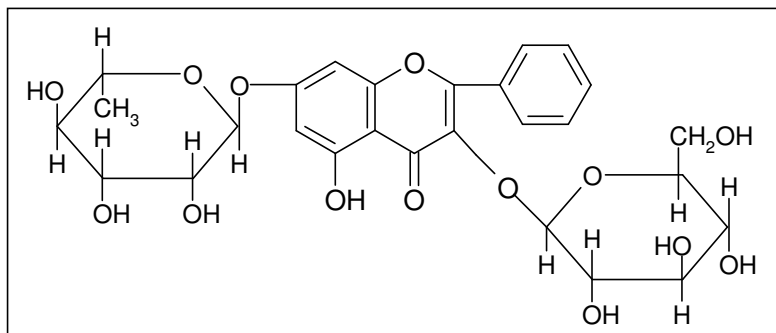
This chapter incorporates the isolation and study of flavonoidal glycoside: PS-I, (yield 0.051%), molecular formula $C_{27}H_{30}O_{14}$, m.p. 150-151°C, $[M^+]$ 578 (FAB-MS). It was obtained by column chromatography of chloroform soluble portion of concentrated ethanolic extract of roots of *Acorus calamus* Linn. Compound PS-I showed important maximum absorption in the UV spectrum at $\lambda_{\max}^{\text{MeOH}}$ nm : 267, 348, $\lambda_{\max}^{\text{AlCl}_3}$ nm: 335, 348, $\lambda_{\max}^{\text{AlCl}_3/\text{HCl}}$ nm: 336, 401, $\lambda_{\max}^{\text{NaOMe}}$ nm: 324, 394, $\lambda_{\max}^{\text{NaOAc}}$ nm: 267, 358, IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3438.4 (Intramolecular hydrogen bonding), 3022.4 (=C–H stretching vibration), 1638.0 (α , β -unsaturated >C=O group), 1560.0, 1525.0, 1460.2 (aromatic ring system), 1216.6 (C-O-C bending vibration), 1089.8 (C-O-C stretching vibration), 768.9, 670.0 (monosubstituted benzene ring) and ^1H NMR signals were recorded at 300 MHz, DMSO) : δ 6.45 (1 H, d, J = 2.4 Hz, H-6), δ 6.76 (1 H, d, J = 2.6 Hz, H-8), δ 7.46 (5 H, m, H-2', 3', 4', 5', 6'), δ 5.39 (1 H, d, J = 7.7 Hz, H-1'', anomeric proton for glucose), δ 3.45 (1H, dd, J = 7.7, 9.1 Hz, H-2''), δ 3.41 (1 H, t, J = 9.1 Hz, H-3''), δ 3.30 (1H, t, J = 9.5 Hz, H-4''), δ 3.22 (1 H, ddd, J = 2.4, 5.5, 9.5 Hz, H-5''), δ 3.90 (1 H, dd, J = 2.0, 11.8 Hz, H-6'' A), δ 3.52 (1 H, dd, J = 1.9, 3.5 Hz, H-6'' B), δ 5.58 (1 H, d, J = 1.9 Hz, H-1''', anomeric proton

of rhamnose), δ 4.02 (1 H, d, J = 1.9, 3.5 Hz, H-2'''), δ 3.81 (1 H, dd, J = 3.5, 9.5 Hz, H-3'''), δ 3.47 (1 H, t, J = 9.5 Hz, H-4'''), δ 3.58 (1 H, dq, J = 5.9, 9.5 Hz, H-5'''), δ 1.24 (3H, d, J = 5.9 Hz, H-6''' for CH₃) . Its ¹³CNMR signals were recorded at (300 MHz, CDCl₃): δ 146.3 (C-2), δ 135.6 (C-3), δ 175.6 (C-4 for C=O), δ 163.3 (C-5), δ 100.7 (C-6), δ 163.6 (C-7), δ 95.9 (C-8), δ 158.0 (C-9), δ 107.5 (C-10), δ 135.5 (C-1'), δ 129.4 (C-2'), δ 128.3 (C-3'), δ 131.7 (C-4'), δ 128.3 (C-5'), δ 129.4 (C-6'), δ 103.4 (C-1''), δ 75.5 (C-2''), δ 77.8 (C-3''), δ 71.5 (C-4''), δ 78.2 (C-5''), δ 62.5 (C-6''), δ 100.2 (C-1'''), δ 71.2 (C-2'''), δ 72.2 (C-3'''), δ 73.3 (C-4'''), δ 71.5 (C-5'''), δ 19.3 (C-6'''). Its FAB-MS showed fragment ions at m/z : 578, 416, 270, 269, 242, 241, 152, 124, 118 and 105.

On acid hydrolysis, PS-I gave aglycone PS-II (A), which on 50% KOH degradation gave phloroglucinol, m.f. C₆H₆O₃, m.p. 115-116°C and benzoic acid, m.f. C₇H₆O₃, m.p. 121-122°C. The sugars obtained by acid hydrolysis of PS-I were identified as D-glucose (R_f 0.19) and L-rhamnose (R_f 0.38) by Co PC and Co TLC.

Periodate oxidation, mild acid hydrolysis, and enzymatic hydrolysis showed that aglycone and sugar were in equimolar ratio and both sugars were in pyranose form. It also showed that D-glucose was liberated first followed by L-rhamnose thereby indicating β -linkage between aglycone and D-glucose and α -linkage between aglycone and L-rhamnose.

Thus the flavonoidal glycoside PS-I was identified as: Galangin-3-O- β -D-glucopyranosyl-7-O- α -L-rhamnopyranoside.



PS-I

(II) Isolation and study of a flavone-C-glycoside PS-II: Luteolin-8-C- β -D-glucopyranoside from the roots of *Acorus calamus* Linn.

This chapter incorporates with the study of the flavone-C-glycoside, (yield 0.0985%), molecular formula $C_{21}H_{20}O_{11}$, m.p. 264-265°C and $[M^+]$ 448 (FAB-MS), which was obtained by column chromatography from the ethyl acetate soluble fraction of concentrated ethanolic extract of the roots of *Acorus calamus* Linn. Its UV spectrum showed maximum absorption at λ_{max}^{MeOH} nm: 270, 334, $\lambda_{max}^{AlCl_3}$ nm: 292, 361, $\lambda_{max}^{AlCl_3/HCl}$ nm: 294, 358, λ_{max}^{NaOMe} nm: 286, 399, $\lambda_{max}^{CH_3COONa}$ nm: 310, 392, $\lambda_{max}^{CH_3COONa/H_3BO_3}$ nm: 282, 439, and IR absorption at ν_{max}^{KBr} 3423.0 (-OH group), 2931.0 (-C-H stretching), 1638.2 (α , β unsaturated $>C=O$ group), 1613.0, 1560.0, 1459.6 (Aromatic ring system), 1218.9 (C-O-C bending vibration), 1035.7 (C-O-C stretching vibration), 770.0, 670.0 (monosubstituted benzene ring). Its ^1H NMR signals were recorded at (300 MHz, DMSO); δ 6.23 (1 H, s, H-3), δ 6.26 (1 H, d, $J = 2.5$, H-6), δ 7.76 (1 H, d, $J=7.5$ Hz, H-2'), δ 6.92 (1 H, d, $J = 7.5$ Hz, H-5'), δ 7.56 (1 H, dd, $J = 7.6$ Hz, 1.8, H-6'), δ 5.60 (1 H, d, $J = 9.5$ Hz, H-1''), δ 2.38 (3H,s,OAc-5), δ 2.42 (3H,s,OAc-7), δ 2.27

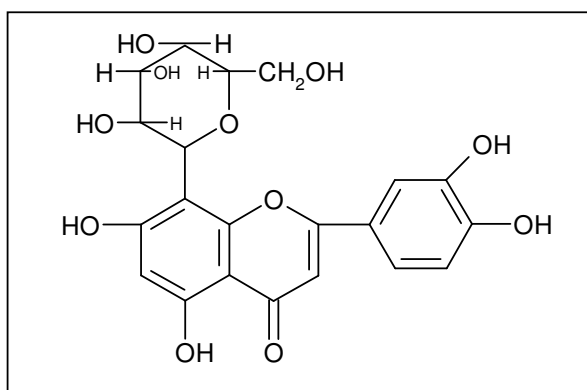
(3H,s,OAc-3'), δ 2.29 (3H,s,OAc-4'), δ 3.40-4.15 (8 H, m, glucose protons) and ^{13}C NMR (300 MHz, CDCl_3) were recorded at δ 163.3 (C-2), δ 102.0 (C-3), δ 181.0 (C-4), δ 159.2 (C-5), δ 97.3 (C-6), δ 163.5 (C-7), δ 103.3 (C-8), δ 159.6 (C-9), δ 102.3 (C-10), δ 121.2 (C-1'), δ 112.2 (C-2'), δ 145.5 (C-3'), δ 148.5 (C-4'), δ 115.0 (C-5'), δ 180.0 (C-6'), δ 74.6 (C-1''), δ 75.0 (C-2''), δ 79.0 (C-3''), δ 70.4 (C-4''), δ 81.3 (C-5''), δ 60.1 (C-6''). Its FAB-MS showed the fragmentation peak at m/z 448, 420, 315, 313, 299, 286, 258, 165, 154, 153, 134, 124 and 123.

On acid hydrolysis and enzymatic hydrolysis, PS-II gave no sugar.

On prolonged boiling with HI and phenol, PS-II gave aglycone. m.f. $\text{C}_{15}\text{H}_{10}\text{O}_6$, m.p. 323-324°C. $[\text{M}^+]$ 286 (FAB-MS)) and sugar moiety which was identified as D-glucose (R_f : 0.19) by Co PC and Co TLC.

The methylated compound on periodate oxidation of showed that aglycone and sugar were in equimolar ratio. The ^1H NMR signal at δ 5.60 (1 H, d, $J = 9.5$, H-1'') showed β -linkage between the aglycone and D-glucose.

Thus the flavone-C-glycoside PS-II was identified as: Luteolin-8-C- β -D-glucopyranoside.



PS-II

(III) Isolation and study of the steroidal saponin PS-III: 24, 25-dihydro-euphol-3-O- β -D-arabinopyranoside from the roots of the *Euphorbia hirta* Linn.

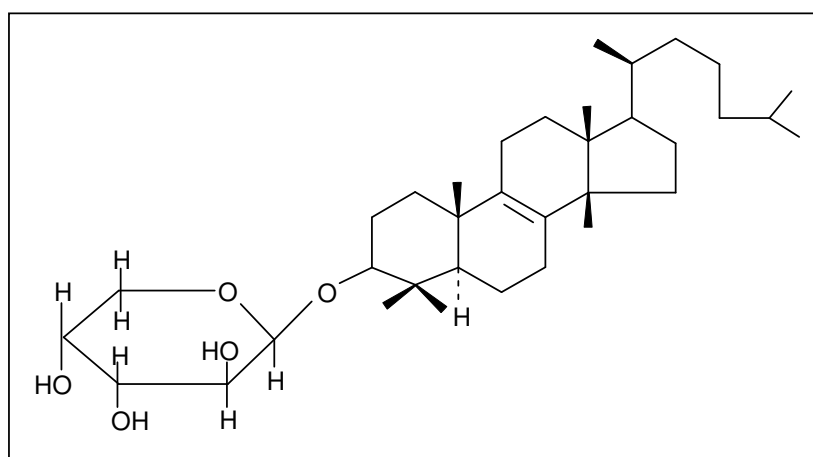
This chapter deals with the study of the steroidal saponin PS-III, (yield 0.062%) molecular formula, $C_{35}H_{60}O_5$, m.p. 262-263°C and $[M^+]$ 560 (FAB-MS), which was obtained by column chromatography from the methanol soluble fraction of concentrated ethanolic extract of the roots of *Euphorbia hirta* Linn. Its IR spectrum showed absorption bands at $\nu_{\max}^{KBr}$ 3402.5 (-OH group), 2928.0 (-CH₃ stretching), 2855.7 (stretching in cyclopentane nucleus), 1397.9, 1121.3, 1037.3 (steroidal nucleus), 1387.2, 1210.0 (angular methyl groups), 1635.4, 884.0 (>C=C<), and ¹HNMR (300 MHz, C₆D₆): δ 1.02 (2H, m, H-1), δ 1.40 (2H, m, H-2), δ 3.25 (1H, dd, J = 4.5, 11.5 Hz, H-3), δ 2.20 (1 H, dd, J = 10.0, 2.0 Hz, H-5), δ 1.90 (2 H, m, H-6), δ 1.23 (2 H, m, H-7), δ 1.30 (2 H, m, H-11), δ 1.18 (2 H, m, H-12), δ 1.35 (2 H, m, H-15), δ 1.38 (2 H, m, H-16), δ 2.50 (1 H, m, H-17), δ 0.67 (3H, s, H-18), δ 1.00 (3H, s, H-19), δ 1.42 (1 H, m, H-20), δ 0.91 (3H, d, J = 7 Hz, H-21), δ 1.53 (2H, m, H-22), δ 2.12 (2H, m, H-23), δ 2.15 (2H, m, H-24), δ 2.23 (1H, m, H-25), δ 0.95 (3H, d, J = 7 Hz, H-26), δ 1.05 (3 H, d, J = 7 Hz, H-27), δ 1.08 (3H, s, H-28), δ 0.89 (3H, s, H-29), δ 1.04 (3H, s, H-30), δ 5.53 (1H, d, J = 3 Hz, H-1' anomeric proton of arabinose), δ 4.65 (1H, dd, J = 10.0, 3.0 Hz, H-2'), δ 4.55 (1H, dd, J = 10.0, 3.0 Hz, H-3'), δ 4.45 (1H, brs, H-4'), δ 4.27 (1H, H-5' α , d, J = 12 Hz), δ 4.11 (1H, dd, J = 12.0, 2.0 Hz, H-5' β).

^{13}C NMR (300 MHz, CDCl_3): δ 36.6 (C-1), δ 30.0 (C-2), δ 77.2 (C-3), δ 39.2 (C-4), δ 50.8 (C-5), δ 22.5 (C-6), δ 26.4 (C-7), δ 139.8 (C-8), δ 139.7 (C-9), δ 35.5 (C-10), δ 16.6 (C-11), δ 26.8 (C-12), δ 42.7 (C-13), δ 49.1 (C-14), δ 30.3 (C-15), δ 30.2 (C-16), δ 48.8 (C-17), δ 15.0 (C-18), δ 18.0 (C-19), δ 35.7 (C-20), δ 18.4 (C-21), δ 35.8 (C-22), δ 23.6 (C-23), δ 37.9 (C-24), δ 27.5 (C-25), δ 22.2 (C-26), δ 22.0 (C-27), δ 23.3 (C-28), δ 27.3 (C-29), δ 15.2 (C-30), δ 99.2 (C-1'), δ 70.3 (C-2'), δ 70.7 (C-3'), δ 71.7 (C-4'), δ 64.4 (C-5') and FAB-MS (m/z) 560, 428, 410, 270, 241, 207 and 203.

On acid hydrolysis PS-III gave aglycone PS-III (A) and sugar moiety, which was identified as D-arabinose (R_f : 0.22) by Co PC and Co TLC.

Periodate oxidation and enzymatic hydrolysis showed that aglycone and sugar was in equimolar ratio and sugar in pyranose form. It also showed that D-arabinose was linked to aglycone via β -linkage.

Thus the steroidal saponin PS-III was identified as 24, 25-dihydro-euphol-3-O- β -D-arabinopyranoside.



PS-III

(IV) This chapter divided into two parts :-

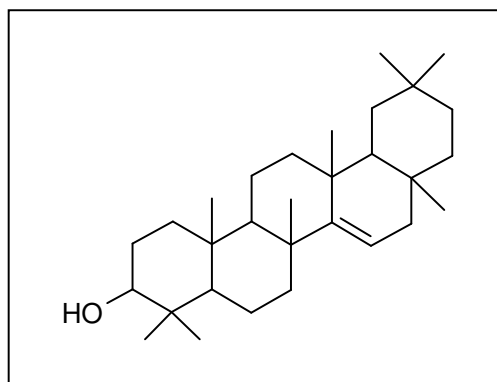
PART-A: Isolation and study of the triterpenoid PS-IV: Taraxerol from the stem of *Euphorbia hirta* Linn.

This chapter incorporates the isolation and study of the triterpene (PS-IV), (yield 0.052%) m.f. $C_{30}H_{50}O$, m.p. 280-282°C, $[M^+]$ 426 (FAB-MS) which was obtained from ethyl acetate soluble portion of the ethanolic extract. Compound PS-IV showed significant bands in the IR spectrum at $\nu_{\max}^{KBr}$ cm^{-1} : 3422.8 (-OH group), 3018.6 (=C-H str.), 2932.4 (C-H str. of CH_3), 2856.6 (-C-H str.), 1635.6, 813.0 ($>C=CH$ trisubstituted), 1461.6 (-C-H bending) 1400.0, 1319.5, 1032.0, (triterpenoidal nature) and ^1H NMR signals were recorded at (300 MHz, DMSO): δ 2.03 (1H, m, H-1a), δ 2.10 (1H, m, H-1b), δ 2.38 (1H, ddd, $J = 15.2, 6.2, 2.4$ Hz, H-2a), δ 2.63 (1H, ddd, $J=16.4, 11.0, 6.6$ Hz, H-2b), δ 3.62 (1H, t, H-3), δ 1.29 (1H, m, H-5), δ 1.71 (1H, m, H-6), δ 1.17 (1H, m, H-7a), δ 1.46 (1H, m, H-7b), δ 1.02 (1H, d, $J=4.8$ Hz, H-9), δ 3.14 (1H, t, $J=4.8$ Hz, H-11), δ 2.80 (1H, d, $J = 4.8$ Hz, H-12), δ 5.53 (1H, dd, $J = 8.0, 3.5$ Hz, H-15), δ 1.69 (1H, dd, $J=14.6, 8.2$ Hz, H-16a), δ 1.89 (1H, dd, $J=14.6, 3.4$ Hz, H-16b), δ 1.17 (1H, m, H-18), δ 1.55 (1H, m, H-19a), δ 1.42 (1H, m, H-19b), δ 0.98 (1H, m, H-22a), δ 1.07 (1H, m, H-22b), δ 1.09 (3H, s, H-23), δ 0.93 (3H, s, H-24), δ 1.22 (3H, s, H-25), δ 1.10 (3H, s, H-26), δ 0.78 (3H, s, H-27), δ 0.85 (3H, s, H-28), δ 0.98 (3H, s, H-29), δ 0.95 (3H, s, H-30).

^{13}C NMR (300 MHz, $CDCl_3$): δ 39.5 (C-1), δ 33.8 (C-2), δ 216.8 (C-3), δ 47.3 (C-4), δ 54.6 (C-5), δ 38.6 (C-6), δ 36.6 (C-7), δ 36.5 (C-8), δ 52.7 (C-9),

δ 338.9 (C-10), δ 51.6 (C-11), δ 58.1 (C-12), δ 37.6 (C-13), δ 156.5 (C-14), δ 119.2 (C-15), δ 38.3 (C-16), δ 35.5 (C-17), δ 48.2 (C-18), δ 20.1 (C-19), δ 28.7 (C-20), δ 33.2 (C-21), δ 35.2 (C-22), δ 21.5 (C-23), δ 26.2 (C-24), δ 16.2 (C-25), δ 26.7 (C-26), δ 19.5 (C-27), δ 30.2 (C-28), δ 33.7 (C-29), δ 29.9 (C-30). Its FAB-MS showed mass fragmentation peak at m/z 426, 408, 393, 284, 264, 204 and 133.

Thus the triterpenoid PS-IV was identified as: Taraxerol.



PS-IV

PART-B: Studies on Anti-asthmatic activity of the isolated compounds

This part deals with anti-asthmatic activity of an isolated compounds isolated from the plants. Compound PS-I and PS-II were isolated from plant *Acorus calamus* Linn. and compound PS-III and PS-IV were isolated from plant *Euphorbia hirta* Linn. These compounds were screened for their anti-asthmatic activity on histamine induced bronchospasms guinea pig.

The compound PS-I and PS-III showed significant protection against the histamine challenge. The compound PS-IV was found to be least potent while the compound PS-II was found to be inactive towards bronchoprotection. Compounds PS-I and PS-III produced bronchoprotective effect due to its H₁-blocking effect.



FIG 1



FIG 2

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Table – 1.2

S.No.	Plants Name	Family Plants	Plant Parts	Compound Isolated	Structure	Reference
1.	<i>Adhatoda vasica</i>	Acanthaceae	Root	Vasicine	1	74
2.	<i>Allium cepa</i>	Liliaceae	Bulb	Thiosulfinates (1-methyl (sulphinyl) propylmethyl disulphide)	2	75
				Cepaenes (α - sulphinyl disulphide)	3	76
3.	<i>Tylophora asthmatica</i>		Leaves	Tylophorine	4	77
				Tylophorinine	5	77
4.	<i>Coleus forskohlii</i>	Labiatae	Roots	Forskoliin (colenol) 7 β -acetoxy-8, 13-epoxy-1 α , 6 β , 9 α – trihydroxy lab-14-en-11- one	6	78, 79
5.	<i>Tridax procumbens</i>	Compositae	Whole herbs	5,7,4' – trihydroxy- 6',3' dimethoxy flavone 5-O- α - L-	7	80

S.N o.	Plants Name	Family Plants	Plant Parts	Compound Isolated	Structu re	Refere nce
				rhamnopyran oside		
6.	<i>Ephedra vulgaris</i>	Genetaceae		Ephedrine	8	70
7.	<i>Atropa belladonna (deadly nightshade)</i>	Solanaceae		Atropine	9	70
8.	<i>Hyosyamus niger (black henbace)</i>	Solanaceae		Hyoscine	10	81
9.	<i>Boswellia serrata</i>	Burseraceae	Resin	β -boswellic acid	11	82
10.	<i>Glycyrrhiza glabra</i>	Leguminosae		Glycyrrhizin	12	83
11.	<i>Solanum dulcamara</i>	Solanaceae		β -solamarine	13	84
12.	<i>Picrorhiza scrophulariaef lora</i>	Scrophulariae flora	Rhizo me	Picracin	14	85
				Dieacetylpicra cin	15	85
13.	<i>Allium sativum</i>	Liliaceae	Bulb	Allicin (Diallyl Thiosulfinate)	16	86
14.	<i>Vitex negundo</i>	Verbenaceae	Leave s	β -sitosterol	17	87
				Sitgmasterol	18	87
15.	<i>Abies pindrow</i>	Pinaceae	Leave s	Taraxerol	19	66
				β -amyrin	20	66

Table – 1.3

S. No .	Name of the Plant	Parts of Plant	Compounds Isolated	Structure	References
1.	<i>Acorus calamus</i>	Rhizomes	Shyobunone	1	99
			6-epi-shyobunone	2	99
			2, 6-diepi-shyobunone	3	99
			Iso-shyobunone	4	99
			Acoragermacrone	5	100
			Acoron	6	100
			Isoacorone	7	100
			Preisocalamendiol	8	100
			Isocalamendiol	9	100
			Calamusenone	10	100
			Isocalamusenone	11	100
			Cadala-1, 4, 9 triene	12	100
			Tropane	13	100
			Acoradin	14	101
			2, 5-di-meo-benzoquinone	15	101
			Galangin	16	101
			β -sitosterol	17	101
			Isoeuguenol methyl ether	18	102
			γ -asarone [1, 2, 4-trimethoxy-5(2-	19	102

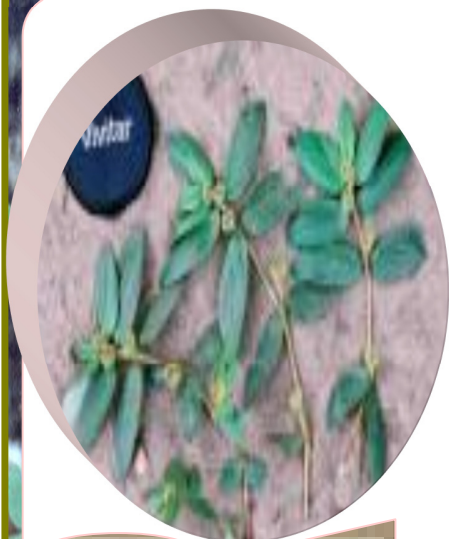
S. No .	Name of the Plant	Parts of Plant	Compounds Isolated	Structure	References
			propenyl) benzene]		
			β -asarone [cis- asarone/cis-[1, 2, 4- trimethoxy-5 (1- propenyl) benzene]	20	102
			α -asarone [trans- asarone/trans 1, 2, 4 – trimethoxy – 5 (1 – propenyl) benzene]	21	102
			Acoramone [1, 2, 4 – trimethoxy – 5 (2 – propanonyl) benzene]	22	102
			Asaraldehyde/ [2, 4, 5- trimethoxy benzaldehyde]	23	102
			Luteolin-6, 8, C- diglycoside	24	103
			Calamol	25	103
			Eugenol	26	103
			1, 8-cineole	27	103
			Calacone	28	104
			Telekin	29	104
			Isotelekin	30	104
			1 β , 2 α , 3 β , 19 α - tetrahydroxyurs-12- en-28-oic acid-28-O {s- β -D- glucopyranosyl(1 $\rightarrow$ 2)}	31	105

S. No .	Name of the Plant	Parts of Plant	Compounds Isolated	Structure	References
			- β -D-galactopyranoside.		
			3 β , 22 α , 24, 29-tetrahydroxyolean-12-en-3-O- { β -D-arabinosyl (1 $\rightarrow$ 3)- β -D-arabinopyranoside.	32	105
2.	<i>Euphorbia hirta</i>	Roots	β -sitosterol	33	97, 106
			β -amyrin (Oleanane)	34	107, 111
			Ingenol triacetate	35	108
			Taraxerol	36	110, 109
			Quercetin	37	110
			Lupeol (Lupane)	38	111
			Euphol	39	110, 112
			Ellagic acid	40	97, 113

A brief Account on Isolated compounds are given below in table-5.5:-

Table – 5.5

S. No.	Plant	Part of the Plant	Isolation compounds	Molecular Formula	Melting point	Structure
1.	<i>Acorus calamus</i> Linn.	Roots	Galangin-3-O- β -D-glucopyranosyl-7-O- α -L-rhamnopyranoside	C ₂₇ H ₃₀ O ₁₄	150-151°C	PS-I
		Roots	Luteolin-8-C- β -D-Glucopyranoside	C ₂₁ H ₂₀ O ₁₁	264-265°C	PS-II
2.	<i>Euphorbia hirta</i> Linn.	Roots	24, 25-dihydro-euphol-3-O- β -D-arabinopyranoside	C ₃₅ H ₆₀ O ₅	262-263°C	PS-III
		Stem	Taraxerol	C ₃₀ H ₅₀ O	280-282°C	PS-IV



You can teach a student a lesson for a day; but if you can teach him to learn by creating curiosity, he will continue the learning process as long



ISOLATION AND STUDY OF THE FLAVONOIDAL GLYCOSIDE PS-I: GALANGIN-3- O- β -D-GLUCO-PYRANOSYL-7-O- α -L-RHAMNOPYRANOSIDE

***This work has been accepted for
publication in the *Journal of Institution
of Chemists (India)*.**

A*corus calamus* Linn¹⁻⁵ belongs to natural order Araceae and is commonly known as 'Bach' in Hindi. It is a hydrophilic and is usually found in wetlands, around the edges of lakes, streams and also around the ponds, ditches and sweeps.

Acorus calamus has long creeping roots that spread just below the soil, and also spread horizontally long upto 2 meters. The leaves of this plant are thick and erect.

This plant possesses various medicinal properties as its roots have been used as a stimulant and combat fatigue. Alcoholic extract of this plant has sedative and analgesic effect and also moderate depression in blood pressure and respiration. Its rhizome is used in emetic, dysentery, colic, remittent fever. It is also used as a nerve tonic in bronchitis and snakebite remedy.

EXTRACTION OF THE FLAVONOL GLYCOSIDE PS-I

The roots of the plant *Acorus calamus* Linn. were collected through the courtesy of the Agricultural Department of M.P. Government at Sagar and was identified by taxonomist of Botany Department of Dr. H.S. Gour University, Sagar (M.P.). The air-dried and powdered roots (3.0 kg.) of this plant were extracted with 95% ethanol. The ethanolic extract was concentrated under reduced pressure and thereafter, light brown syrupy mass was obtained, which was then sequentially and successively partitioned

with pet- ether (60-80°C), benzene, chloroform, ethyl acetate, acetone and methanol. Except the chloroform soluble fraction other fractions as: pet-ether (60-80°C) benzene, ethyl acetate, acetone and methanol were not studied phytochemically because they yielded very small amount on concentration which were insufficient for any substantive study.

The chloroform soluble fraction was concentrated under reduced pressure. The concentrated chloroform extract was subjected to TLC examination using n-butanol: acetic acid: water (4:1:5) as a solvent system and I₂ vapours as a visualizing agent which displayed two spots. The mixture was then subjected to column chromatography over silica gel G and gradiently eluted with CHCl₃: MeOH in varying proportions.

Eluates collected from CHCl₃: MeOH (9:1) were found to have same R_f values and therefore were mixed. On removal of the solvent, it gave yellow compound. This compound was found to be homogeneous on TLC examination using n-butanol: acetic acid: water (4:1:5) as a solvent system and I₂ vapours as a visualizing agent. It was crystallised from methanol as a yellow crystals PS-I (0.051%) (FIG 2.1).

STUDY OF FLAVONOL GLYCOSIDE PS-I

The glycoside PS-I was found to be soluble in methanol and ethanol. It analysed for molecular formula C₂₇H₃₀O₁₄, m.p.150-151°C and [M⁺] 578 (FAB-MS).

It responded to all characteristic colour reactions of flavonoids^{6, 7}. It gave positive Molisch's test and did not reduce the Fehling solution, thereby showing it to be glycosidic in nature.

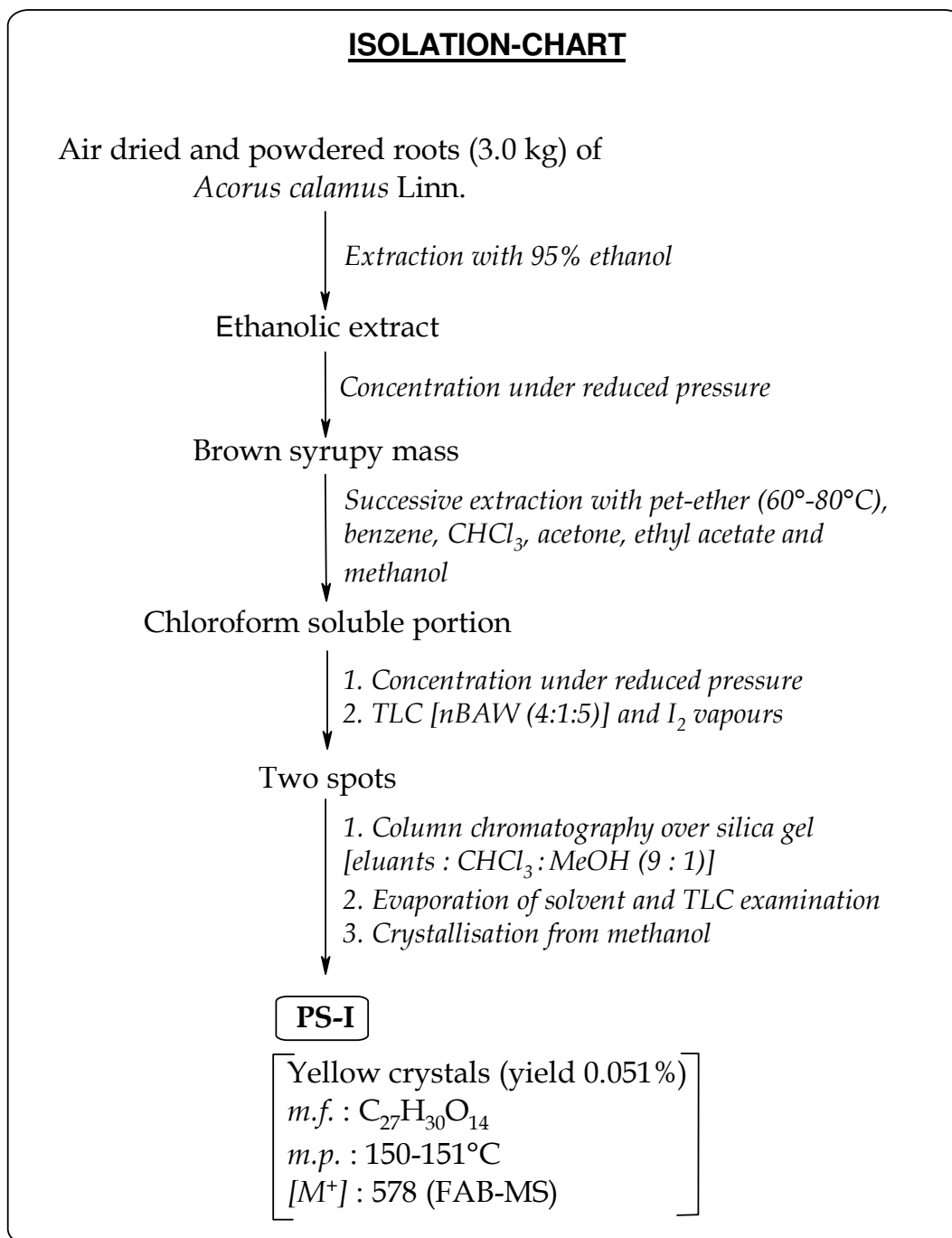


FIG. 2.1: Fractionation protocol for isolation of compound PS–I
from plant *Acorus calamus* Linn.

UV SPECTRUM OF THE FLAVONOL GLYCOSIDE PS-I

The wavelength of maximum absorption with various shift reagents as observed are filed below in table-2.1.

Table – 2.1

UV spectrum of the Flavonol Glycoside PS-I

S.No.	Solvent + (Shift Reagents)	Shifts in nm Band	Band I
1.	MeOH	267	348
2.	MeOH + AlCl ₃	335	348
3.	MeOH +AlCl ₃ /HCl	336	401
4.	MeOH + NaOAc	324	394
5.	MeOH + CH ₃ COONa	267	358

IR SPECTRUM OF THE FLAVONOL GLYCOSIDE PS-I

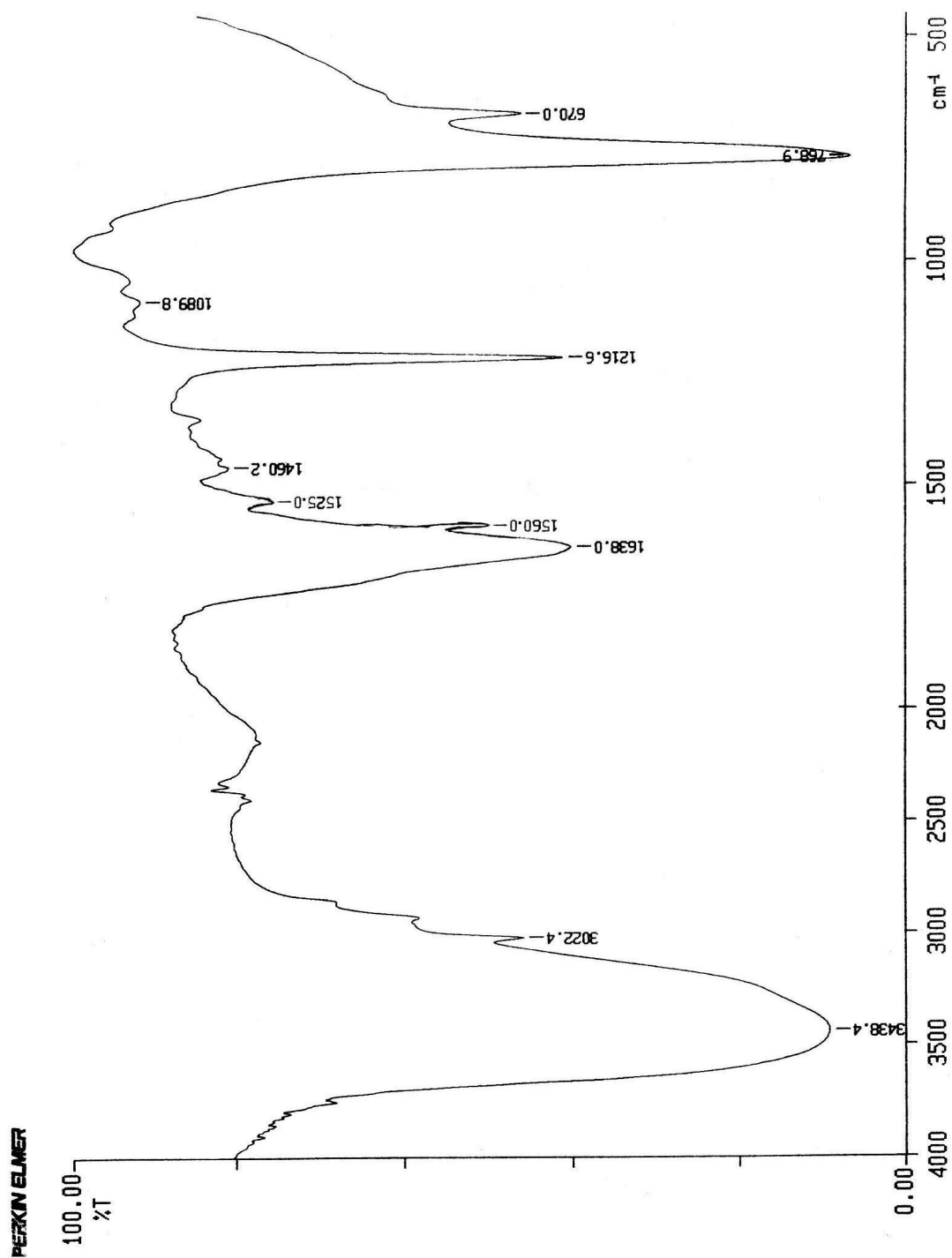
The characteristic bands appeared in the IR spectrum of the flavonol glycoside PS-I [FIG. 2.2] and functional groups ascribed to the molecule with the support of the available literature ⁸⁻¹¹ are given in table-2.2.

Table – 2.2

IR spectrum of the Flavonol Glycoside PS-I

S.No.	Wave Number	Assignments
1.	3438.4	-OH group(s) (Intramolecular hydrogen bonding)
2.	3022.4	= C-H Stretching vibration
3.	1638.0	α , β - Unsaturated >C= O Group
4.	1460.2, 1560.0, 1525.0	Aromatic ring System
5.	1216.6	C-O-C bending Vibration
6.	1089.8	C-O-C Stretching Vibration
7.	768.9, 670.0	Monosubstituted benzene ring

IR SPECTRUM OF THE FLAVONOL GLYCOSIDE PS-I



04/02/25 10:36 AM, CODE-PS-I
X: 4 scans, 4.0cm-1, flat, smooth, abex

PRESENCE OF –OH GROUP (S) IN THE FLAVONOL GLYCOSIDE PS-I

Significant IR absorption band at $\nu_{\text{max}}^{\text{KBr}}$ 3438.4 cm^{-1} in the IR spectrum of the PS-I indicated the presence of hydroxyl groups in it, which on acetylation with acetic anhydride/ pyridine furnished an acetylated product. The product analysed for molecular formula $\text{C}_{40}\text{H}_{44}\text{O}_{21}$, m.p. 176-177°C and $[\text{M}^+]$ 860 [FAB-MS]. The percentage of acetyl group in the acetylated product was determined by Weisenberger method¹² as described by Belcher and Godbert¹³. The results showed the presence of seven hydroxyl groups in the glycoside PS-I (35.00%).

Appearance of the band at $\nu_{\text{max}}^{\text{KBr}}$ 3438.4, in the IR spectrum of the acetylated product indicated still the presence of the hydroxyl group in the acetylated product thereby indicating that some hydroxyl group(s) must have escaped acetylation. Such group must be hydroxyl group at position C-5.

The position of various hydroxyl groups and structure of the glycoside was elucidated by its hydrolysis and subsequently identifying aglycone and sugar moieties.

ACID HYDROLYSIS OF THE FLAVONOL GLYCOSIDE PS-I

Glycoside PS-I on acid hydrolysis of with aqueous 7% H_2SO_4 afforded an aglycone PS-I (A), which was precipitated out. It was filtered and washed. The sugar moiety (ies) remains in the hydrolysate. Aglycone PS-I (A) and sugars were studied separately.

STUDY OF THE AGLYCONE PS-I (A)

The precipitate obtained after acid hydrolysis of glycoside was subjected to TLC examination using the solvent system n-butanol: acetic acid: water (4: 1:5) and I₂ vapours as a spraying agent when, it was found to be homogenous. Aglycone PS-I (A) analysed for molecular formula, C₁₅H₁₀O₅, m.p. 213- 214⁰ C and [M⁺] 270 [FAB-MS]. It gave various colour reactions of flavonoids^{6, 7}.

UV SPECTRUM OF AGLYCONE PS-I (A)

The wavelength of the maximum absorbance as observed with various shift reagents are tabulated below in table – 2.3.

Table – 2.3

UV spectrum of the Aglycone PS-I (A)

S.No.	Solvent + (Shift Reagents)	Shifts in nm Band	Band I
1.	MeOH	269	366
2.	MeOH + AlCl ₃ /HCl	268	422
3.	MeOH + NaOAc	280	386
4.	MeOH + NaOMe	280	412

IR SPECTRUM OF THE AGLYCONE PS-I (A)

The prominent bands observed in the IR spectrum of the aglycone PS-I (A) [FIG.2.3] and structural units inferred with the help of available literature¹⁴⁻¹⁸ are given in table-2.4.

Table – 2.4

IR spectrum of the Aglycone PS-I (A)

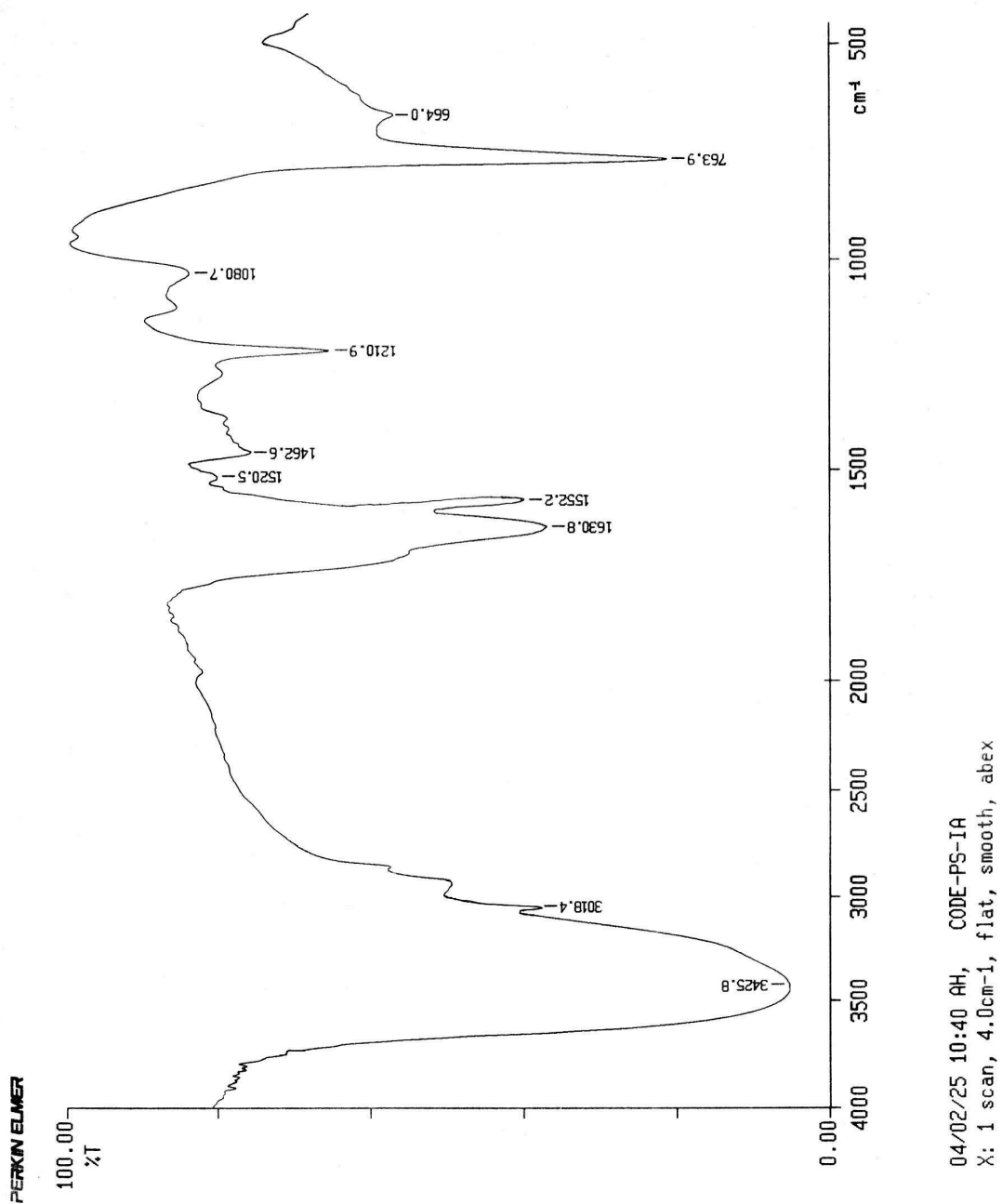
S.No.	Wave Number	Assignments
1.	3425.8	–OH group(s) (Intramolecular hydrogen bonding)
2.	3018.4	=C-H Stretching vibration
3.	1630.8	α , β - Unsaturated –C= O group
4.	1462.6,1552.2,1520.5	Aromatic ring system
5.	1210.9	C-O-C bending vibration
6.	1080.7	C-O-C- Stretching vibration
7.	763.9, 664.0	Monosubstituted benzene ring

PRESENCE OF (- OH) GROUP (S) IN THE AGLYCONE PS-I (A)

The characteristic band at $\nu_{\max}^{\text{KBr}}$ 3425.8 cm^{-1} in the IR spectrum of the aglycone indicated the presence of –OH group(s) in it, which on acetylation with NaOAc and Ac_2O afforded an acetylated product. The acetylated derivative analysed for the molecular formula, $\text{C}_{19}\text{H}_{14}\text{O}_7$ m.p. 175-176°C and $[\text{M}^+]$ 354 [FAB-MS].

The percentage of acetyl group was estimated by the Weisenberger method¹² as described by Belcher and Godbert¹³, which indicated the presence of two hydroxyl groups (24.15%) in it. The band appeared at 3425.8 cm^{-1} in the IR spectrum of acetylated product, indicated still the presence of -OH group in it. It must be C-5 –OH, which must have escaped on acetylation, due to the strong hydrogen bonding with the oxygen of carbonyl at C-4.

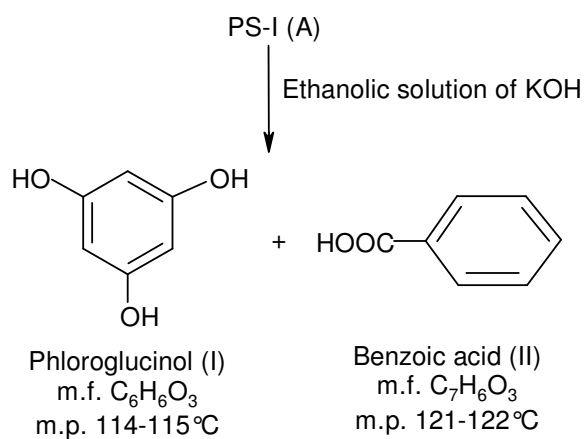
IR SPECTRUM OF THE AGLYCONE PS-I (A)



ALKALINE DEGRADATION OF THE AGLYCONE PS-I (A)

Aglycone PS-I (A) on fusion with ethanolic solution of potassium hydroxide afforded two products

(1) Phloroglucinol, m.f. $C_6H_6O_3$, m.p. 115-116°C and (2) Benzoic acid, m.f. $C_7H_6O_2$, m.p. 121-122°C confirmed by CoPC and CoTLC.



POSITION OF HYDROXYL GROUP (S) IN AGLYCONE PS-I (A)

The position of the –OH groups in the aglycone were ascertained by UV spectral data and various shift reagents.

Position of Hydroxyl Group at C-3

(1) Aglycone under UV spectrum produced yellow fluorescent colour, which after fuming with NH_3 didn't change, thereby indicating the presence of free –OH group at C–3 positions.^{21, 22}

- (2) The bathochromic shift at 46 nm in band I with NaOMe relative to the methanol indicated the presence of free –OH group in the aglycone, at position C–3 that was engaged in glycoside²¹.

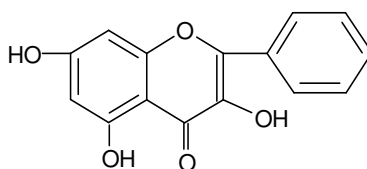
Position of Hydroxyl Group at C-5

- (1) Aglycone PS-I (A) produced bright yellow colour with boric acid in the presence of citric acid²³ and a green colour with aqueous ferric chloride. Thereby, indicated the presence of hydroxyl group at position C-5.²⁴
- (2) The bathochromic shift of 56nm in band–I in AlCl₃/HCl relative to the methanol confirmed the presence of hydroxyl group at C-5.^{25, 26}
- (3) The presence of hydroxyl group at C-5 position was also confirmed by the partial acetylation of aglycone. Only diacetyl derivative was formed instead of triacetyl derivative, which depicted that hydroxyl group at position C-5 was not acetylated because, it was strongly associated with intramolecular hydrogen bonding with keto group at C-4.^{25, 26}

Position of Hydroxyl Group at C-7

- (1) The UV spectral data showed a bathochromic shift of 11 nm in band II with NaOAc relative to methanol, which indicated the presence of –OH group at position C–7.^{27, 28}
- (2) The formation of phloroglucinol and benzoic acid by alkaline degradation of aglycone further confirmed the presence of 5,7 dihydroxy discipline.²⁹

On the basis of above deliberations, the following structure was assigned to the aglycone PS-I (A), thereby establishing its identity as 3, 5, 7-tri-hydroxy flavone (Galangin).³⁰



PS-I (A)

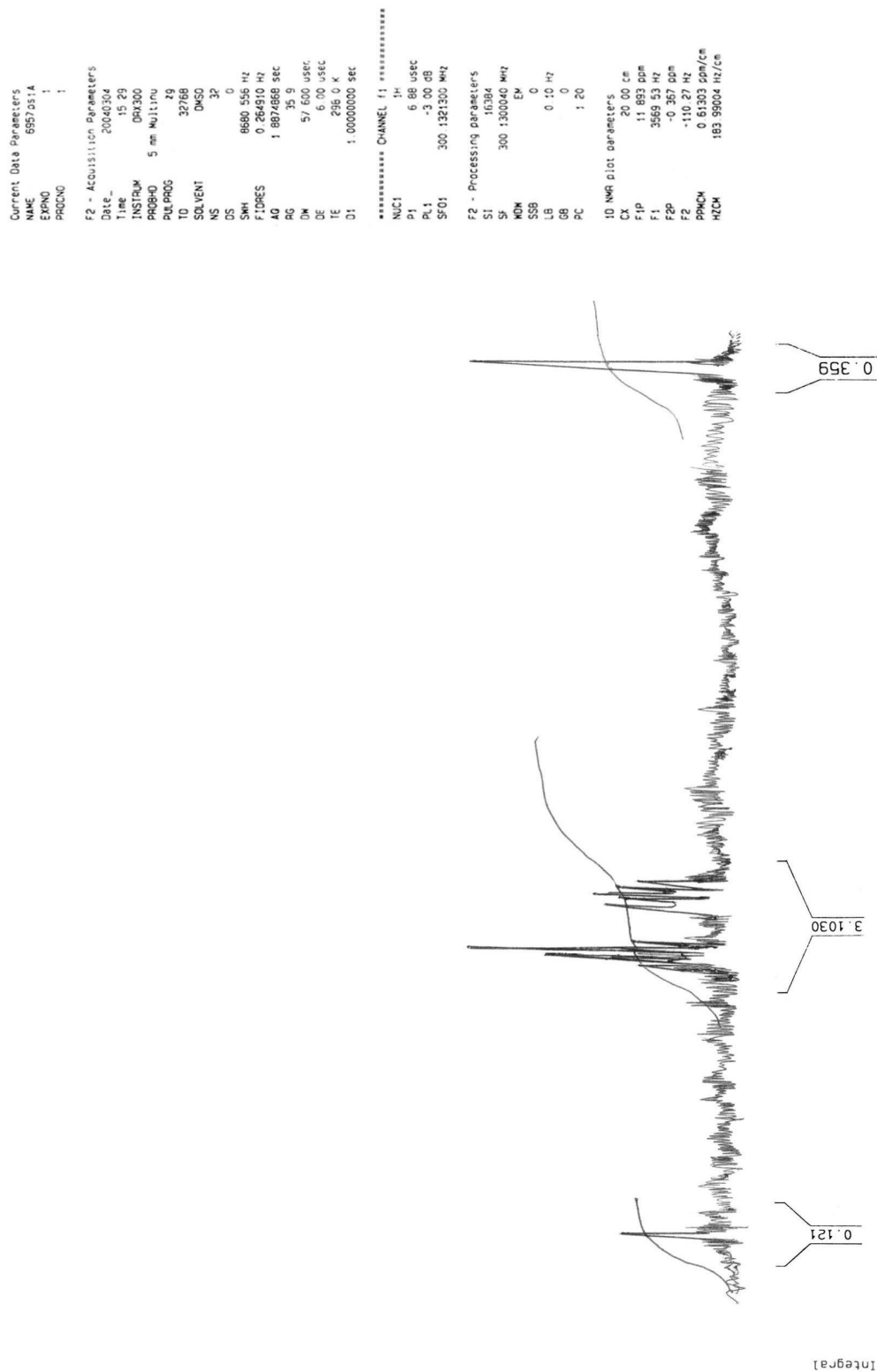
¹H-NMR SPECTRUM OF AGLYCONE OF PS-I (A)

The significant chemical shifts observed in the ¹H-NMR of PS-I [FIG. 2.4] (A) and the structural units ascertained are recorded with the help of literature^{19, 20} are recorded in table-2.5.

Table – 2.5
¹H-NMR spectrum of Aglycone of PS-I (A)

S. No.	δ Value	Patter n	J value Hz	No. of Proton	Assignments
1.	6.30	S	---	1	OH-3
2.	6.47	D	2.6	1	H-6
3.	11.11	S	--	1	OH-7
4.	6.77	D	2.6	1	H-8
5.	7.45	M	-	5	H-2', 3', 4', 5'6'

¹H-NMR SPECTRUM OF AGLYCONE OF PS-I (A)



PS-1A

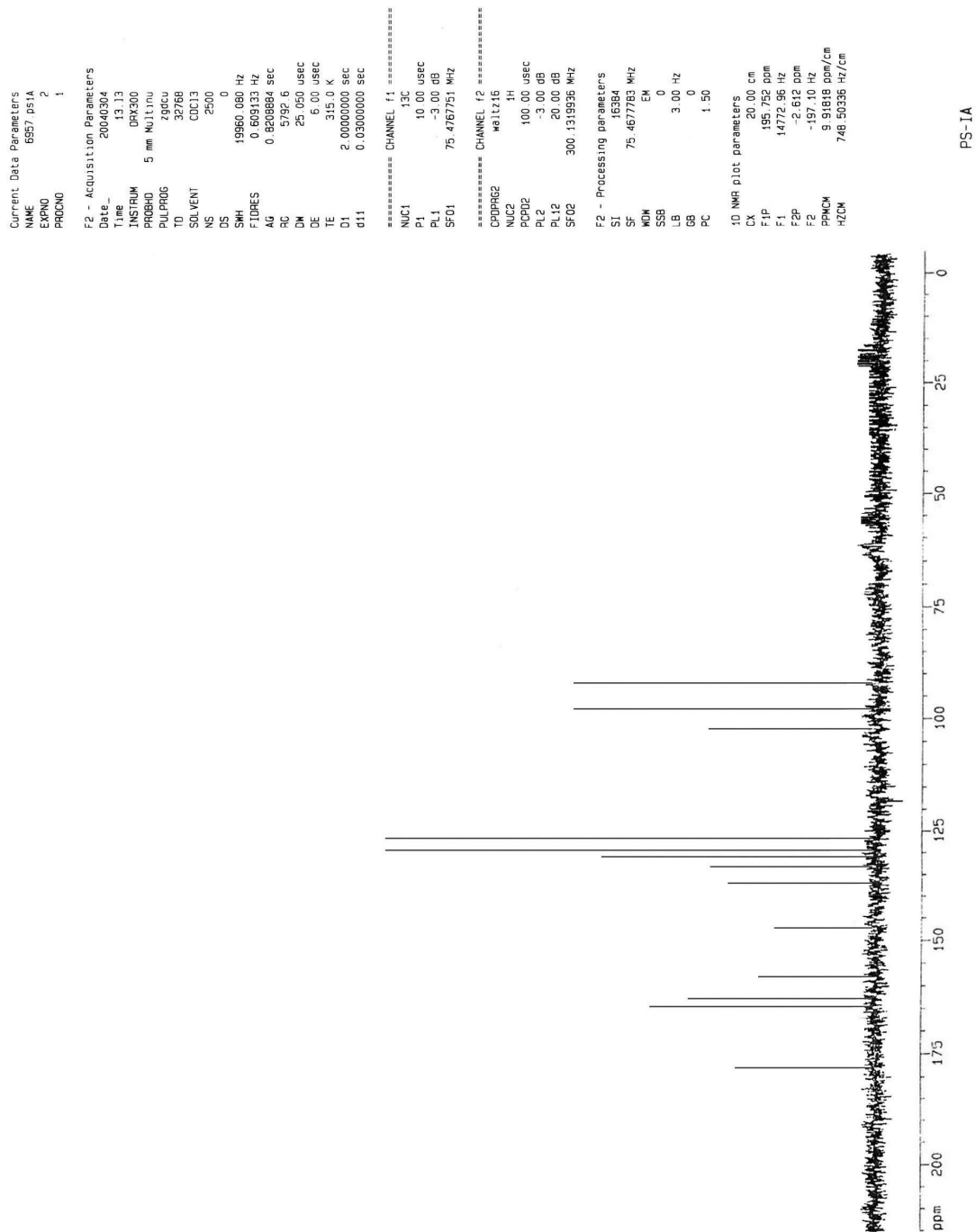
¹³C-NMR SPECTRUM OF THE AGLYCONE PS-I (A)

The various signals observed in the ¹³C-NMR spectrum of the aglycone PS-I (A) [FIG. 2.5] and structural units assigned with the help of available literature^{45-47, 51} are filed below in table-2.6.

Table – 2.6
¹³C-NMR spectrum of Aglycone PS-I (A)

S.No.	δ _c Value	Carbon No.	Carbon Type
1.	145.3	C-2	C
2.	134.6	C-3	C
3.	174.5	C-4	C= O
4.	163.2	C-5	C
5.	100.1	C-6	CH
6.	163.5	C-7	C
7.	95.8	C-8	CH
8.	157.0	C-9	C
9.	107.4	C-10	C
10.	134.5	C-1'	C
11.	128.4	C-2'	CH
12.	128.2	C-3'	CH
13.	131.6	C-4'	CH
14.	127.3	C-5'	CH
15.	128.3	C-6'	CH

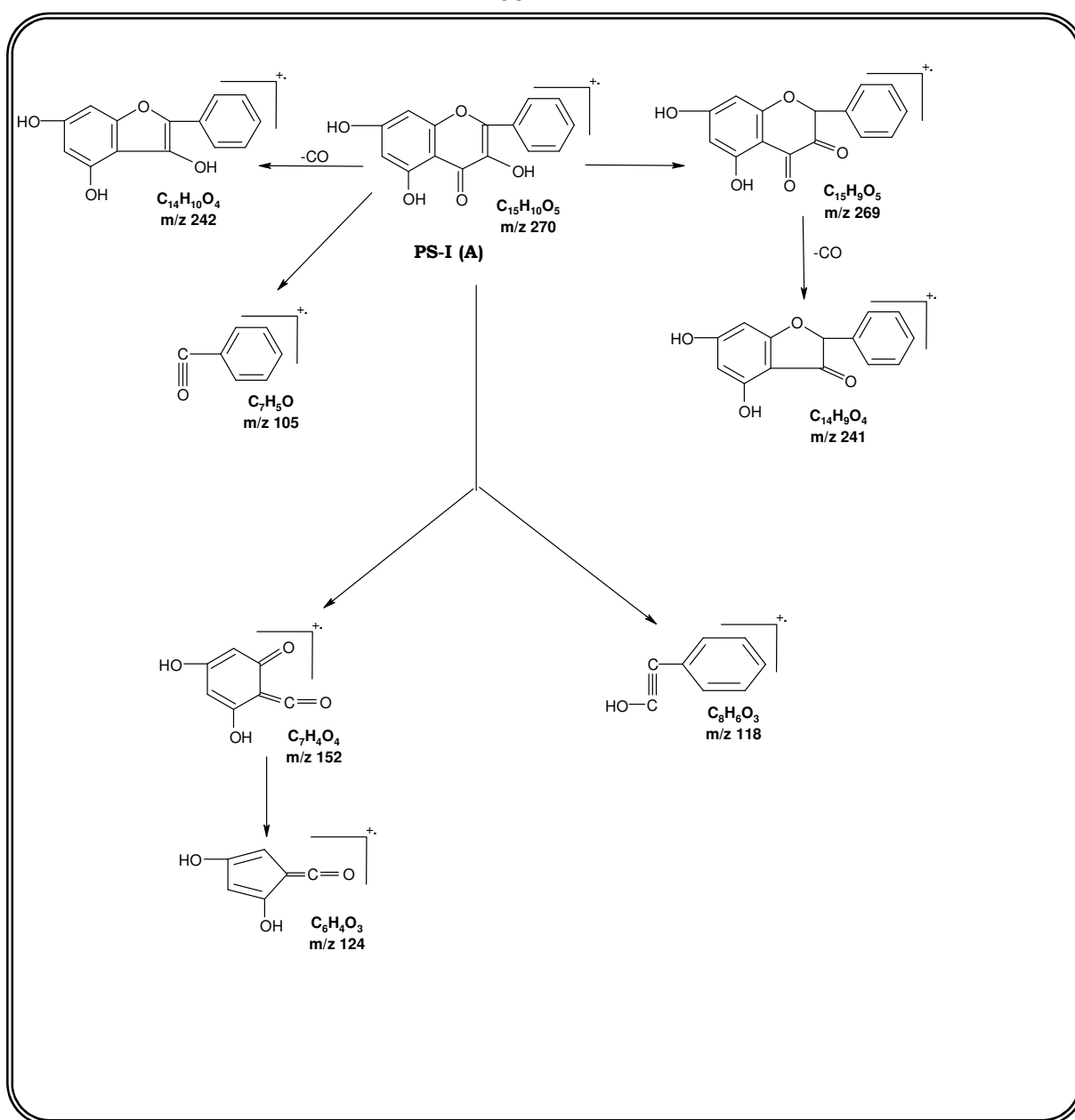
¹³C-NMR SPECTRUM OF THE AGLYCONE PS-I (A)



MASS SPECTRUM OF THE AGLYCONE PS-I (A)

The important fragment ion peaks obtained in aglycone PS-I (A) as: m/z 270 [M^+] 269, 242, 241, 152, 124, 118 and 77 are shown in scheme-I.

SCHEME-I



STUDY OF THE HYDROLYSATE

The filtrate obtained after the acid hydrolysis of glycoside PS-I was neutralised with BaCO_3 and BaSO_4 was filtered off. The filtrate obtained, reduced Fehling solution and also gave the colour with aniline hydrogen phthalate. The filtrate was concentrated to syrupy mass, which was subjected to paper chromatography, thereby indicating the presence of sugars; L-rhamnose and D-glucose (R_f 0.38 and R_f 0.19).³¹⁻³⁵

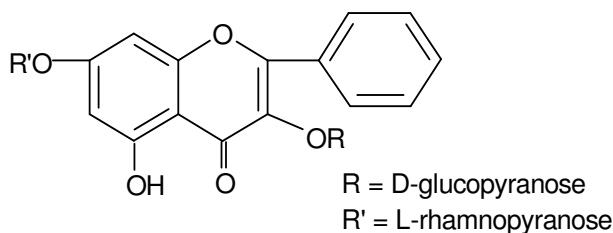
QUATITATIVE ESTIMATION OF SUGAR (S)

Quantitative estimation of the sugars in the glycoside PS-I was done by the method of Mishra and Rao³⁶ which showed the presence of aglycone PS-I (A), D-glucose and L-rhamnose in ratio 1:1:1.

PERIODATE OXIDATION OF THE FLAVONOL GLYCOSIDE PS-I

The glycoside PS-I on periodate oxidation³⁷ with sodium meta periodate consumed 3.02 moles of periodate and produced 1.01 moles of formic acid, thereby showing the presence of both sugars (D-glucose and L- rhamnose) and the aglycone PS-I (A) as one mole each and also confirmed the pyranose³⁸ form of sugars.

Thus, a tentative structure given below was assigned to the glucoside PS-I;



POSITION OF ATTACHMENT OF THE SUGAR (S) TO THE AGLYCONE PS-I (A)

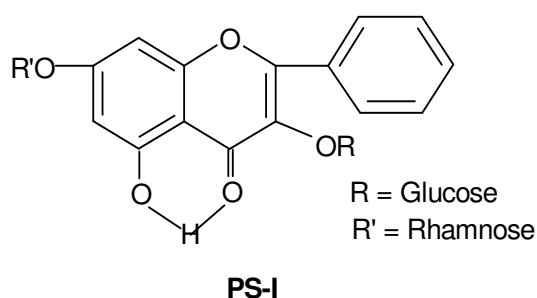
The point of attachment of sugar moiety (ies) was assigned by a comparative study of colour reactions and UV spectral shift of aglycone and glycoside PS-I.

Since the –OH group at positions C–5 formed a intramolecular hydrogen bonding with oxygen of the carbonyl group at C-4 which suggested that the glycosylation must be either at C-3 position or at C-7 position. This was further cleared by the facts given below.²⁶

- (1) In the UV spectrum^{21, 22} a bathochromic shift of 46nm in band–I with NaOMe relative to the methanolic solution suggested that the position–3 was blocked in the glycoside and free in aglycone which was also confirmed by the fact that the glycoside PS-I showed a hypsochromic shift by 18nm in band–I comparative to the aglycone. Thereby suggesting that C-3 –OH group in the glycoside PS-I was involved in the glycosylation.
- (2) A bathochromic shift of 11nm in band–II with NaOAc relative to the MeOH suggested that the position C-7 of ring A was engaged in glycoside PS-I and free in aglycone PS-I (A). This was also confirmed by the colour reaction

in which aglycone, produced red colour with p-toluene sulphonic acid, while glycoside did not respond the test. Thereby indicating that C-7 –OH of the PS-I was involved in the glycosidic linkage.^{18,27, 28}

Viewing all the above facts the tentative structure to the glycoside PS-I was assigned as;



SEQUENCE OF THE SUGAR MOIETY (IES)

The sequence of sugar moiety (ies) was established by mild hydrolysis of glycoside PS-I which, liberated D-glucose and proaglycone PS-I (B).

STUDY OF THE PROAGLYCONE PS-I (B)

Glycoside PS-I on mild acid hydrolysis with 1N HCl refluxed for 10 to 20 min.afforded proaglycone and sugar, which were separatedby filtration and studied separately.³⁹PS-I (B) analysed for molecular formula $C_{20}H_{20}O_9$, m.p. 230-231°C and $[M^+]$ 404 (FAB-MS).

ACID HYDROLYSIS OF PROAGLYCONE PS-I (B)

The proaglycone PS-I (B) on acid hydrolysis furnished aglycone PS-I (A) and L-rhamnose in the hydrolysate (CoPC, CoTLC). Quantitative

estimation of sugar by the procedure of Mishra and Rao³⁶ suggested that in proaglycone PS-I(B), the aglycone PS-I(A) (Galangin) and L-rhamnose were present in equimolar ratio (1:1).

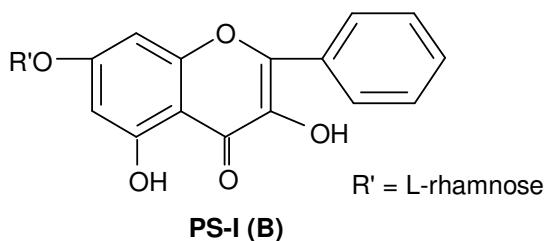
POSITION OF ATTACHMENT OF SUGAR TO THE PROAGLYCONE PS-I (B)

The FAB-MS of PS-I indicated peak at $[M^+] = 416$ (scheme-II) which indicated that in proaglycone PS-I (B), the L-rhamnose was directly attached to C-7 hydroxyl group of the aglycone PS-I (A).

PERMETHYLATION AND HYDROLYSIS OF PROAGLYCONE PS-I (B)

The proaglycone PS-I (B) on permethylation by Kuhn⁴⁰ method followed by acid hydrolysis yielded aglycone and methylated sugar. The methylated sugar was identified as 2''', 3''', 4'''-tri-O-methyl- L-rhamnose confirmed by CoPC and CoTLC which indicated that the C-1''' of L-rhamnose was involved in glycosylation and also confirmed that the L-rhamnose was present in the pyranose form.

Thus by keeping in view to the above points, the tentative structure was assigned to proaglycone PS-I (B) as Galangin-7-O- α -L-rhamnopyranoside.



PERMETHYLATION AND HYDROLYSIS OF THE GLYCOSIDE PS-I

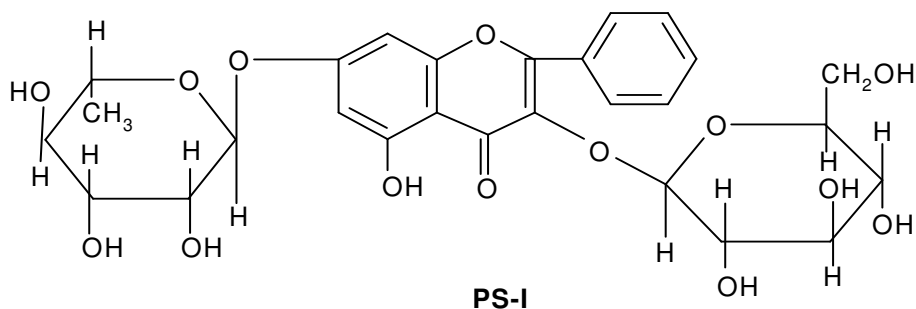
The glycoside PS-I on permethylation by Kuhn⁴⁰ procedure followed by acid hydrolysis yielded aglycone and methylated sugars 2", 3", 4", 6"-tetra-O-methyl-D-glucose and 2"', 3"', 4"'-tri-O-methyl-L-rhamnose (CoPC and CoTLC) confirmed that C-1" of D-glucose and C-1"' of L-rhamnose was involved in the formation of glycosidic linkage and also suggested that both the sugars were present in pyranose form.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE PS-I

The glycoside PS-I on enzymatic hydrolysis with enzyme almond emulsion⁴¹ yielded proaglycone PS-I (B) and D-glucose confirmed by (CoPC) which suggested β -linkage between PS-I (A) and D-glucose. The enzymatic hydrolysis of proaglycone PS-I (B) with Takadiastase⁴² enzyme afforded an aglycone PS-I (A) and L-rhamnose, which indicated the presence of α -linkage between aglycone and L-rhamnose.

On the basis of the all above deliberations, it was concluded that the hydroxyl group at C-3 position of the aglycone was linked to C-1" of D-glucose through β -linkage and C-7 –OH group of aglycone was attached to C-1"' of L-rhamnose through α - linkage.

Thus when all the abstracts put together, the tentative structure was assigned to the glycoside PS-I as Galangin-3-O- β -D-glucopyranosyl-7-O- α -L-rhamnopyranoside.



The structure of glycoside PS-I was further assisted by its $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and mass spectral studies.

$^1\text{H-NMR}$ SPECTRUM OF THE GLYCOSIDE PS-I

The prominent chemical shifts observed in the glycoside PS-I [FIG. 2.6] and the structural units inferred with the available literature^{19, 20, 43, 44} are given in the table-2.7.

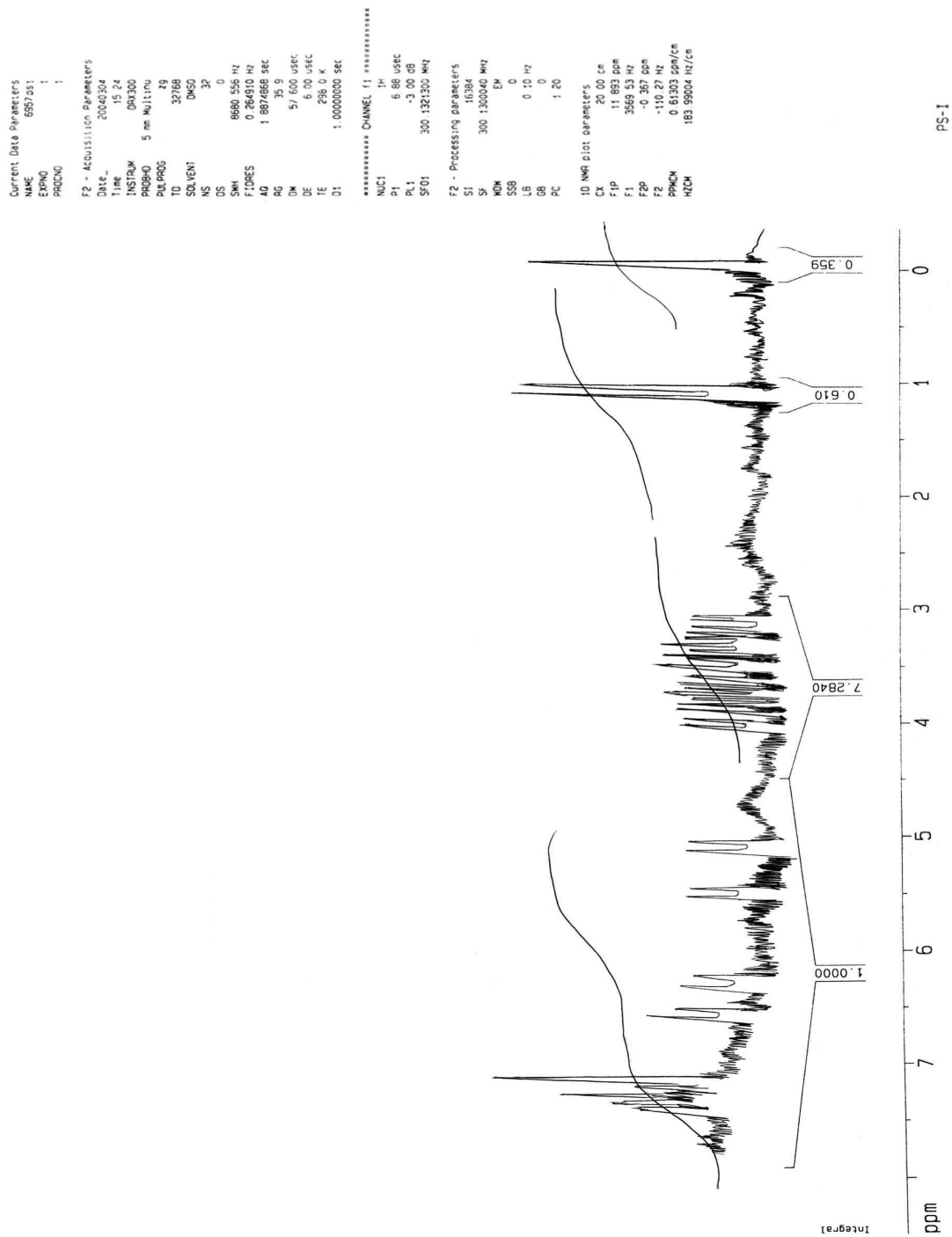
Table – 2.7

$^1\text{HNMR}$ spectrum of the Glycoside PS-I

S. No.	δ Value	Pattern	J value Hz	No. of Proton	Assignments
1.	6.45	d	2.4	1	H-6
2.	6.76	d	2.6	1	H-8
3.	7.46	m	-	5	H-2', 3', 4', 5', 6'
4.	5.39	d	7.7	1	H-1'', anomeric proton for Glucose
5.	3.45	dd	7.7,9.1	1	H-2''
6.	3.41	t	9.1	1	H-3''
7.	3.30	t	9.5	1	H-4''

S. No.	δ Value	Pattern	J value Hz	No. of Proton	Assignments
8.	3.22	ddd	2.4, 5.5, 9.5	1	H-5"
9.	3.90	dd	2.0, 11.8	1	H-6"A
10	3.52	dd	1.9, 3.5	1	H-6"B
11.	5.58	d	1.9	1	H-1''' anomeric proton of rhamnose
12.	4.02	d	1.9, 3.5	1	H-2'''
13.	3.81	dd	3.5, 9.5	1	H-3'''
14.	3.47	t	9.5	1	H-4'''
15.	3.58	dq	5.9, 9.5	1	H-5'''
16.	1.24	d	5.9	3	H-6''' for CH ₃

¹H-NMR SPECTRUM OF THE GLYCOSIDE PS-I



¹³C-NMR SPECTRUM OF GLYCOSIDE PS-I

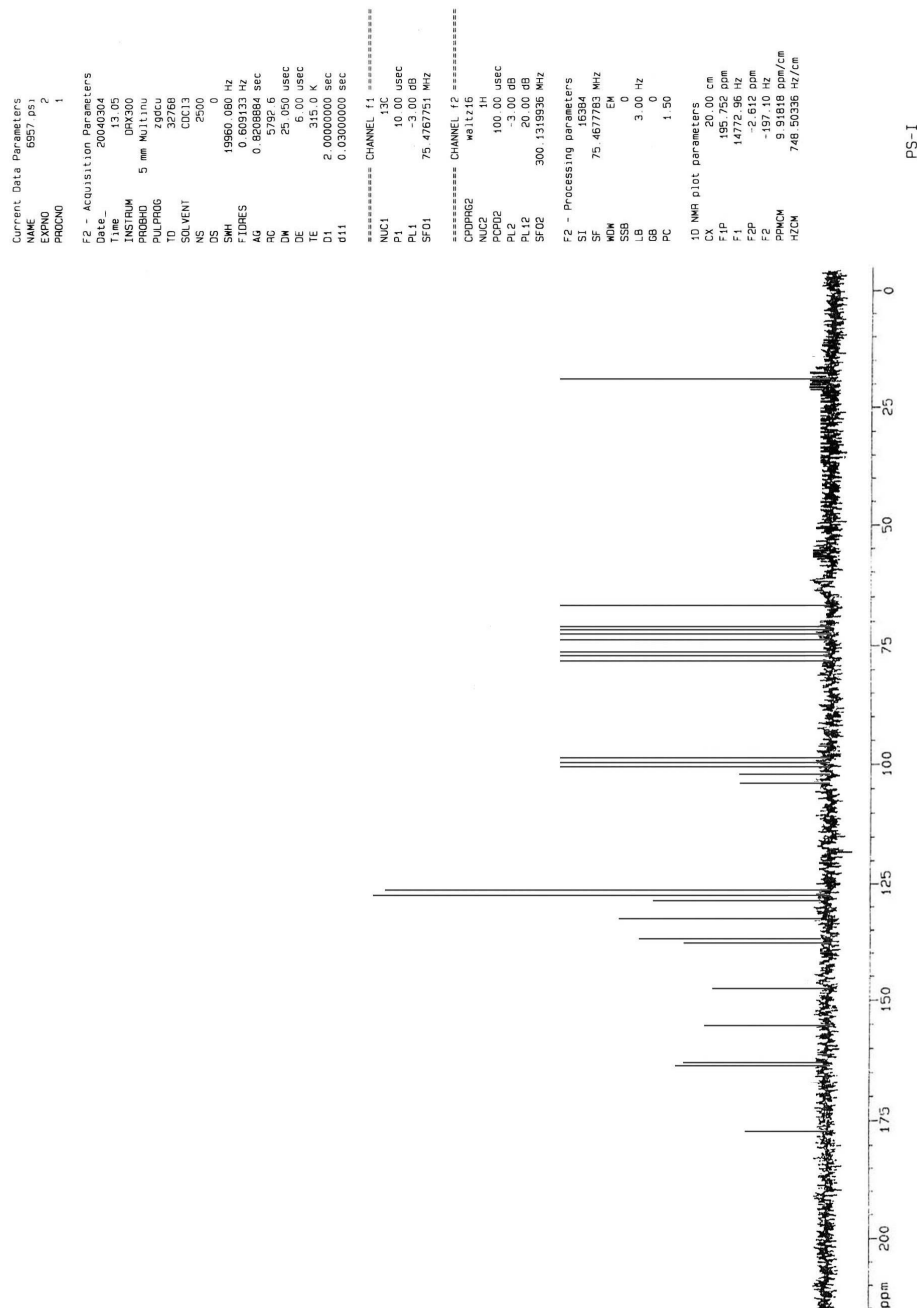
The various signals observed in the ¹³C-NMR spectrum of the glycoside PS-I [FIG. 2.7] and structural units assigned with the help of available literature^{45-47, 51} are filed below in table-2.8.

Table – 2.8
¹³C-NMR spectrum of Glycoside PS-I

S.No.	δ _c Value	Carbon No.	Carbon Type
1.	146.3	C-2	C
2.	135.6	C-3	C
3.	175.6	C-4	C= O
4.	163.3	C-5	C
5.	100.7	C-6	CH
6.	163.6	C-7	C
7.	95.9	C-8	CH
8.	158.0	C-9	C
9.	107.5	C-10	C
10.	135.5	C-1'	C
11.	129.4	C-2'	CH
12.	128.3	C-3'	CH
13.	131.7	C-4'	CH
14.	128.3	C-5'	CH

S.No.	δ_c Value	Carbon No.	Carbon Type
15.	129.6	C-6'	CH
16	103.4	C-1"	CH
17.	75.5	C-2"	CH
18.	77.8	C-3"	CH
19.	71.5	C-4"	CH
20.	78.2	C-5"	CH
21.	62.5	C-6"	CH
22.	100.2	C-1'''	CH
23.	71.2	C-2'''	CH
24.	72.2	C-3'''	CH
25.	73.3	C-4'''	CH
26.	71.5	C-5'''	CH
27.	19.3	C-6'''	CH ₃

¹³C-NMR SPECTRUM OF GLYCOSIDE PS-I

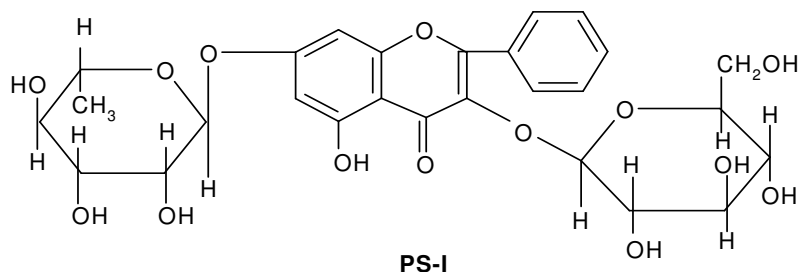


MASS SPECTRUM OF THE GLYCOSIDE PS-I

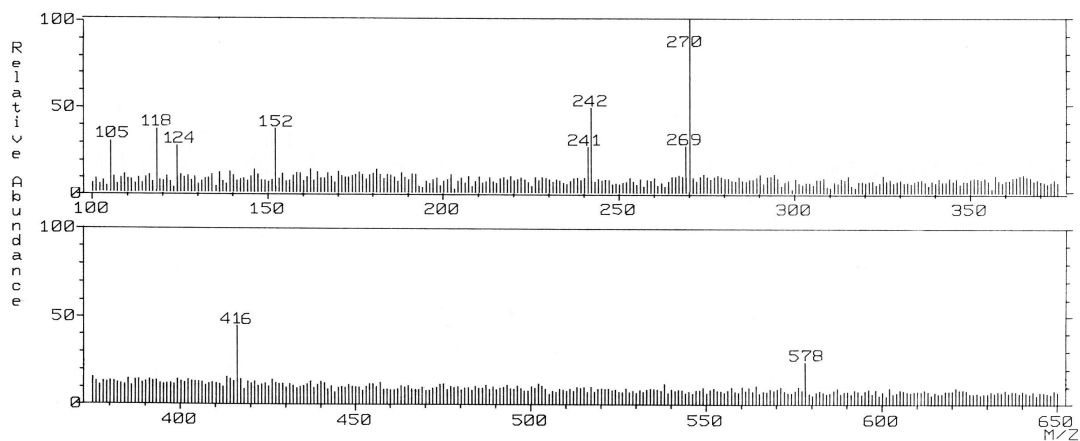
The characteristic fragment ion peaks⁴⁸⁻⁵⁰ obtained in the FAB-MS of glycoside PS-I [FIG.2.8] are given below:

m/z = 578, $[M^+]$ 416, 270, 269, 242, 241, 152, 124, 118 and 105.

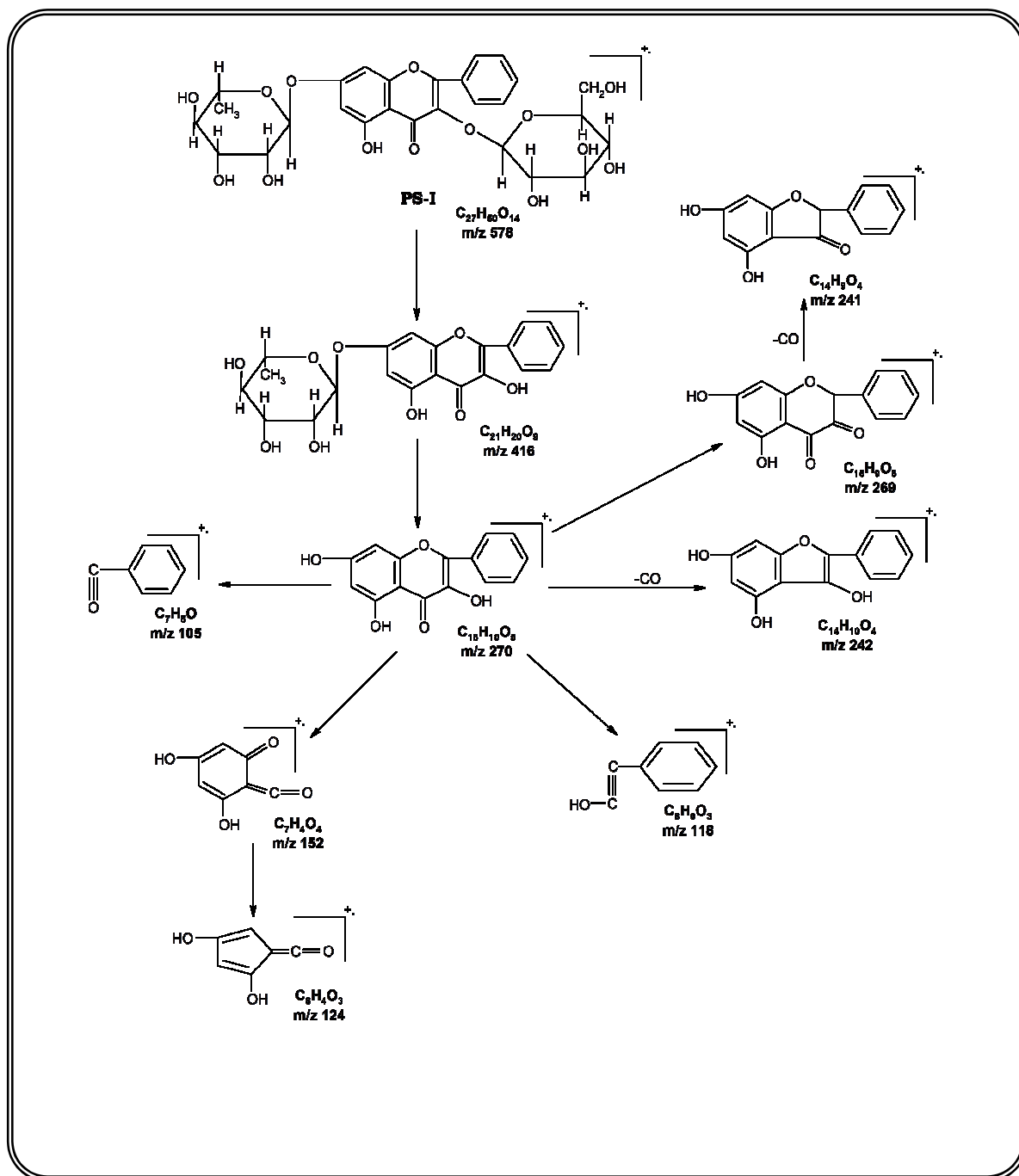
The different species obtained during the fragmentation are depicted in scheme-II and further supported the identity of the glycoside PS-I as Galangin-3-O- β -glucopyranosyl-7-O- α -L-rhamno-pyranoside.



MASS SPECTRUM Data File: 4EAP06I 6-APR- 4 11:36
Sample: PS-I PROF V K SAXENA SAGAR #6957
RT 0.12" FAB(Pos.) GC 1.4c BP: m/z 270.0000 Int. 46.2242 LV 0.00
Scan# (1 to 5)



SCHEME-II



EXPERIMENTAL

PLANT MATERIAL:

The roots of the plant *Acorus calamus* Linn were collected through the courtesy of the Agricultural Department of M.P. Government at Sagar and was identified by taxonomist of Botany Department of Dr. H.S. Gour University Sagar (M.P.). The herbarium specimen no. XXXIV has been deposited in the room no. 36 of the Chemistry Department of Dr. H.S. Gour University Sagar.

EXTRACTION OF THE FLAVONOL GLYCOSIDE PS-I

The air-dried and powdered roots (3.0kg) of the plant *Acorus calamus* Linn.were extracted with 95% ethanol in a round bottomed flask fitted with water condenser. The total ethanolic extract was concentrated under reduced pressure to get brown syrupy mass. The concentrated extract was then sequentially and successively partitioned with various solvents as: Petroleum ether (60-80°C), benzene, chloroform, ethyl acetate, acetone and methanol.

Except the chloroform soluble fraction other fractions as pet-ether (60-80°C) benzene, ethyl acetate, acetone and methanol were not studied phytochemically because they yielded a very small amount on concentration for any substantive study.

The chloroform soluble fraction was concentrated under reduced pressure. The concentrated chloroform extract was subjected to

chromatographic resolution over silica gel column for purification using CHCl_3 : MeOH mixtures in varying proportions viz. 8:1, 9:1 and 10:1.

COLUMN CHROMATOGRAPHY

S.No.	Parameter	Quantity
1.	Length of the column	160 cm.
2.	Diameter of the column	5.0 cm.
3.	Weight of the crude extract	6.0 g
4.	Weight of the silica gel G	140 g

The results obtained from the column chromatography are educe below:

S.No.	Fraction	Eluants	TLC Spot	Remarks
1.	1-5	CHCl_3 : MeOH (8:1)	Nil	No solid mass
2.	6-14	CHCl_3 : MeOH (9:1)	One	Compound PS-I
3.	15-25	CHCl_3 : MeOH (10:1)	Nil	No solid mass

STUDY OF THE FRACTIONS (6-14)

Eluates from CHCl_3 : MeOH (9:1) were collected and were found to have same R_F values and so were combined together. On evaporation of the solvent, it gave yellow compound. The compound was found to be homogenous on TLC examination. This was done by using solvent system: n butanol: acetic acid: water (4:1:5) and I_2 vapours as visualizing agent. It was crystallised from methanol as yellow crystals PS-I (yield 0.051%).

STUDY OF FLAVONOL GLYCOSIDE PS-I

The compound PS-I analysed for molecular formula, $C_{27}H_{30}O_{14}$, m.p.150-151°C and $[M^+]$ 578 (FAB-MS) .It was found to be soluble in water, ethanol and methanol.

The compound PS-I positively responded to Molisch's test for glycoside and all the characteristic colour reactions for flavonoids:

- (1) It produced green colour with $FeCl_3$.
- (2) It produced pink colour with Mg/HCl .
- (3) It gave yellow green fluorescent with liquid NH_3 .
- (4) It produced Molisch's test (violet ring) specific for glycoside.
- (5) It gave orange colour with Zn/HCl .

Elemental Analysis:

Found	Calculated For $C_{27}H_{30}O_{14}$
C= 56.04%	C= 56.05%
H=5.18%	H=5.19%
Molecular weight 578 by FAB-MS	

ACETYLATION OF THE FLAVONOL GLYCOSIDE PS-I

In a 100ml round bottomed flask, 70mg of compound was refluxed with 5.0ml of acetic anhydride and 20mg of sodium acetate for 7-8 hours on sand bath. The total reaction mixture after cooling was poured in ice-cold water, resulting in to brown precipitate when separated by filtration. The precipitate was mixed with water and then extracted with solvent ethanol

while shaking in a separating funnel which developed into two separate layers: ethanolic layer and aqueous layer. The ethanolic layer was separated, washed and dried over anhydrous sodium sulphate. After the removal of solvent, it yielded an acetylated derivative. The product obtained analysed for molecular formula $C_{40}H_{44}O_{21}$, m.p. 126-127°C and $[M^+]$ 860 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{40}H_{44}O_{21}$
C = 55.82%	C = 55.81%
H = 5.10%	H = 5.11%
% of acetyl group = 35.02%	% of acetyl group = 35.00%
Molecular weight 860 by FAB-sMS	

ACID HYDROLYSIS OF THE FLAVONOL GLYCOSIDE PS-I

In a round-bottomed flask of 250ml fitted with water condenser, 300mg of glycoside PS-I was taken, to which 10ml of 7% aqueous H_2SO_4 was added. Then the total reaction mixture was allowed to reflux for 7-8 hours. The contents of the flask were extracted with water. The aqueous layer was shaken with solvent ether solvent in a separating funnel. The ethereal layer was separated, washed and dried over anhydrous sodium sulphate. After evaporation of the solvent ether, yellow crystals of aglycone were obtained. The aqueous layer in hydrolysate was worked up separately for identification of sugar(s).

STUDY OF THE AGLYCONE PS-I (A)

The aglycone PS-I (A) was analysed for m.f. $C_{15}H_{10}O_5$, m.p. 213-214°C and $[M^+]$ 270 (FAB-MS).

It responded to all colour reactions of flavonoids as described on page 76 of this thesis. It did not respond to Molish's test.

Elemental Analysis: Found	Calculated For $C_{15}H_{10}O_5$
C = 66.66%	C = 66.67%
H = 3.70%	H = 3.71%
Molecular weight 270 by FAB-MS	

ACETYLATION OF THE AGLYCONE PS-I (A)

The acetylation of the aglycone PS-I (A) was performed in a similar manner as described for glycoside on page no. 76 of this thesis.

It forms a diacetate derivative because hydroxyl group at position C-5 was not acetylated due to the formation of intramolecular hydrogen bonding.

The aglycone analysed for molecular formula $C_{19}H_{16}O_7$, M.P. 141-142°C and $[M^+]$ 356 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{19}H_{16}O_7$
C = 64.03%	C = 64.04%
H = 4.48%	H = 4.49%
% of acetyl group = 24.14%	% of acetyl group = 24.15%
Molecular weight 356 by FAB-MS	

AKALINE DEGRADATION OF THE AGLYCONE PS-I (A)

In a 250 ml of B-14 ground joint flask, 30 mg of the aglycone PS-I (A) was treated with 20 ml of 50% of ethanolic KOH and refluxed for 8 hrs. The total reaction mixture after cooling was acidified with HCl and degraded product was extracted with ether solvent in a separating funnel. The ethereal layer was separated, washed with water and then divided into two parts.

- (1) The first fraction of the ethereal layer was treated with 10% NaOH solution, when afforded compound (I). The compound (I) was identified as phloroglucinol, molecular formula $C_6H_6O_3$, m.p. 180-191°C, (by mixed m.p. and CoTLC).
- (2) The second fraction of the ethereal layer was treated with 50% of $NaHCO_3$ solution when yielded a compound (II), molecular formula $C_7H_6O_2$, m.p. 121-122°C, and identified as benzoic acid (by mixed m.p. and CoTLC with authentic sample)

MILD ACID HYDROLYSIS OF GLYCOSIDE PS-I

The glycoside 50 mg was taken in a conical flask (250 ml) to which 1N HCl was added. The mixture was refluxed for 10 to 20 min. at room temperature. The contents of the flask were cooled and extracted with ethanol. The ethanol soluble portion on TLC examination showed two spots. The mixture was chromatographed over silica gel column. The column was eluted with $CHCl_3$: CH_3OH in various proportions. The details of column chromatography are tabulated below:-

S.No.	Parameter	Quantity
1.	Length of the column	150 cm.
2.	Diameter of the column	3.0 cm.
3.	Weight of the crude extract	65mg
4.	Weight of the silica gel G	100 g

The results obtained from the column chromatography are educe below:

S.No.	Fraction	Eluants	TLC Spot	Remarks
1.	1-8	CHCl ₃ : MeOH (7:0)	Nil	No solid mass
2.	6-14	CHCl ₃ : MeOH (7:1)	Nil	No solid mass
3.	15-25	CHCl ₃ : MeOH (7:4)	One	Compound PS-I

STUDY OF THE FRACTIONS (15-25)

Eluates from CHCl₃: MeOH (7:4) were collected and were found to have same R_F values and so were combined together. Evaporation of the solvent, gave proaglyconePS-I(B), which was crystallized from methanol. The proaglycone analysed for molecular formula C₂₀ H₂₀ O₉, m.p. 230-231°C and [M⁺]=404 (FAB-MS).

Elemental Analysis:

Found	Calculated For C ₂₀ H ₂₀ O ₉
C = 60.03%	C = 59.80%
H = 4.92%	H = 4.90%
Molecular weight 404 by FAB-MS	

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE PS- I (B)

In a 150 ml conical flask, proaglycone PS- I (B) (20 mg) was refluxed with methyl iodide (3 ml) and Ag_2O (20 mg) in a dimethyl formamide (50ml). The precipitate obtained was filtered and filtrate was hydrolysed with 7% H_2SO_4 and refluxed for 7-8 min. The resultant mixture was further extracted with CHCl_3 and gave aglycone and methylated sugars.

The aqueous hydrolysate after separation from aglycone was neutralised with BaCO_3 and BaSO_4 was filtered off. After concentration it was examined on paper chromatography on Whatmann filter paper No. 1 using solvent system: nBAW (4:1:5) and visualizing agent as aniline hydrogen phthalate. Then sugar was identified as 2'', 3'', 4''-tri-O-methyl-L-rhamnose (CoPC and CoTLC)).

IDENTIFICATION OF THE SUGAR (S) AFTER HYDROLYSIS

The aqueous hydrolysate obtained after the acid hydrolysis of the glycoside PS-I was neutralised with BaCO_3 and BaSO_4 was filtered off. The resultant filtrate was concentrated and then subjected to the paper chromatography using solvent system n butanol: acetic acid: water (4:1:5) and aniline hydrogen phthalate as visualizing agent. The development of chromatogram depicted one spot.

The results of paper chromatography are given below in table-2.9: -

Table – 2.9
Identification of Sugars

S. No.	<i>Solvent system</i>	R _f Value		<i>Sugar identified</i>
		Reported	Found	
1.	n- butanol: acetic acid: water (4:1:5)	0.18	0.19	D-Glucose
		0.38	0.36	L-rhamnose

PERMETHYLATION OF THE GLYCOSIDE PS-I

In a 100 ml conical flask, 100 mg of glycoside PS-I was refluxed with methyl iodide (6 ml) and Ag₂O (20 mg) in a DMF (5ml). The total reaction mixture was stand for 4 days. The contents of the flask were filtered and filtrate was washed with DMF. After concentration under reduced pressure, it was hydrolysed with 1N HCl and refluxed for 10-15 min. It yielded aglycone PS-I (A) and methylated sugar.

The aglycone PS-I (A) was separated and the aqueous hydrolysed was neutralised with BaCO₃ and BaSO₄ was filtered off. The filtrate was concentrated under reduced pressure and then subjected to paper chromatography using Whatmann filter paper No.1, solvent system: n-butanol: acetic acid: water (4: 1: 5) and visualizing agent as aniline hydrogen phthalate for identification of sugar. The methylated sugars were identified as 2", 3", 4", 6"-tetra-O-methyl-D-glucose and 2", 3", 4",-tri-O-methyl-L-rhamnose (CoPC and CoTLC).

PERIODATE OXIDATION OF THE GLYCOSIDE PS- I

In a 250 ml conical flask, fitted with glass stopper, glycoside PS-I (25 mg) was dissolved in methanol (15 ml) and treated with sodium meta periodate (3ml). The reaction mixture was then left for 48 hours at room temperature. Simultaneously a blank titration was run with same method. The quantity of sodium meta periodate used up and formic acid produced was estimated by Jone's procedure.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE PS-I

25 mg of the glycoside PS-I was dissolved in (25 ml) MeOH and treated with almond emulsion (20 ml) in a 100 ml conical flask. The total reaction mixture was allowed to stand at room temperature for 48 hrs and then filtered. The concentrated hydrolysate was examined over paper chromatography using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as visualizing agent .The sugar was identified as D- glucose (R_f 0.019).

The glycoside was dissolved in (20 ml) MeOH and was mixed with an equal volume of Takadiastase enzyme solution in a 150 ml conical flask. The contents were allowed to stand for 48 hrs and then filtered. The aqueous hydrolysate was concentrated under reduced pressure. The concentrated hydrolysate was examined for sugar by paper chromatography. The sugar was identified as L-rhamnose (R_F 0.38) using solvent system n-BAW (4: 1: 5) and visualizing agent as aniline hydrogen phthalate.

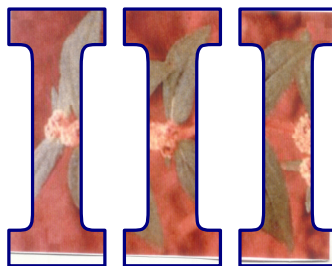
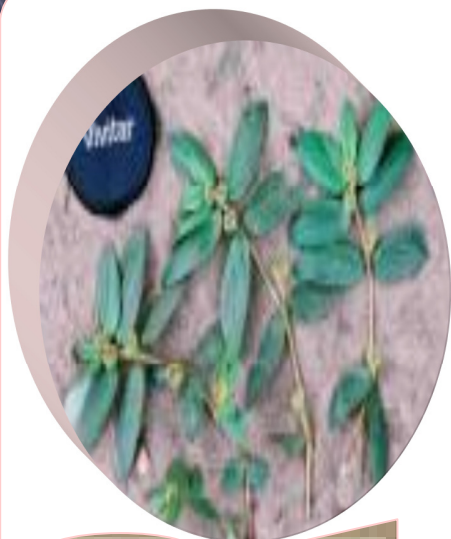
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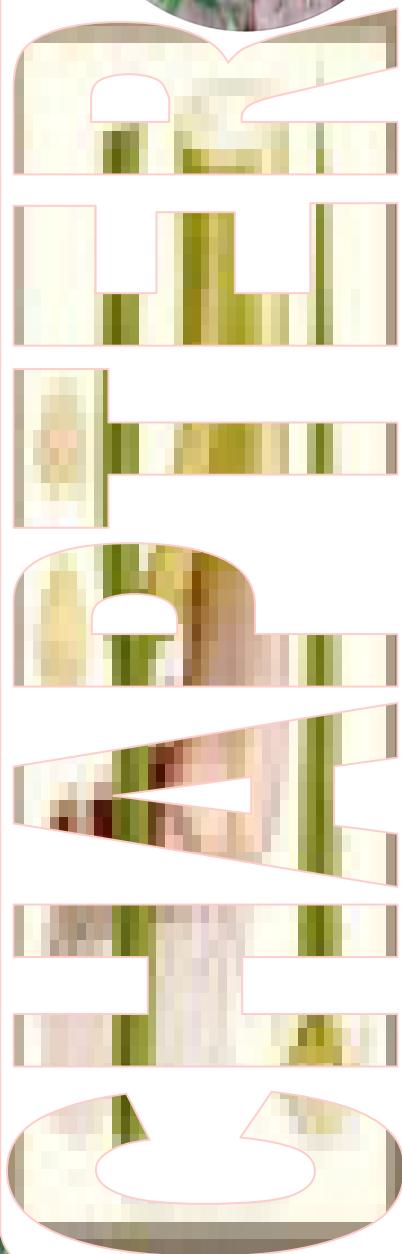
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*If you aspire to the highest place, it
is no disgrace to reach at the second
or even the third place.*



ISOLATION AND STUDY OF A
FLAVONE-C-GLYCOSIDE PS-II:
LUTEOLIN-8-C- β -D-
GLUCOPYRANOSIDE FROM
THE ROOTS OF *ACORUS*

*This work has been communicated for
publication in the *Journal of Institution of
Chemists (India)*.

A*corus calamus*¹⁻⁶ belongs to natural order Araceae and is commonly known as 'Bach' in Hindi. It is found mainly in marshy area.

The roots of the *Acorus calamus* have been reported to possess anti-asthmatic properties.

Since, no systematic phytochemical examination has been reported on the roots of this plant and also, in view of its medicinal importance it was thought worthwhile to carryout further phytochemical investigation on the roots of this plant.

ISOLATION OF THE FLAVONE-C-GLYCOSIDE

Air-dried and powdered roots (2.0 kg) of the plant *Acorus calamus* were extracted with 95% ethanol. The ethanolic extract was concentrated under reduced pressure to get a brown viscous mass. The concentrated extract was partitioned between diethyl ether and water. The aqueous layer was extracted repeatedly with ethyl acetate. The concentrated ethyl acetate extract on TLC examination using solvent system: CHCl₃: MeOH (89:11) and visualizing agent: 10% aqueous H₂SO₄ displayed a mixture of three compounds. It was therefore, purified by column chromatography over silica gel G using CHCl₃: MeOH as an eluants in varying proportions i.e.2: 1, 3: 1 and 3: 2. Eluants collected from CHCl₃: MeOH (3: 2) were found to have same R_f values and therefore were mixed together which on evaporation of the solvent gave a yellow amorphous mass. Its homogeneity was confirmed by TLC [solvent system nBuOH: HOAc: H₂O (4:1:5) and I₂ vapours as

visualising agent]. The amorphous mass on crystallisation from methanol afforded a yellow crystalline compound PS-II (0.0985%) (FIG. 3.1).

STUDY OF THE FLAVONE-C-GLYCOSIDE PS-I

The compound was crystallised from methanol as yellow crystals. It analysed for the molecular formula $C_{21}H_{20}O_{11}$, m.p. 264-265°C and $[M^+]$ 448 (FAB-MS).

It responded to all characteristics colour reactions of flavonoids⁷⁻⁹ and positive Molisch's test for glycoside. It was found to be soluble in chloroform, methanol and ethanol.

UV SPECTRUM OF THE GLYCOSIDE PS-II

The flavone-C-glycoside PS-II gave following bands in UV spectrum, which are tabulated in table- 3.1.

Table - 3.1

S.No.	Solvent+(Shift Reagent) λ_{Max}	Shift in nm + λ	
		II	I
1.	MeOH	270	334
2.	MeOH + AlCl ₃	292	361
3.	MeOH +AlCl ₃ /HCl	294	358
4.	MeOH + NaOMe	286	399
5.	MeOH + CH ₃ COONa	310	392
6.	MeOH + CH ₃ COONa/H ₃ BO ₃	282	439

SCHEME OF EXTRACTION AND ISOLATION

ISOLATION-CHART

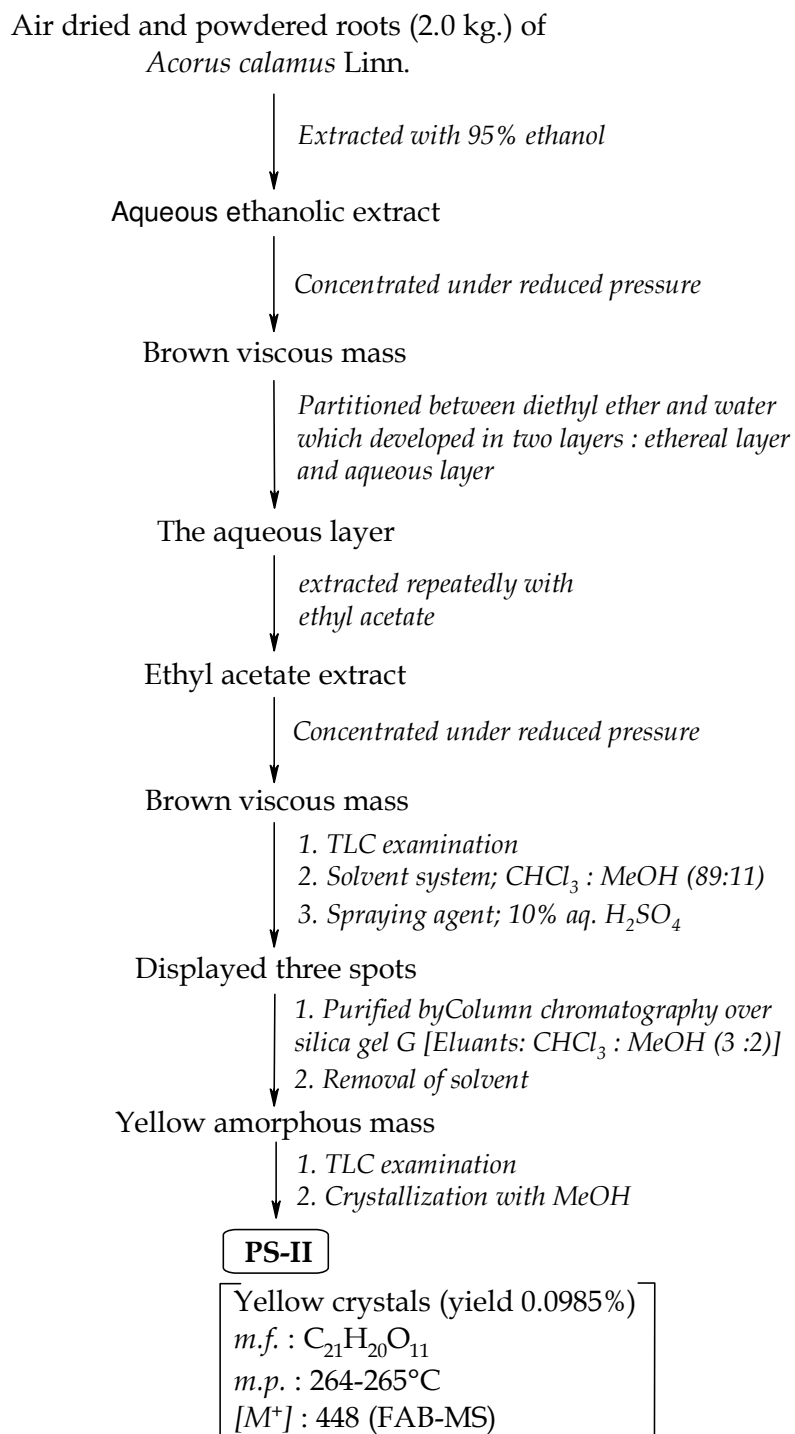


FIG. 3.1: Fractionation protocol for isolation of compound PS–II

from Plant *Acorus calamus*

IR SPECTRUM OF THE GLYCOSIDE PS-II

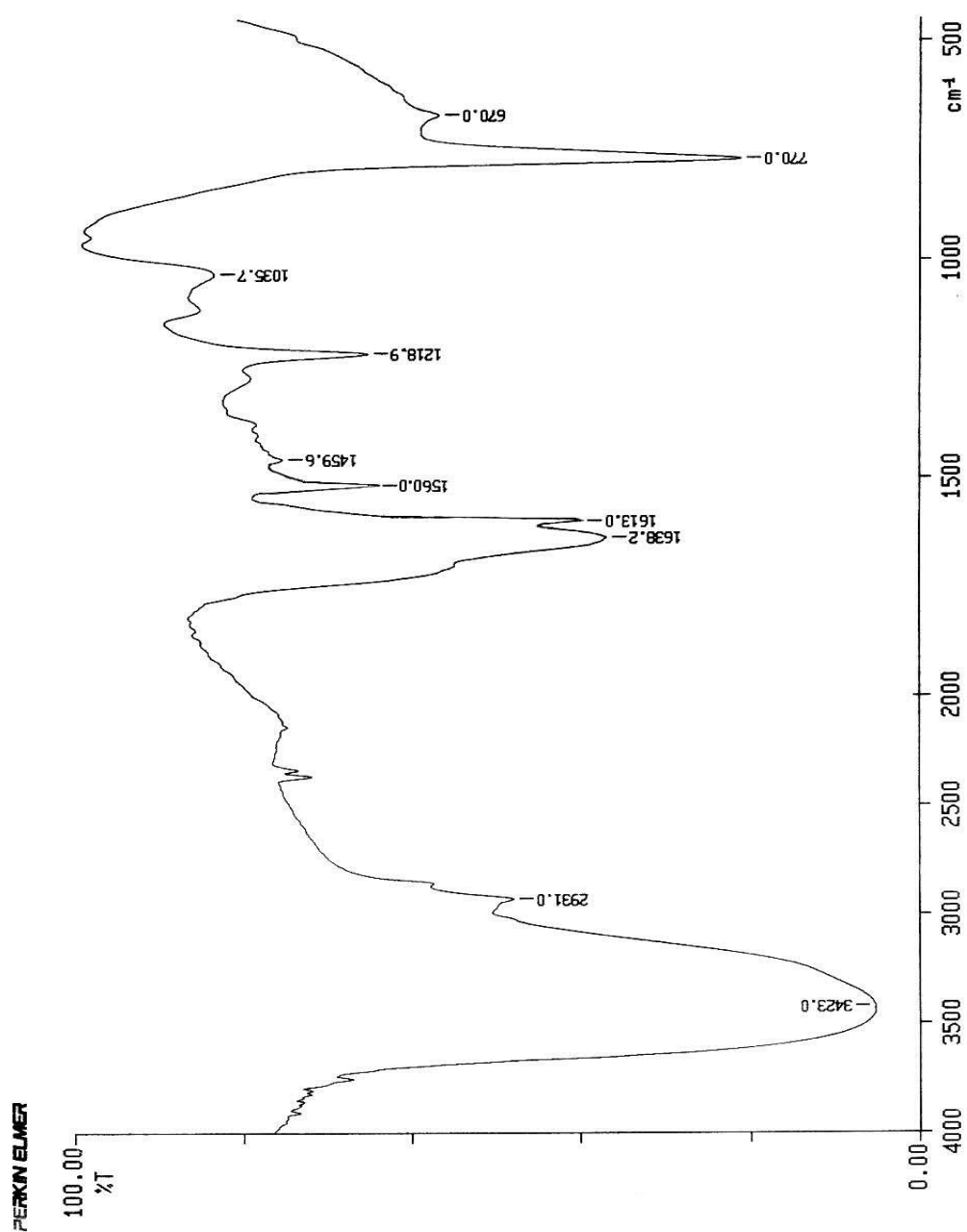
The significant bands obtained in the IR spectrum of the glycoside PS-II [FIG. 3.2] and structural units assigned to the molecule with the help of the available literature¹⁰⁻¹³ are tabulated below in table-3.2: -

Table- 3.2

IR spectrum of the Glycoside PS-II

S.No.	Wave Number cm ⁻¹	Assignment
1.	3423.0	-OH group(s)
2.	2931.0	-C-H Stretching vibration
3.	1638.2	α , β Unsaturated C = O group
4.	1613.0, 1560.0, 1459.6	Aromatic ring system
5.	1218.9	C-O-C bending vibration
6.	1035.7	C-O-C Stretching vibration
7.	770.0, 670.0	Monosubstituted benzene ring

IR SPECTRUM OF THE GLYCOSIDE PS-II



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X: 1 scan, 4.0cm-1, flat, smooth, abex

PRESENCE OF HYDROXYL GROUP (S) IN THE GLYCOSIDE PS-II

The characteristic IR band in the IR spectrum of PS-II obtained at $\nu_{\max}^{\text{kBr}}$ 3423.0 cm^{-1} , which showed the presence of –OH group(s) in it. The numbers of hydroxyl group(s) were quantitatively estimated by the acetylation of the compound with Ac_2O /pyridine, when, it furnished an acetylated product, which analysed for molecular formula $\text{C}_{37}\text{H}_{36}\text{O}_{19}$, m.p. 144-145°C and $[\text{M}^+]$ 784 (FAB-MS).

The percentage of acetyl group was determined by Weisenberger¹⁴ method as described by Belcher and Godbert¹⁵ and found to be 42.80%. Thereby indicating the presence of eight acetylatable hydroxyl groups in the glycoside.

Further the acetylatable product did not show any band at 3423.0 cm^{-1} , which revealed that all the hydroxyl groups(s) had under gone acetylation.

METHYLATION¹⁶ OF THE GLYCOSIDE PS-II

The glycoside PS-II was methylated with ethereal solution of the diazomethane, which was prepared by the action of 50% aq. solution of KOH on nitroso-methyl urea (9.0 gm) in ether solvent.

The total reaction mixture was allowed to stand for whole day and ethereal layer obtained was washed with NaHCO_3 and then dried over anhydrous sodium sulphate. After crystallisation from ether solvent colourless prisms were obtained.

The methylated product analysed for the molecular formula, $\text{C}_{25}\text{H}_{28}\text{O}_{11}$, m.p. 273-274°C and $[\text{M}^+]$ 504 (FAB-MS).

ACID HYDROLYSIS OF THE GLYCOSIDE PS-II

The glycoside PS-II on acid hydrolysis with 7% aqueous H_2SO_4 for 7 hours at 100°C afforded no sugar and aglycone thus indicated the glycoside to be C-glycosyl compound. This was further supported by the presence of two bands¹⁷ at $\nu_{\text{max}}^{\text{kBr}}$ 1047 cm^{-1} and 1015 cm^{-1} in the IR spectrum of glycoside PS-II. A significant peak in the mass spectrum at m/z 299 of the glycoside showed the presence of the glycosyl unit in the glycoside as C-glycosyl moiety.¹⁸

HYDRIODIC ACID FISSION¹⁹ OF THE GLYCOSIDE PS-II

The glycoside PS-II on prolonged boiling with hydriodic acid in phenol yielded a yellow precipitate, which was separated by filtration. Further, the precipitate on crystallisation from methanol afforded yellow crystals of the aglycone and simultaneously caused the decomposition of the sugar moiety.

STUDY OF THE AGLYCONE PS-II (A)

The aglycone was crystallised from methanol as yellow crystals. It analysed for the molecular formula, $\text{C}_{15}\text{H}_{10}\text{O}_6$, m.p. $323\text{--}324^\circ\text{C}$ and $[\text{M}^+]$ 286 (FAB-MS).

It gave all characteristic colour reactions of the flavonoids⁷⁻⁹ and was found to be readily soluble in aqueous Na_2CO_3 , and aqueous NaOH giving deep yellow solution. It was also found to be soluble in methanol and ethanol.

UV SPECTRUM OF THE AGLYCONE PS-II (A)

The wavelengths of maximum absorption as observed with the various shift reagents are recorded below in table-3.3:-

Table–3.3

S.No.	Solvent + (Shift reagents)	Shift in (nm) + $\lambda_{\max}$	
		II	I
1	MeOH	276	337
2	MeOH + AlCl ₃ /HCl	292	364
3	MeOH + NaOMe	294	~330
4	MeOH + CH ₃ COONa	291	384
5	MeOH + CH ₃ COONa/H ₃ BO ₃	285	354

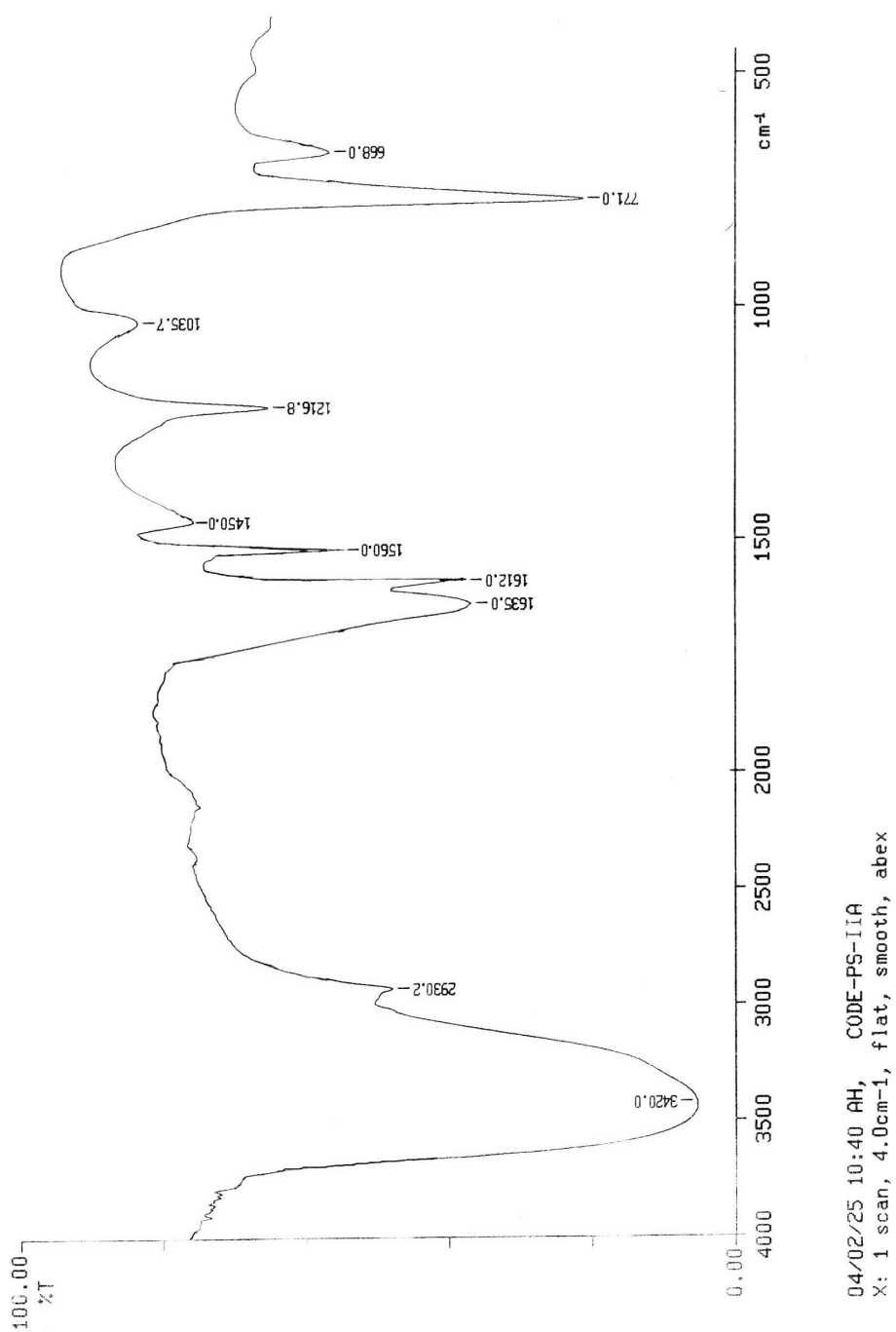
IR SPECTRUM OF THE AGLYCONE PS-II (A)

The bands obtained in the IR spectrum of the aglycone PS-II (A) [FIG.3.3] and structural units assigned to the molecules with the help of the available literature²⁰⁻²² are tabulated below in table-3.4 :-

Table-3.4

S.No.	Wave Number cm ⁻¹	Assignment
1	3420.0	-OH group(s)
2	1635.0	α , β unsaturated C=O group
3	1612.0, 1560.0, 1450.0	Aromatic ring system.
4	2930.2	C-H stretching mode
5	1216.8	C-O-C bending vibration
6	1035.7	C-O-C stretching vibration
7	771.0, 668.0	Monosubstituted benzene ring.

IR SPECTRUM OF THE AGLYCONE PS-II (A)



PRESENCE OF –OH GROUP (S) IN THE AGLYCONE PS-II (A)

The characteristic band appeared at $\nu_{\max}^{\text{KBr}}$ 3420.0 in the IR spectrum, of aglycone PS-II (A) indicated the presence of –OH group in it. The aglycone on acetylation with acetic anhydride and pyridine yielded an acetylated product.

The acetylated product analysed for the molecular formula $\text{C}_{23}\text{H}_{18}\text{O}_{10}$, m.p. 221-222°C and $[\text{M}^+]$ 454 (FAB-MS).

The percentage of the acetyl group was determined by Weisenberger¹⁴ method as described by Belcher and Godbert¹⁵ which revealed the presence of four hydroxyl groups in the acetylated product (37.18%).

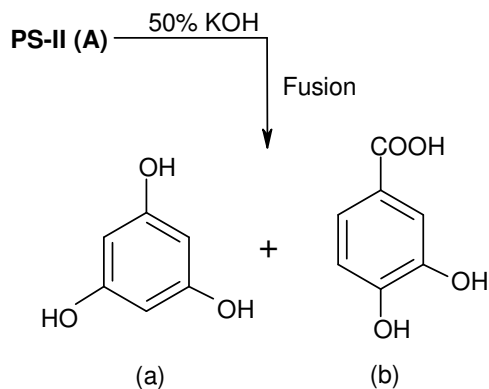
POSITION OF THE –OH GROUPS IN THE AGLYCONE PS-II (A)

The positions of various hydroxyl groups were established by its alkaline degradation, various specific colour reactions and various shifts in UV spectrum of the aglycone PS-II (A).

ALKALINE DEGRADATION OF THE AGLYCONE PS-II (A)

The aglycone PS-II (A) on alkaline degradation²³ with 50% ethanolic KOH afforded two compounds, which were identified as:-

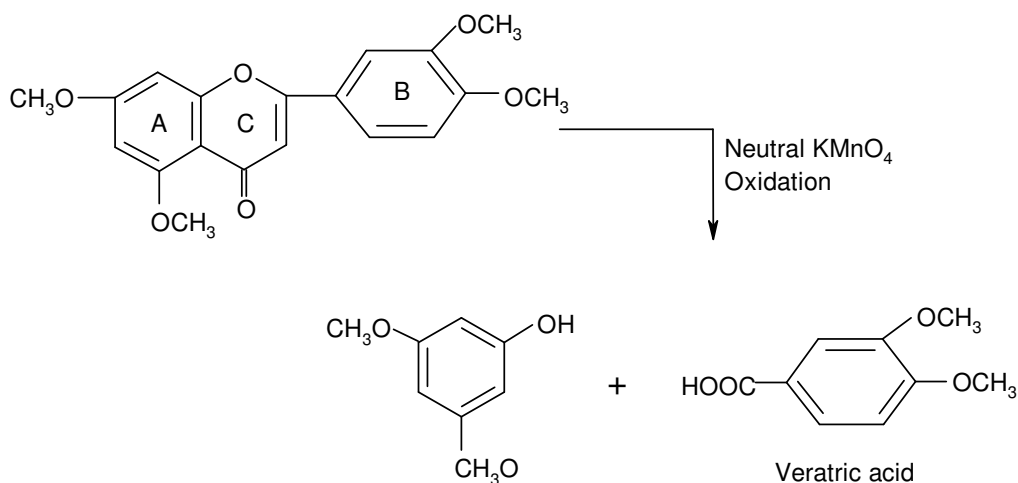
1. Phloroglucinol (a), m.f. $\text{C}_6\text{H}_6\text{O}_3$, m.p. 114-115°C, $[\text{M}^+]$ 126.
2. Protocatechuic acid (b), m.f. $\text{C}_7\text{H}_6\text{O}_4$, m.p. 202-203°C, $[\text{M}^+]$ 154.



The two products formed indicated the presence of –OH groups at position. C-5, 7, 3', 4' in the aglycone.

POTASSIUM PERMANGNATE OXIDATION OF THE METHYLATED AGLYCONE

Tetra-O-methyl ether of the aglycone when mixed with neutral KMnO_4 afforded a compound, which was identified as veratric acid, molecular formula $\text{C}_9\text{H}_{10}\text{O}_4$, m.p. $179\text{--}180^\circ\text{C}$ and $[\text{M}^+]$ 182 (FAB-MS).



The formation of veratric acid indicated that the ring B was deficit glycosyl unit.

POSITION OF HYDROXYL GROUPS IN PS-II (A):

The positions of all the hydroxyl groups were ascertained by observing and comparing the UV spectral data in various shift reagents.

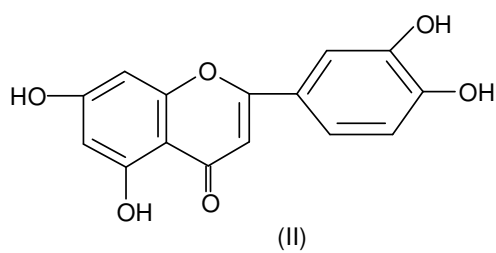
POSITION OF HYDROXYL GROUPS AT C-5 AND C-7 POSITION:

- (a) The formation of phloroglucinol on alkaline degradation of the aglycone confirmed the presence of -OH group(s) at C-5 and C-7 position.
- (b) A bathochromic shift of 27 nm in band-I with AlCl_3/HCl relative to MeOH in UV spectrum of the aglycone suggested the presence of hydroxyl group at position C-5.⁸
- (c) A bathochromic shift of 15 nm in band-II with NaOAc relative to the band-II in MeOH confirmed the presence of a hydroxyl group at position C-7⁸ which was further confirmed by viewing a shoulder at 330 nm in band-I with NaOMe.
- (d) The aglycone PS-II (A) gave yellow fluorescent colour under UV spectrum, which showed the presence of free hydroxyl group at position C-5.⁴⁸

POSITION OF HYDROXYL GROUPS AT C-3' AND C-4' POSITION

- (a) The formation of protocatechuic acid on alkaline degradation showed the presence of -OH group at position C-3' and C-4'
- (b) A bathochromic shift of 17nm in the UV spectrum of the aglycone with NaOAc/ H_3BO_3 in band-I relative to MeOH in Band-I indicated the presence of free O-dihydroxy substitution in ring-B, thereby confirming the presence of hydroxyl groups at position C-3' and C-4' in ring-B.⁸

On the basis of the above evidences, the tentative structure to the aglycone PS-II (A) was assigned as 5, 7, 3', 4' tetrahydroxyflavone.



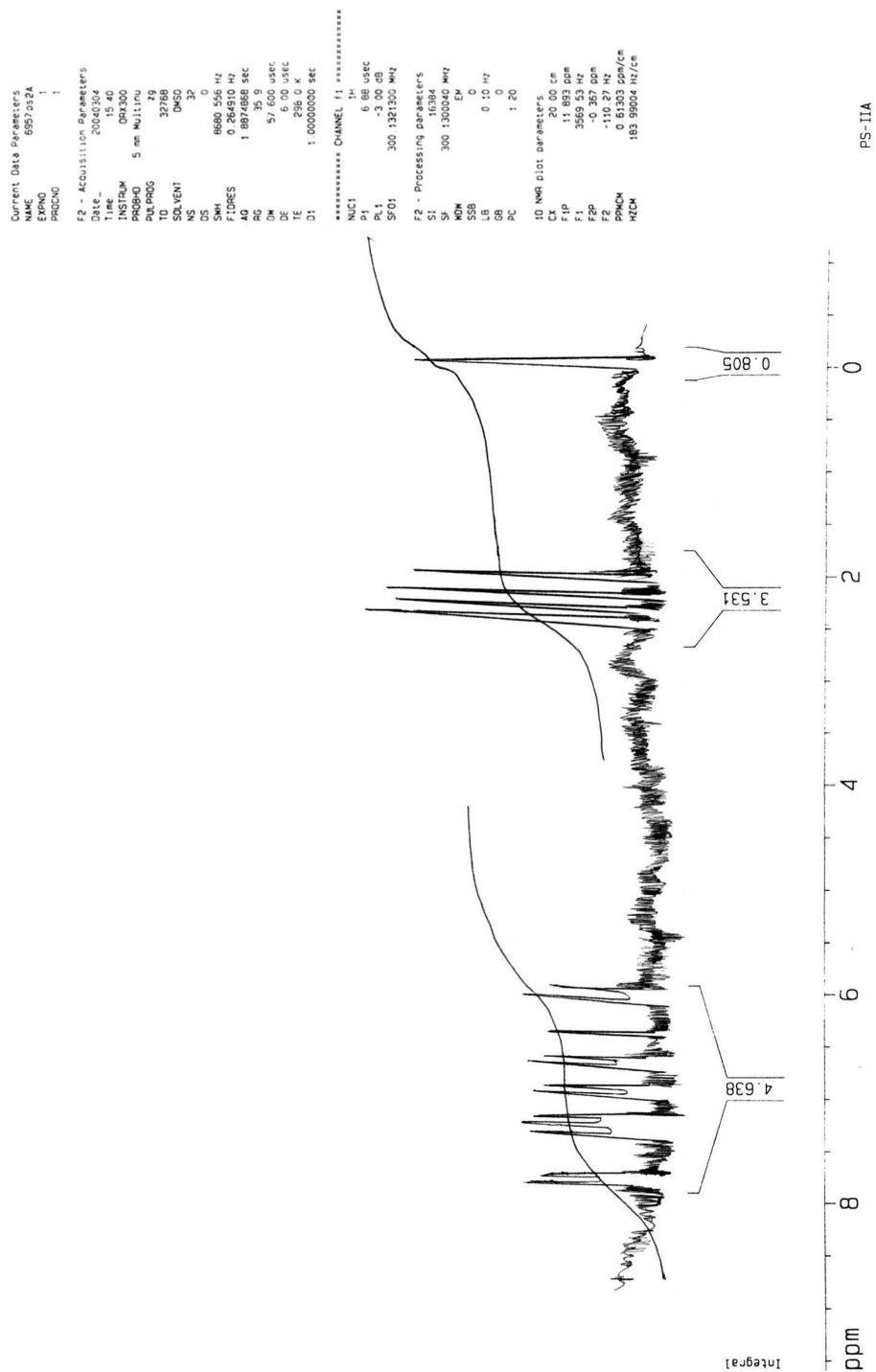
¹H-NMR SPECTRUM OF THE AGLYCONE PS-II (A)

The chemical shifts obtained in the ¹H-NMR spectrum of the aglycone PS-II (A) [FIG.3.4] and the structural units inferred with the help of available literature²⁴⁻²⁶ are given below in table –3.5.

Table-3.5

S.No.	δValue	Pattern	J-Value (Hz)	No. of Protons	Assignments
1.	6.50	s	--	1	H-3
2.	6.26	d	2.4	1	H-6
3.	6.75	d	2.4	1	H-8
4.	7.74	d	7.5	1	H-2'
5.	6.93	d	7.5	1	H-5'
6.	7.56	dd	7.6,1.8	1	H-6'
7.	2.41	s	-	3	OAc-5
8	2.42	s	-	3	OAc-7
9.	2.28	s	-	3	OAc-3'
10.	2.30	s	-	3	OAc-4'

¹H-NMR SPECTRUM OF THE AGLYCONE PS-II (A)



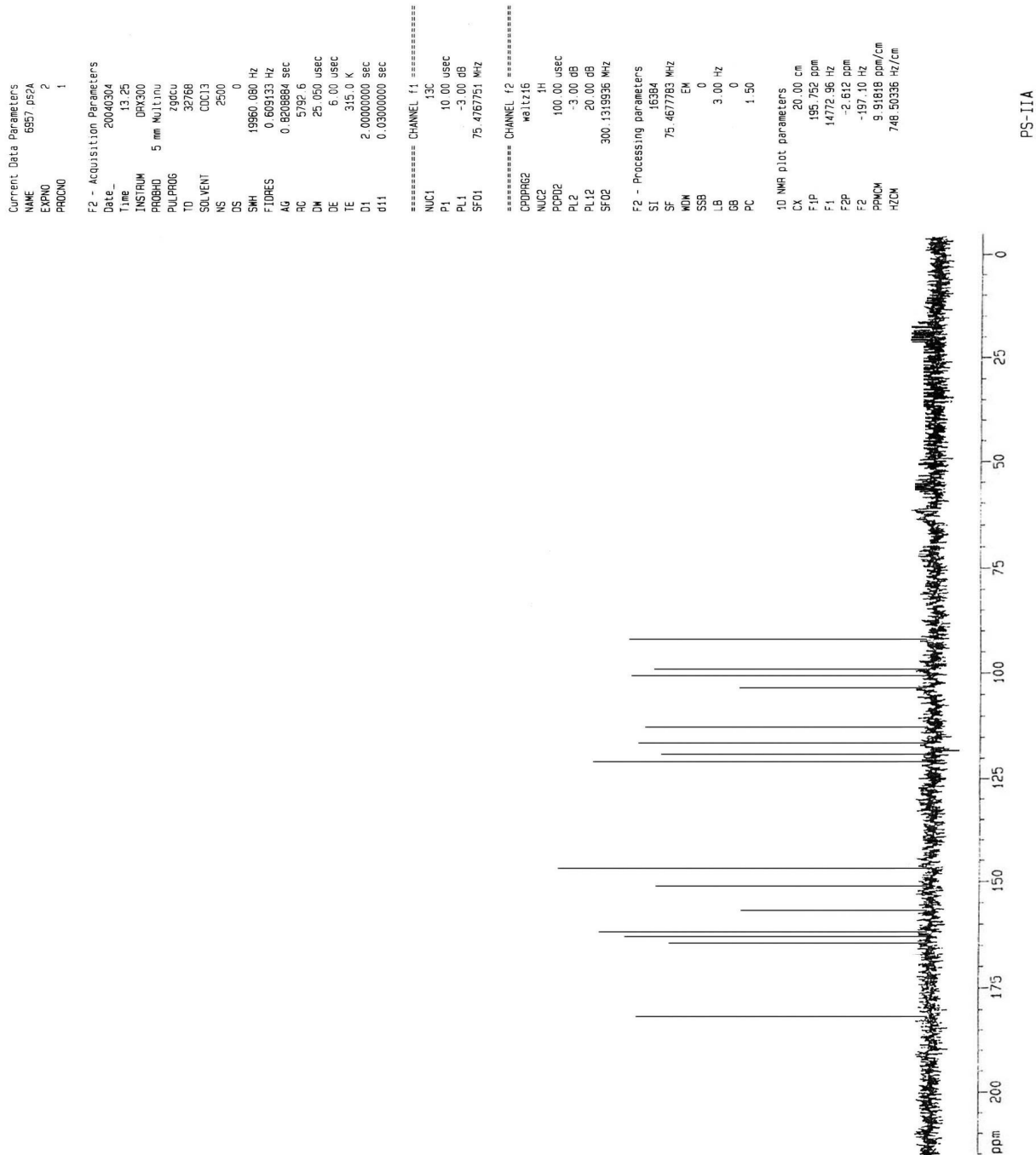
¹³C-NMR SPECTRUM OF THE AGLYCONE PS-II (A)

The significant chemical shifts obtained in the ¹³C-NMR spectrum of the aglycone PS-II (A) [FIG. 3.5] and the structural units inferred with the help of available literature^{8, 34,, 47}are given in table-3.6 :-

Table – 3.6

S.No.	δ Value	Carbon No.	Carbon Type
1.	164.2	C-2	C
2.	102.2	C-3	CH
3.	182.2	C-4	C=O
4.	162.1	C-5	C
5.	99.2	C-6	CH
6.	164.3	C-7	C
7.	94.2	C-8	C
8.	157.6	C-9	C
9.	104.2	C-10	C
10.	122.0	C-1'	C
11.	113.7	C-2'	CH
12.	146.3	C-3'	CH
13.	150.1	C-4'	CH
14.	116.4	C-5'	CH
15.	119.2	C-6'	CH

¹³C-NMR SPECTRUM OF THE AGLYCONE PS-II (A)

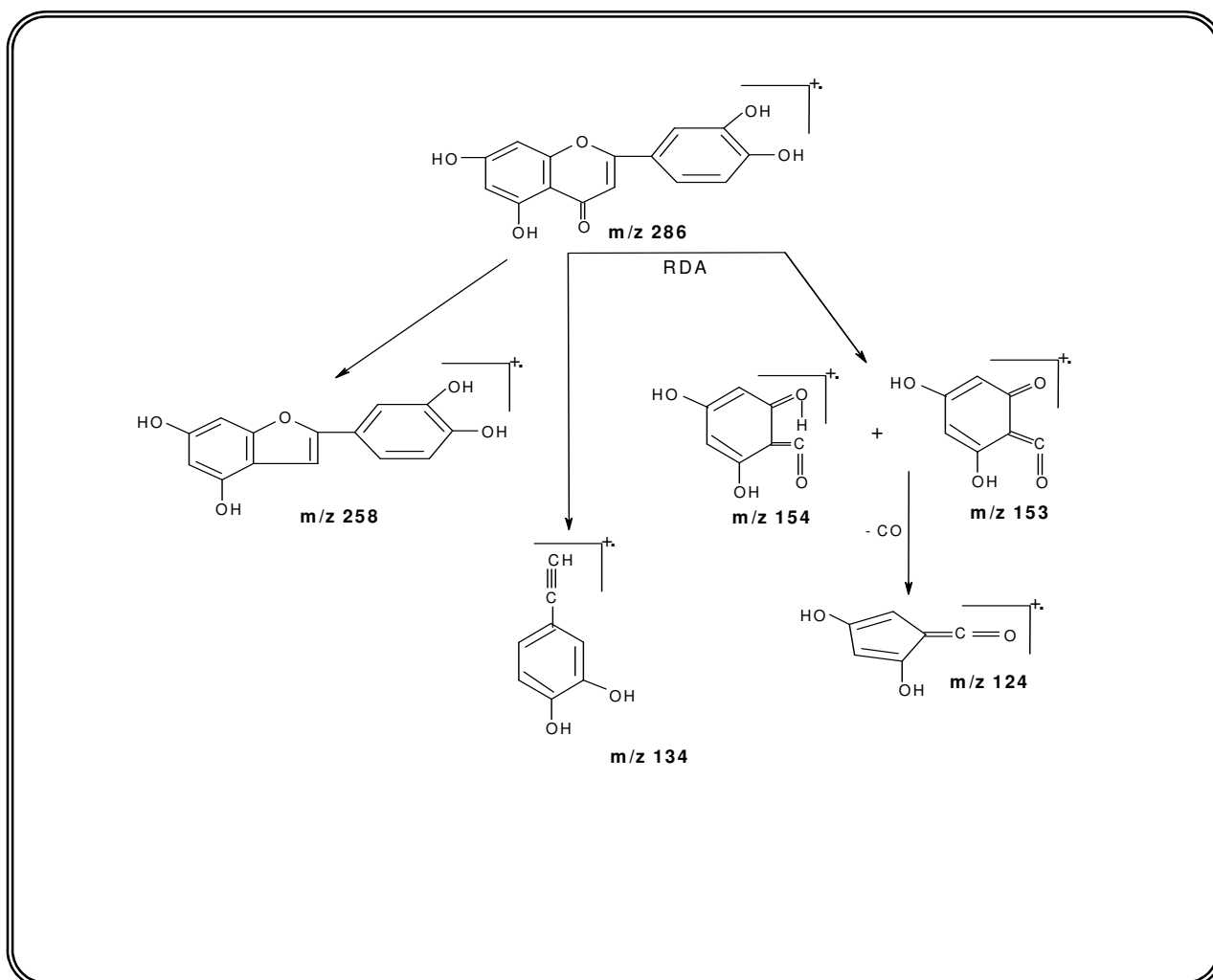


MASS SPECTRUM OF THE AGLYCONE PS-II (A)

The fragment ion peaks obtained in the FAB-MS of the aglycone PS-II (A) are as follows in scheme I.

m/z 286, $[M^+]$ 258, 153, 152, 134 and 124.

SCHEME-I



OXIDATION OF THE GLYCOSIDE PS-II WITH FERRIC CHLORIDE

50mg of the glycoside PS-II on oxidation²⁷ with FeCl_3 (45mg) in water (2ml) when refluxed at 115°C for 8 hours afforded a dark coloured mixture. The mixture when extracted with water was separated by filtration. The filtrate was extracted several times with amyl alcohol to give yellow coloured solution which when passed through a column of silica gel G using water as an eluant to remove completely all the iron and chloride ions. The syrupy product was obtained was examined by paper chromatography on Whatmann filter paper No.1 using a solvent system as n-BuOH: pyridine: water (6: 4: 3) and aniline: hydrogen phthalate as visualizing agent. It showed the presence of D-glucose and D-arabinose, thereby confirming the presence of D-glucose (R_F 0.24) unit in the glycoside as identified by comparison with an authentic sample.

PERIODATE OXIDATION OF METHYLATED GLYCOSIDE AND HYDROLYSIS OF OXIDATION

PRODUCT

Periodate oxidation of the methylated glycoside (120mg) with NaIO_4 (3ml) and MeOH (15ml) when allowed to stand for overnight afforded an oxidised product, which was hydrolysed with 1N HCl. It was then separated by filtration. The filtrate when treated with 2, 4-dinitrophenyl hydrazine afforded a yellow precipitate, which on TLC examination displayed two spots (R_F 0.41 and 0.11). These spot's were identified as 2, 4-dinitrophenyl hydrazone derivatives of glyceraldehyde and pyruvaldehyde. Further they were purified by column chromatography using eluants as benzene:

ethylacetate (4: 1). Thereby confirming the presence of glucose moiety in the glycoside.^{28, 29}

PERIODATE OXIDATION OF THE METHYLATED GLYCOSIDE FOLLOWED BY SODIUM

BOROHYDRIDE REDUCTION

Periodate oxidation of the methylated glycoside followed by the reduction of the oxidized product with sodium borohydride (NaBH_4)³⁰ afforded a product. The product obtained was hydrolyzed with 1N HCl, when it furnished a glycerol (Co PC, Co TLC). This suggested that the sugar moiety was present in pyranose form in the glycoside.^{31, 32}

PERIODATE OXIDATION OF THE METHYLATED GLYCOSIDE

(50mg) Methylated glycoside on periodate oxidation with NaIO_4 ³³ consumed 2.1 moles of periodate and in turns produced 1.06 moles of formic acid, thereby suggested that the D-glucose and aglycone were present in equimolar ratio (1:1) which also confirmed that the sugar was present in the form of pyranose structure.

POSITION OF ATTACHMENT OF THE SUGAR TO THE AGLYCONE PS-II (A)

The position of attachment of the sugar moiety at C-8 position in the ring A was confirmed by following details of aglycone PS-II (A) to that of glycoside (PS-II).

(A) The aglycone gave green colour with FeCl_3 , which showed the presence of $-\text{OH}$ group at C-5 position.⁸

- (B) The aglycone gave bright yellow colour with boric acid, which suggested the presence of –OH group at position C-5.⁸
- (C) The formation of phloroglucinol on alkaline degradation confirmed the presence of –OH group at C-5 and C-7 position.^{8, 46}
- (D) The bathochromic shift of 27nm in band I with AlCl₃ / HCl relative to MeOH indicated the presence –OH group at C-5 whereas the bathochromic shift of 15nm in band II, on addition of NaOAc relative to MeOH indicated the presence of free –OH group at C-7 position in the aglycone.^{45, 46}
- (E) The methylated glycoside on oxidation with KMnO₄ afforded a veratric acid which suggested that the ring B was devoid of glycosylation.^{33,46}
- (F) After considering the above views it has been suggested that the position C-6 and C-8 of the condensed benzene ring were the only sites of glycosylation and C-5 and C-7 which have already been occupied by the hydroxyl groups as described above in points (a), (b), (c) and (d).
- (G) The ¹H-NMR spectrum of the glycoside depicted chemical shift at δ 6.26 (d, J = 2.5, H-6) which showed the position of aromatic proton at C-6 position in ring A thereby concluding the possibility of glycosylation at C-8 position of the glycoside.³⁴⁻³⁶

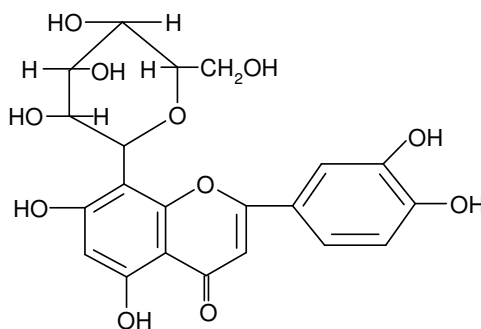
ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE PS-II

The glycoside PS-II on enzymatic hydrolysis with emulsion solution yielded no aglycone and sugar. This suggested that the C-glycoside was stable against enzymatic hydrolysis.³³

STEREOCHEMISTRY OF THE LINKAGE BETWEEN THE AGLYCONE AND THE SUGAR MOIETY

A doublet at δ 5.60 (1H, d, $J = 9.5$ Hz, H-1") for the anomeric proton in the ^1H -NMR spectrum of the glycoside (PS-II) suggested the presence of β -linkage between the D-glucose and the aglycone.²⁷

So on the basis of all the above abstractions, the tentative structure to the glycoside PS-II was identified as 8-C- β -D-glucopyrano 5, 7, 3, 4'- tetrahydroxy flavone.



¹H-NMR SPECTRUM OF THE GLYCOSIDE PS-II

The ¹H-NMR spectrum of the glycoside PS-II was found to be in complete conformity with the above structure. The characteristic chemical shifts obtained in the ¹H-NMR spectrum of PS-II [FIG. 3.6] with the help of available literature³⁷⁻⁴³ are filed below in table-3.7 :-

Table-3.7

S.No.	δ Value	Pattern	J value (Hz)	No. of Protons	Assignment
1.	6.23	s	--	1	H-3
2.	6.26	d	2.5	1	H-6
3.	7.76	d	7.5	1	H-2'
4.	6.92	d	7.5	1	H-5'
5.	7.56	dd	7.6,1.8	1	H-6'
6	5.60	d	9.5	1	H-1"
7.	2.38	s	-	3	OAc-5
8.	2.42	s	-	3	OAc-7
9.	2.27	s	-	3	OAc-3'
10.	2.29	s	-	3	OAc-4'
11.	3.40-4.15	m	-	8	Sugar (glucose) protons

¹H-NMR SPECTRUM OF THE GLYCOSIDE PS-II

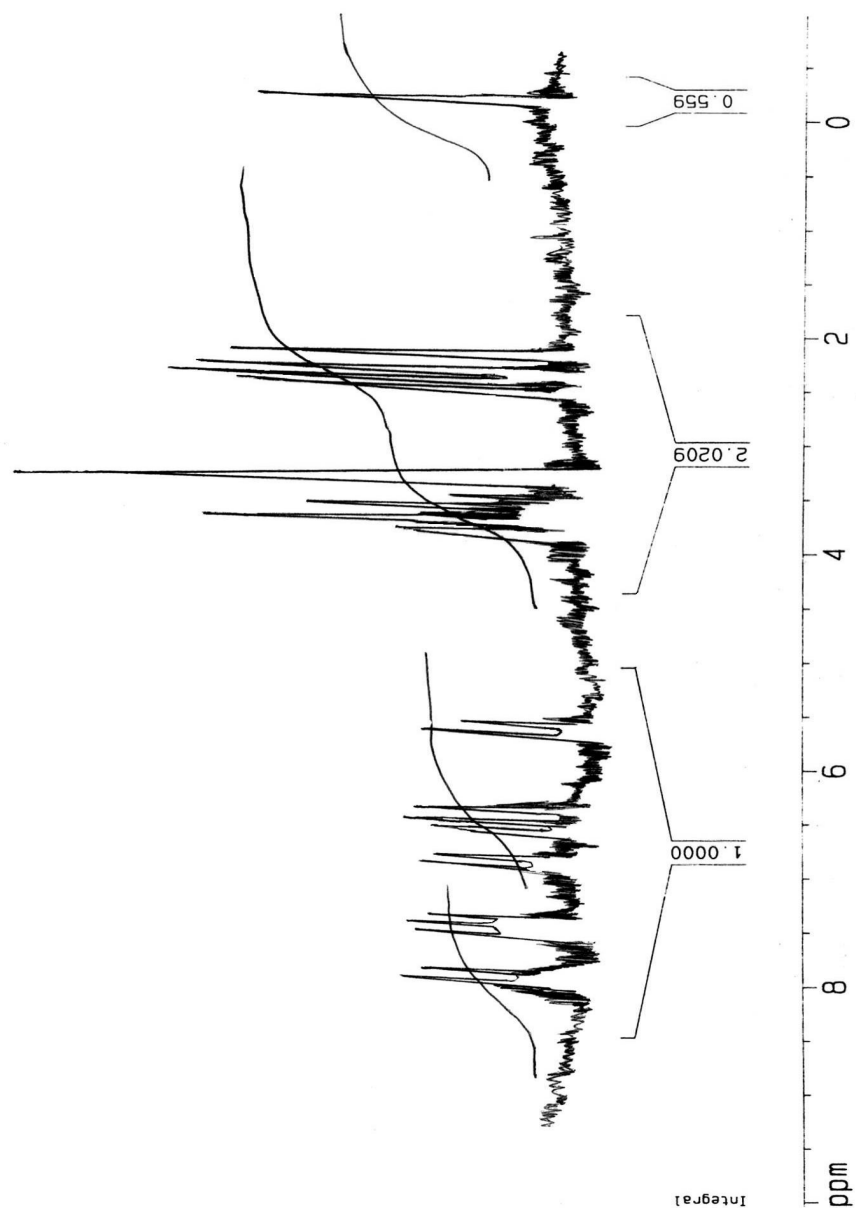
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EXPNO 1
PROCNO 1

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FIDRES 0.264910 Hz
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RG 35.9
DM 57.600 usec
DE 6.00 usec
TE 298.0 K
D1 1.0000000 sec

***** CHANNEL f1 *****
NUC1 ¹H
P1 6.88 usec
PL1 -3.00 dB
SFO1 300.1321300 MHz

F2 - Processing parameters
SI 16384
SF 300.1300040 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.20

1D NMR plot parameters
CX 20.00 cm
FIP 11.893 ppm
F1 3565.53 Hz
F2 -0.357 ppm
F2 -110.27 Hz
PPHOM 0.61303 ppm/cm
HZCM 183.9904 Hz/cm



PS-II

¹³C-NMR SPECTRUM OF THE GLYCOSIDE PS-II

The significant chemical shifts obtained in the ¹³C-NMR spectrum of the glycoside PS-II [FIG. 3.7] and the structural units inferred with the help of available literature^{8, 34, 47} are given in table-3.8 :-

Table-3.8

S.No.	δ Value	Carbon No.	Carbon type
1.	163.3	C-2	C
2.	102.0	C-3	C-H
3.	181.0	C-4	C=O
4.	159.2	C-5	C
5.	97.3	C-6	CH
6.	163.5	C-7	C
7.	103.3	C-8	C
8.	159.6	C-9	C
9.	102.3	C-10	C
10.	121.2	C-1'	C
11.	112.2	C-2'	CH
12.	145.5	C-3'	C
13.	148.5	C-4'	C
14.	115.0	C-5'	CH
15.	180.0	C-6'	CH
16.	74.6	C-1''	CH
17.	75.0	C-2''	CH
18.	79.0	C-3''	CH

S.No.	δ Value	Carbon No.	Carbon type
19.	70.4	C-4"	CH
20.	81.3	C-5"	CH
21.	60.1	C-6"	CH

¹³C-NMR SPECTRUM OF THE GLYCOSIDE PS-II

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Current Data Parameters
NAME      6957 ps3
EXPNO     2
PROCNO    1

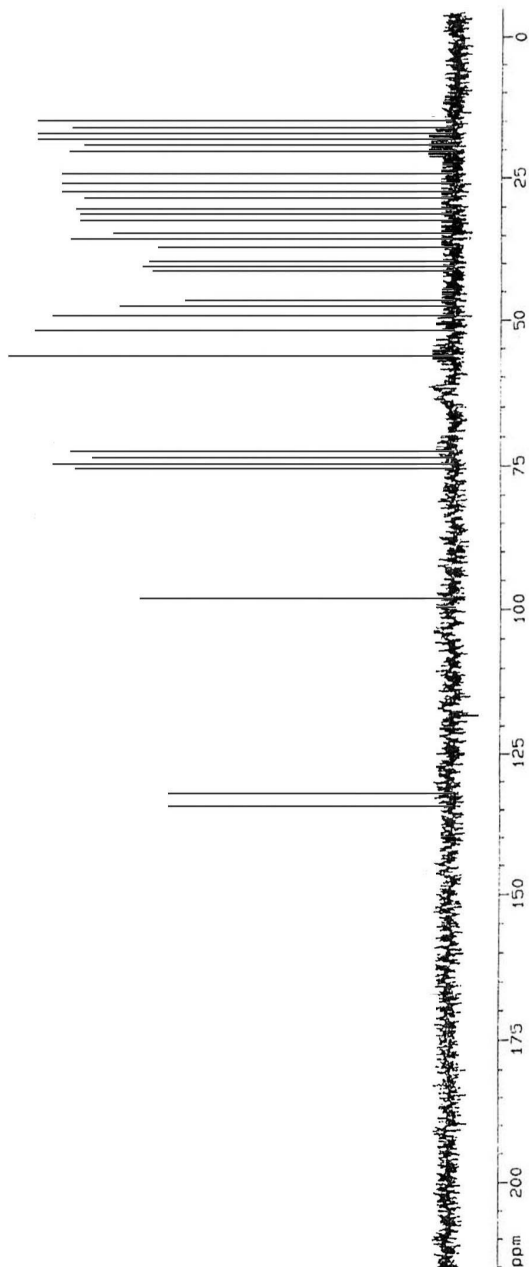
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SWH        19960.080 Hz
FIDRES     0.609133 Hz
AQ         0.8208884 sec
RG         5792.6
DM         25.050 usec
DE         6.00 usec
TE         315.0 K
D1         2.00000000 sec
d11        0.03000000 sec

===== CHANNEL f1 =====
NUC1       13C
P1         10.00 usec
PL1        -3.00 dB
SFO1       75.4767751 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2       1H
P2         10.00 usec
PL2        -3.00 dB
SFO2       300.1319936 MHz

F2 - Processing parameters
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SF         75.4677783 MHz
WDW        EM
SSB        0
LB         3.00 Hz
GB         0
PC         1.50

1D NMR plot parameters
CX         20.00 cm
F1P        195.752 ppm
F1         14772.96 Hz
F2P        -2.612 ppm
F2         -197.10 Hz
PPMCM      9.91818 ppm/cm
HZCM       746.50336 Hz/cm
    
```



PS-III

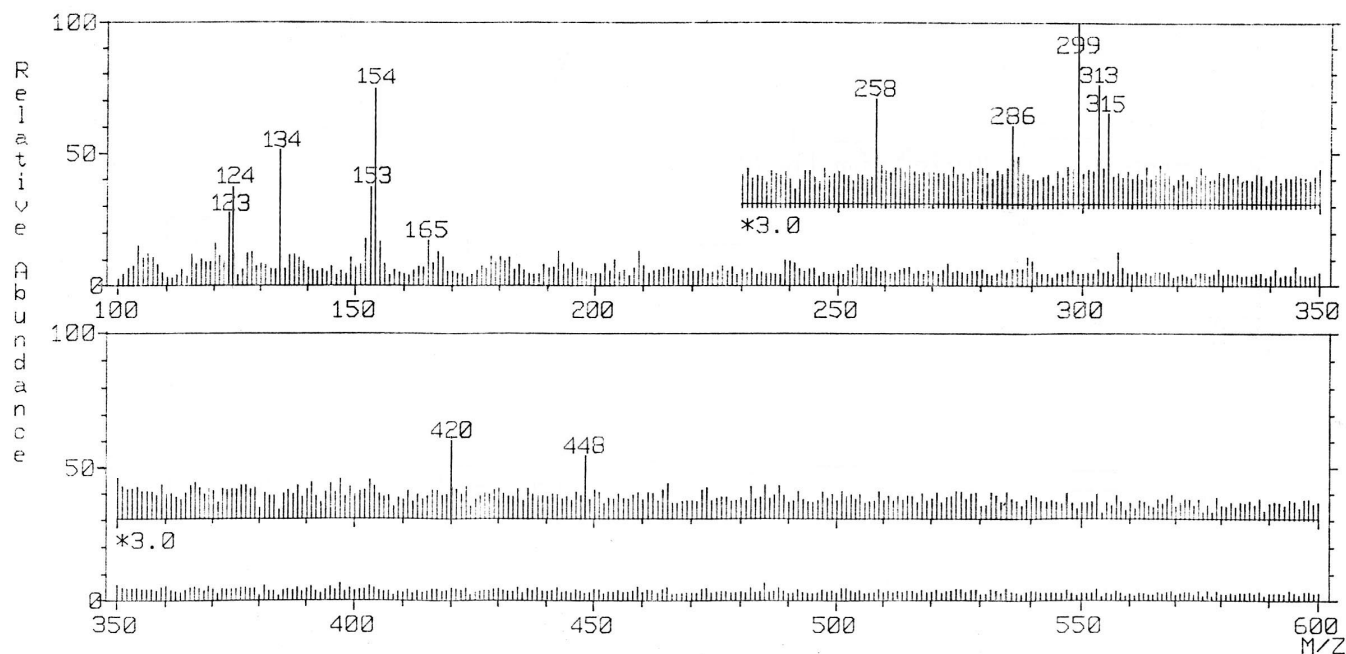
MASS SPECTRUM OF THE GLYCOSIDE PS-II

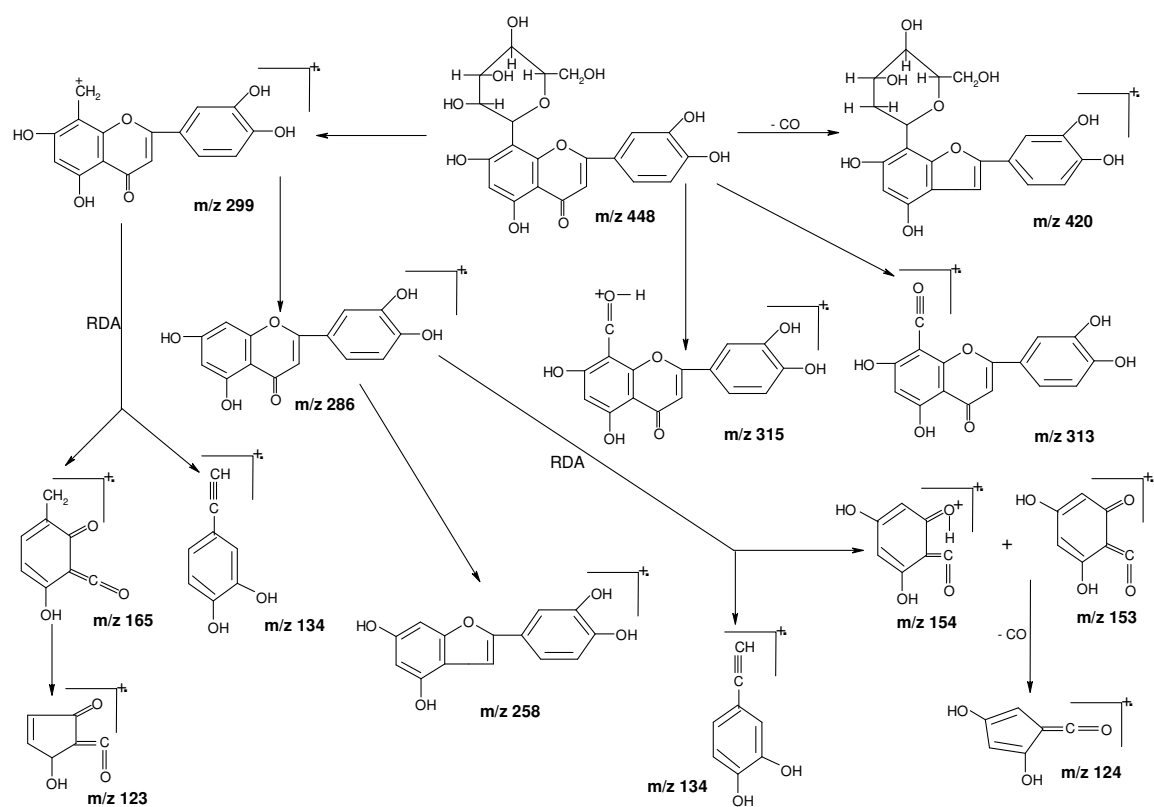
The important fragmentation pattern obtained in the mass spectrum of the glycoside PS-II [FIG. 3.8] is given below: -

[M⁺] 448, 420, 315, 313, 299, 286, 258, 165, 154, 153, 134, 124 and 123.

The various species obtained during the fragmentation⁴⁸⁻⁵⁰ are shown in the scheme-II which further supported the structure of the glycoside PS-II as: Luteolin-8-C- β -D-glucopyranoside.

MASS SPECTRUM Data File: 4EAP06J 6-APR- 4 11:48
Sample: PSII PROF V K SAXENA SAGAR#6957
RT 0'12" FAB(Pos.) GC 1.4c BP: m/z 299.0000 Int. 64.2799 LV 0.00
Scan# (2 to 3)





EXPERIMENTAL

PLANT MATERIAL

The roots of *Acorus calamus* were procured through the courtesy of Agricultural Department, Sagar and were authenticated by the Department of Botany, Dr. H.S. Gour University Sagar (M.P.). The herb specimen no. XXXIV has been deposited in the room no. 36 of the Chemistry Department, Dr. H.S. Gour University, Sagar (M.P.).

ISOLATION OF THE FLAVONE-C-GLYCOSIDE PS-II

2.0 kg roots of the *Acorus calamus* were air dried and crushed into powdered form. This powder was extracted with 95% ethanol. As a result, brown viscous mass was obtained. The concentrated ethanolic extract was partitioned between diethyl ether and water. The aqueous layer was extracted repeatedly with ethyl acetate and ethyl acetate extract was concentrated under reduced pressure to get brown viscous mass which on TLC examination using a solvent system CHCl_3 : MeOH (89:11) and visualizing agent: 10% aqueous H_2SO_4 showed it to be the mixture of three compounds. It was therefore purified by column chromatography over silica gel 'G' using eluants as CHCl_3 –MeOH in varying proportions. The details of the column chromatography are recorded below in the table below: -

Column Chromatography

S.No	Parameter	Amount
1.	Length of column	155 cm
2.	Diameter of column	4.0 cm
3.	Weight of crude extract	5.0 g
4.	Weight of the silica gel G	150 gm

The results of the column chromatography are given below in table 3.9:-

Table-3.9

S. No.	Fraction No.	Eluants	Spot On TLC	Remarks
1.	1-5	CHCl ₃ : MeOH(2:1)	Three	Mix. Rejected
2.	6-11	CHCl ₃ –MeOH (3:1)	Three	Mix. Rejected
3.	12-18	CHCl ₃ –MeOH (3:2)	One	Compound (PS-II)

STUDY OF THE FRACTIONS (12-18)

Eluates collected from the fractions (12-18) were found to have same R_f value and hence were combined. Evaporation of the solvent gave yellow amorphous mass. Its homogeneity was confirmed by TLC examination. The amorphous mass on crystallization from methanol afforded yellow crystals compound PS-II.

STUDY OF THE FLAVONE-C-GLYCOSIDE PS-II

The compound PS-II analysed for the molecular formula $C_{21}H_{20}O_{11}$, m.p. 264-265°C and $[M^+]$ 448 (FAB-MS). It responded to Molisch's test positively specific for glycoside and also responded to following characteristic reaction for flavonoids.

1. With alcoholic $FeCl_3$, it produced olive green colour.
2. It gave a reddish pink colour with Mg and HCl.
3. It gave red colour with sodium amalgam and hydrochloric acid (Na-Hg/HCl).
4. It produced yellow green fluorescent under UV light.
5. It produced orange colour with Zn/HCl.
6. It gave yellow colour with fluorescence with concentrated sulphuric acid.

Elemental Analysis:

Found	Calculated For $C_{20}H_{21}O_{11}$
C= 52.23%	C=56.26%
H=4.40%	H=4.46%
Molecular weight = 448 (FAB-MS)	

ACETYLATION OF THE FLAVONE-C-GLYCOSIDE PS-II

50 mg. of the compound was refluxed with 5ml of pyridine and 9ml of acetic anhydride in a round bottomed flask for about 8hrs at 110°C. The total reaction mixture was cooled and quenched into ice-cold water. When the sticky mass settled down in the beaker it was separated by decantation. Thus the mass was crystallised from ethanol to acetylated product. It was analysed for m.f. $C_{37}H_{36}O_{19}$, m.p. 144-145°C and $[M^+]$ 784 (FAB-MS).

Elemental Analysis:

Found	Calculated for $C_{37}H_{36}O_{19}$
C=56.56%	C=56.55%
H=4.59%	H=4.57%
% of acetyl group= 42.80%	% of acetyl group = 42.75%
Molecular weight = 784 (FAB-MS)	

METHYLATION OF THE FLAVONE-C-GLYCOSIDE PS-II

The compound (400mg) was dissolved in (95mg) of ether in a 250 ml round bottomed flask, to which freshly prepared ethereal solution of diazomethane was added. This solution was prepared by the action of 50% aqueous solution of KOH (20ml) on nitroso-methyl urea (9.0g) in ether solvent (100ml). The total contents of the flask were kept cooled in ice during the addition of diazomethane, and were allowed to stand for whole day in a refrigerator. The addition of glacial acetic acid (20ml) to the solution

neutralized the excess of diazomethane. The ethereal layer was first washed with aqueous NaHCO_3 and then with water followed by drying over anhydrous sodium sulphate. Evaporation of the solvent afforded white residue, which on crystallisation from solvent ether afforded colourless prisms.

The methylated product obtained analysed for molecular formula $\text{C}_{25}\text{H}_{28}\text{O}_{11}$, m.p. 273-274°C and $[\text{M}^+]$ 504 (FAB-MS).

Elemental Analysis:

<i>Found</i>	Calculated For $\text{C}_{25}\text{H}_{28}\text{O}_{11}$
C = 59.40%	C = 59.41%
H= 5.32%	H= 5.30%
% of Methyl group = 24.58%	% of Methyl group = 24.55%
Molecular weight = 504 (FAB-MS)	

ACID HYDROLYSIS OF FLAVONE-C-GLYCOSIDE PS-II

100 mg of the glycoside PS-II was hydrolysed with 25ml 7% H_2SO_4 aqueous for 7hrs at 100°C in a 150ml round bottomed flask. The total contents of the flask were diluted with water and then extracted with ethyl acetate. The contents were then allowed to cool and then separated by filtration.

The aqueous portion was treated with excess of BaCO_3 ; and the precipitate (BaSO_4) was filtered off. The filtrate was concentrated and examined by paper chromatography using a solvent system n butanol: acetic acid: water (4 : 1 : 5) upper layer as developer and aniline hydrogen phthalate

as the spraying agent. It did not show any spot and was found to be devoid of any sugar moiety. The glycoside was recovered unchanged.

HYDRIODIC ACID FISSION OF THE FLAVONE–C-GLYCOSIDE PS-II

40 mg of the glycoside was placed in a 150ml conical flask, to which 5mg of phenol was added, and hydriodic acid (HI) was gradually poured with cooling the flask. The total reaction mixture was refluxed for 8hrs. The cooled mixture was poured into an aqueous solution of NaHSO₃ (sodium bi sulphate) with stirring. The yellow precipitate was separated by filtration and crystallised from methanol to get yellow crystals.

STUDY OF THE AGLYCONE PS-II (A)

The aglycone PS-II (A) analysed for the molecular formula C₁₅H₁₀O₆, m.p. 323-324°C and [M⁺] 286 [FAB-MS].

It responded to all characteristic colour reactions of the flavonoids.

1. It gave green colour with alcoholic ferric chloride.
2. It produced pink colour with Na-Hg/HCl.
3. It gave an orange red colour with Zn.
4. It gave yellow colour with green fluorescence with con. H₂SO₄.

Elemental Analysis:

Found	Calculated For $C_{15}H_{10}O_6$
C = 62.90%	C = 62.87%
H = 3.49%	H = 3.48%
Molecular weight = 286 (FAB-MS)	

ACETYLATION OF THE AGLYCONE PS-II (A)

The acetylation of the aglycone (30mg) was done in a same manner as described for the glycoside (PS-II) in chapter of this thesis. This resulted in a formation of tetra-acetate derivative, which analysed for molecular formula $C_{23}H_{18}O_{10}$, m.p. 221-222°C and $[M^+]$ 454 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{23}H_{18}O_{10}$
C = 60.70%	C = 60.68%
H = 3.88%	H = 3.89%
% of Acetyl group = 36.91%	% of Acetyl group = 37.18%
Molecular weight = 454 (FAB-MS)	

METHYLATION OF THE AGLYCONE PS-II (A)

The methylation of the aglycone (40mg) was done in a similar way as described for glycoside PS-II in chapter. The tetra-O-methyl-ether of the aglycone analysed for molecular formula $C_{19}H_{18}O_6$, m.p. 262-263°C and $[M^+]$ 342 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{19}H_{18}O_6$
C = 66.66%	C = 66.65%
H = 5.20%	H = 5.22%
% of methoxyl group = 36.10%	% of methoxyl group = 36.15%
Molecular weight = 342 (FAB- MS)	

ALKALINE DEGRADATION OF THE AGLYCONE PS-II (A)

60mg of the aglycone PS-II (A) was refluxed with 50% ethanolic KOH (20ml) for 5 hrs in a B-14 ground joint flask. The total contents of the flask were cooled and then acidified with dil. HCl. Degraded products were extracted with solvent ether in a separating funnel. The ethereal layer was washed with water and divided into two portions.

- (1) The First part of the ethereal layer was treated with 10% NaOH solution, which on acidification yielded a compound (a), m.p. 114-115°C, m.f. $C_6H_6O_3$ and $[M^+]=126$, identified as phloroglucinol (by m.m.p., Co PC and Co TLC).

- (2) The second part of the ethereal layer was treated with 50% NaHCO_3 solution and then acidified by dil. HCl . The total reaction mixture gave a compound (b), m.p. $202-203^\circ\text{C}$, m.f. $\text{C}_7\text{H}_6\text{O}_4$ and $[\text{M}^+] = 154$, identified as protocatechuic acid (by m.m.p., Co-Pc and Co-TLC).

POTASSIUM PERMANGNATE OXIDATION OF THE AGLYCONE PS-II (A)

60mg of the methylated glycoside was placed in a B-14 ground joint flask to which 50 ml of methanol and 10% of potassium permanganate was added to it, till the colour persisted. The total reaction mixture was allowed to refluxed for 4-5 hrs. After cooling, the excess of MnO_2 was removed by adding sodium bisulphite (NaHSO_3). The resulting solution was filtered off to remove the suspended impurities. It was washed with 35 ml of 3% NaOH . The filtrate obtained was acidified with con HCl and extracted with CHCl_3 . The CHCl_3 layer was washed with distilled water and dried over anhydrous Na_2SO_3 when afforded a colourless compound , m.f. $\text{C}_9\text{H}_{10}\text{O}_4$ m.p. $179-180^\circ\text{C}$ and $[\text{M}^+] 182$ (FAB-MS).

OXIDATION OF THE GLYCOSIDE PS-II BY FeCl_3

The glycoside (50 mg) was refluxed with FeCl_3 (45 mg) in 2 ml of water in a 150 ml of round bottomed flask for 8 hrs at 115°C , when it afforded a dark coloured mixture. The mixture was allowed to cool and diluted with water. The mixture was diluted with distilled water and the P^{H} adjusted to 8 by gradually addition of aq. NaOH solution. The dark coloured mixture obtained was filtered off. Dil HCl was added to the filtrate to adjust the pH to 7.0. The mixture was extracted with amyl alcohol and this resulted into

yellow coloured solution which passed through a column of silica gel G, using water as an eluants to remove the iron and chloride ions. The neutral solution obtained was evaporated and afforded a syrupy mass which was examined by paper chromatography on Whatmann filter paper no. 1 using following solvent system as (i) and (ii) and aniline hydrogen phthalate as spraying agent. The results are filed below in table-3.10:-.

Table-3.10

Identification of Sugars

S. No.	Solvent system	R _f Value		Sugar identified
		Reported	Found	
1.	n-butanol : acetic acid : water (4 : 1 : 5 v/v)	0.18	0.17	D-glucose
		0.21	0.20	D-arabinose
2.	n-butanol : pyridine : water (6 : 4 : 3 v/v)	0.68	0.67	D-glucose
		0.72	0.70	D-arabinase

PERIODATE OXIDATION OF TETRA-O-METHYL-ETHER OF THE GLYCOSIDE AND HYDROLYSIS OF THE OXIDATION PRODUCT

120mg of the methylated ether of the glycoside was dissolved in 15ml of MeOH and treated with sodium meta periodate (3ml) in a 150ml conical flask fitted with a glass stopper. The mixture was allowed to stand for over night at room temperature, which resulted into an oxidised product. Then the product was hydrolysed with 1N HCl followed by filtration. The filtrate obtained, when treated with 2-4-dinitrophenyl hydrazine, furnished a yellow precipitate, which on TLC examination, using a solvent system as; benzene: ethyl acetate (4:1) and 10% H₂SO₄ as spraying agent, showed two spots (R_f

0.41 & 0.11). Therefore, the mixture was subjected to column chromatography using eluants as benzene: ethyl acetate (4: 1) ratio.

1. The eluates collected from the first (1-5) fraction were found to be of same R_F values and so were mixed together. Evaporation of the solvents afforded a compound m.p. 166-168°C, which was found to be homogenous on TLC examination (R_F 0.11).
2. The eluates collected from fraction (6-11) were found to have same R_f values (0.41) and hence were combined. On evaporation of the solvent; it afforded an orange-coloured needles, m.p. 301-302°C.

PERIODATE OXIDATION FOLLOWED BY SODIUM BOROHYDRIDE REDUCTION OF THE METHYLATED GLYCOSIDE

25mg of the methylated glycoside was placed in a 100ml of conical flask, to which sodium meta periodate was added. The total reaction mixture was allowed to reflux for 6hrs. The solution of sodium borohydride (20mg) in 5ml of water was added to the reaction mixture, and the contents of the flask were allowed to stand for overnight at room temperature. The reaction mixture was hydrolysed with 1N HCl at 115°C 15min and was filtered. Sugar was determined by identified the filtrate on the paper chromatography on Whatmann filter paper no. 1 using the solvent system, ethyl acetate: pyridine: water (10:4:3) and a spraying reagent [a mixture of aq. NaIO_4 (2%, 4 parts) and alkaline KMnO_4 (1% KMnO_4 in 2% aq. Na_2CO_3 , 1 part)]. The results displayed a yellow spot on a pink back-ground with R_f 0.37 and glucose treated in a same way gave R_f (0.38).

PERIODATE OXIDATION OF METHYLAED GLYCOSIDE

In a 250ml of round bottomed flask, 50 mg of the methyl ether of the glycoside was dissolved in 25 ml (dioxane), to which a solution of NaIO_4 . (25ml) solution and 100ml of distilled water was added.

The total reaction mixture was allowed to stand for a whole day at room temperature. Simultaneously a blank was also run in a same manner. The quantity of consumed NaIO_4 and liberated formic acid were estimated volumetrically using the procedure of Bhatia et al.

ENZYMATIC HYDROLYSIS⁴³ OF THE GLYCOSIDE (PS-II)

25mg of the glycoside was dissolved in methanol (25ml) and then treated with almond emulsion (25ml) in a 100ml conical flask. The mixture was allowed to keep at room temperature for 48hrs and then filtered. The hydrolysate was concentrated and subjected to the paper chromatography⁴⁴ on Whatmann filter paper no. 1 using a solvent system as n-butanol; acetic acid : water (4 : 1 : 5) as moving phase and aniline hydrogen phthalate as spraying agent. On examination, it did not reveal any spot for the sugar identification.

REFERENCES

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Euphorbia hirta Linn.¹ belong to natural order Euphorbiaceae and is commonly known as ‘Dudhi’ in Hindi.

The plant *Euphorbia hirta* Linn.²⁻⁵ has been reported to possess significant medicinal values. Its juice is used in dysentery, colic and worm infection. Its roots are anti-emetic and used in bone-fracture, emphysema, measles, nausea and tumors. The decoction of its leaves is used in bronchial asthma and chronic coughs. This plant has been found to possess anti-asthmatic, anti-histaminic and spasmolytic properties.

In view of its medicinal importance, it was worthwhile to carry out systematic phytochemical investigation on the roots of this plant.

ISOLATION OF STEROIDAL SAPONIN PS-III

The roots of the plant *Euphorbia hirta* (Linn.) were collected locally and were identified by taxonomist of Botany Department, Dr. H.S. Gour University, Sagar (M.P.). A herb specimen no. XXXV has been deposited at room no. 36 at Department of Chemistry, Dr. H.S. Gour University, Sagar.

3.0kg of the air-dried and powdered roots of *Euphorbia hirta* Linn. were extracted with 95% hot ethanol. The ethanolic extract was concentrated under reduced pressure to get a brown viscous mass, which was successively extracted with petroleum ether (60-80°C), benzene, chloroform, acetone, ethyl acetate and methanol.

Except the methanol soluble fraction, all other fractions could not be studied phytochemically because on evaporation of the solvent they yielded very small quantity of residue which was insufficient for the phytochemical study.

STUDY OF THE METHANOL SOLUBLE PART

The methanol soluble portion was concentrated under reduced pressure to a brown viscous mass. It showed two spots on TLC examination using solvent system; n-butanol: acetic acid: water (4:1:5) and I₂ vapours as visualizing agent, thereby showing it to be a mixture of two compounds. As such this mixture was subjected to column chromatography over silica gel G and eluted with CHCl₃: MeOH in varying proportion of 13:1, 15 : 1 and 19 : 1 respectively.

STUDY OF THE ELUATES FROM CHLOROFORM: METHANOL (19:1)

The eluates collected from chloroform: methanol (19:1) were found to have same R_F values and so were combined together. On removal of the solvent, it gave an amorphous mass (0.062%). The homogeneity of the mass was confirmed by TLC examination when only one spot was observed. The amorphous mass was crystallised from methanol when it gave colourless needles.

STUDY OF STEROIDAL GLYCOSIDE PS-III

The compound PS-III was crystallised from methanol as colourless needles and found to be soluble in methanol and ethanol. It analysed for molecular formula, $C_{35}H_{60}O_5$, m.p 262-263°C and $[M^+]$ 560 (FAB-MS).

It responded to Molisch's test for glycoside and also responded to all the characteristic colour reactions of steroidal saponin.⁶⁻¹¹

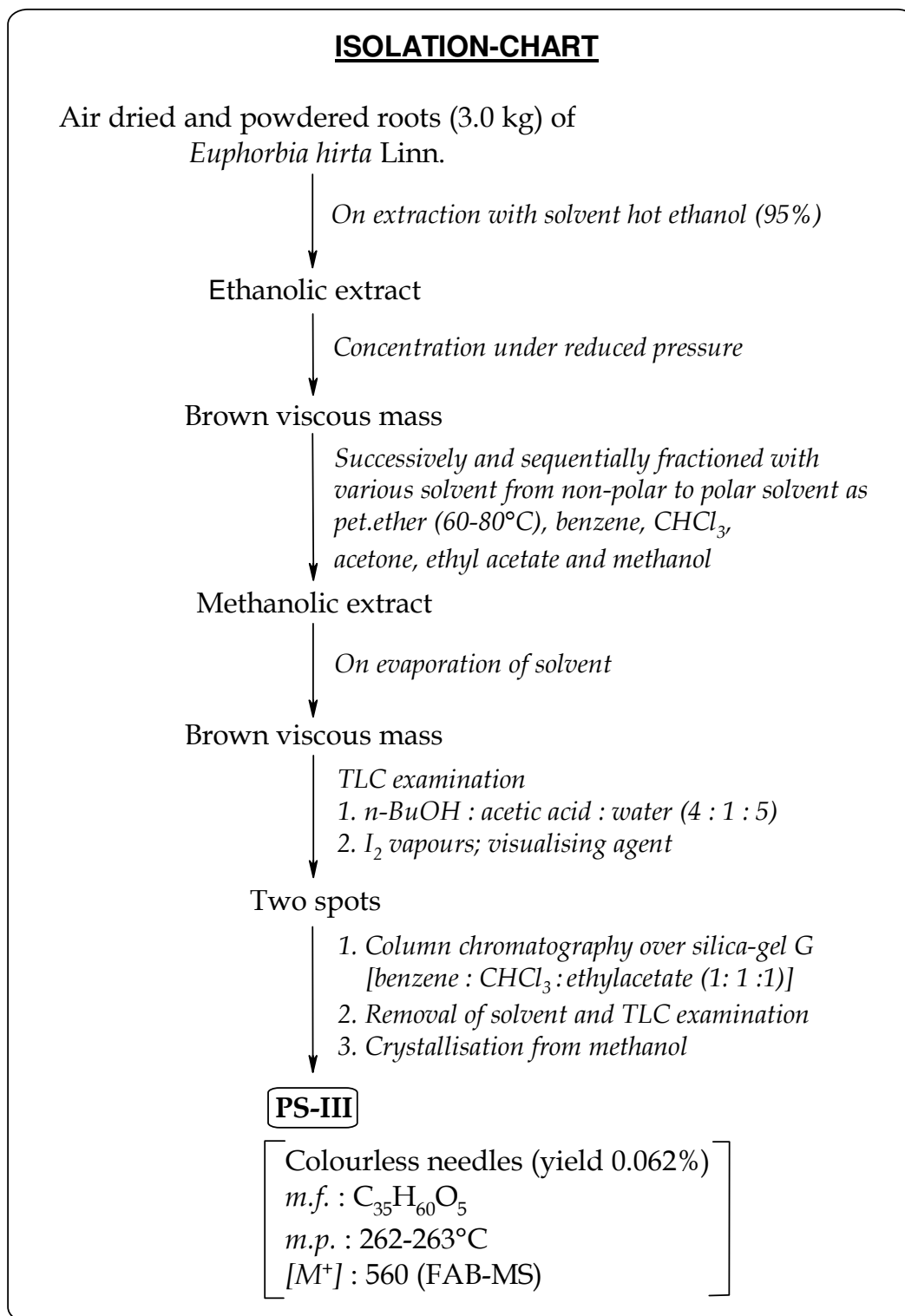


FIG. 4.1 : Fractionation protocol for isolation of compound PS–III from plant *Euphorbia hirta* Linn.

IR SPECTRUM OF COMPOUND PS-III

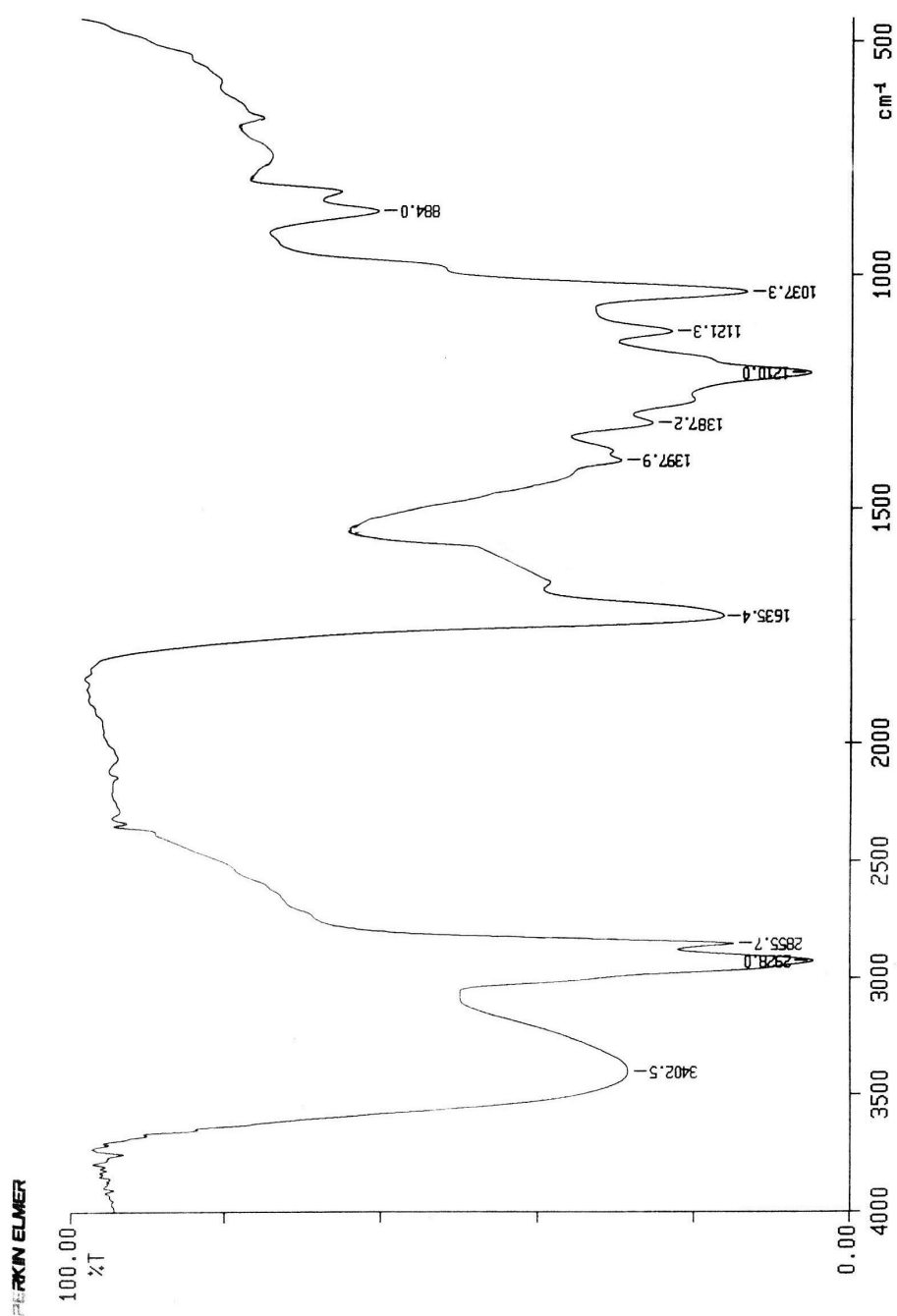
The characteristic bands observed in the IR spectrum of the glycoside PS-III (FIG. 4.2) and functional groups assigned to the molecule with the help of available literature¹²⁻¹⁶ are given in table-4.1:-

Table – 4.1
IR spectrum of glycoside

S.No.	Wave Number cm ⁻¹	Assignments
1.	3402.5	-OH group
2.	2928.0	C-H stretching vibration in CH ₃
3.	2855.7	C-H stretching in cyclopentane nucleus
4.	1634.7, 884.0	>C = C< bond
5.	1397.9, 1121.3, 1037.3, 1210.0	Twisting and wagging of C-H in steroidal nucleus
6.	1387.2,	Angular methyl group between five and six member ring

Characteristic bands at $V_{\max}^{\text{KBr}}$ 1635.4 and 884.0 cm⁻¹ in the IR spectrum of PS-III showed the presence of double bond in the glycoside.¹⁷

IR SPECTRUM OF COMPOUND PS-III



04/02/25 10:45 AM, CODE-PS-III
X: 4 scans, 4.0cm-1, flat, smooth, abex

PRESENCE OF –OH GROUPS IN PS-III

The characteristic band appeared at $\nu_{\text{max}}^{\text{KBr}}$ 3402.5 cm^{-1} in the IR spectrum of saponin PS-III and indicated the presence of –OH group(s) in it.

The number of hydroxyl groups was quantitatively estimated by acetylation of PS-III with Ac_2O /pyridine, when an acetylated product was formed. The acetylated product analysed for molecular formula, $\text{C}_{41}\text{H}_{66}\text{O}_8$ and $[\text{M}^+]$ 686 (FAB-MS).

The percentage of acetyl group in the acetylated product was determined by Weisenberger¹⁸ method as described by Belcher and Godbert¹⁹ and was found to be 18.80% which indicated the presence of three hydroxyl groups in the saponin PS-III.

PRESENCE OF DOUBLE BOND IN PS-III

The presence of double bond in the saponin PS-III was indicated because solution of saponin in CCl_4 produced yellow colour with tetra nitro methane²⁰. This was further confirmed by band at 1635.4 and 884.0 cm^{-1} in the IR spectrum of PS-III.

PRESENCE OF METHYL GROUPS IN PS-III

IR bands at wave number 2928.0 cm^{-1} and 1387.2 cm^{-1} in the IR spectrum of PS-III indicated the presence of methyl group(s) in it. The percentage of the methyl groups was quantitatively estimated by Zeisel's

method²¹ and found to be 17.44%, thereby showing the presence of eight methyl groups in the saponin.

To assign the positions of different methyl and hydroxyl groups in order to elucidate the structure of glycoside PS-III and also for the identification of sugar moiety (ies), the saponin, PS-III was subjected to acid hydrolysis.

ACID HYDROLYSIS OF COMPOUND PS-III (A)

The saponin PS-III was hydrolysed with 7% H₂SO₄ when at afforded sapogenin (aglycone) PS-III (A) and sugar moiety (ies) which were separated by filtration and studied separately.

STUDY OF THE SAPOGENIN PS-III (A)

The sapogenin obtained from the acid hydrolysis of PS-III was subjected to TLC examination, when it was found to be homogenous.

The saogenin PS-III (A) analysed for m.f. C₃₀H₅₂O, m.p. 127-128°C and [M⁺] 428 (FAB-MS). It positively responded to all the characteristic colour reactions of steroids and did not give Molisch's test for glycoside.

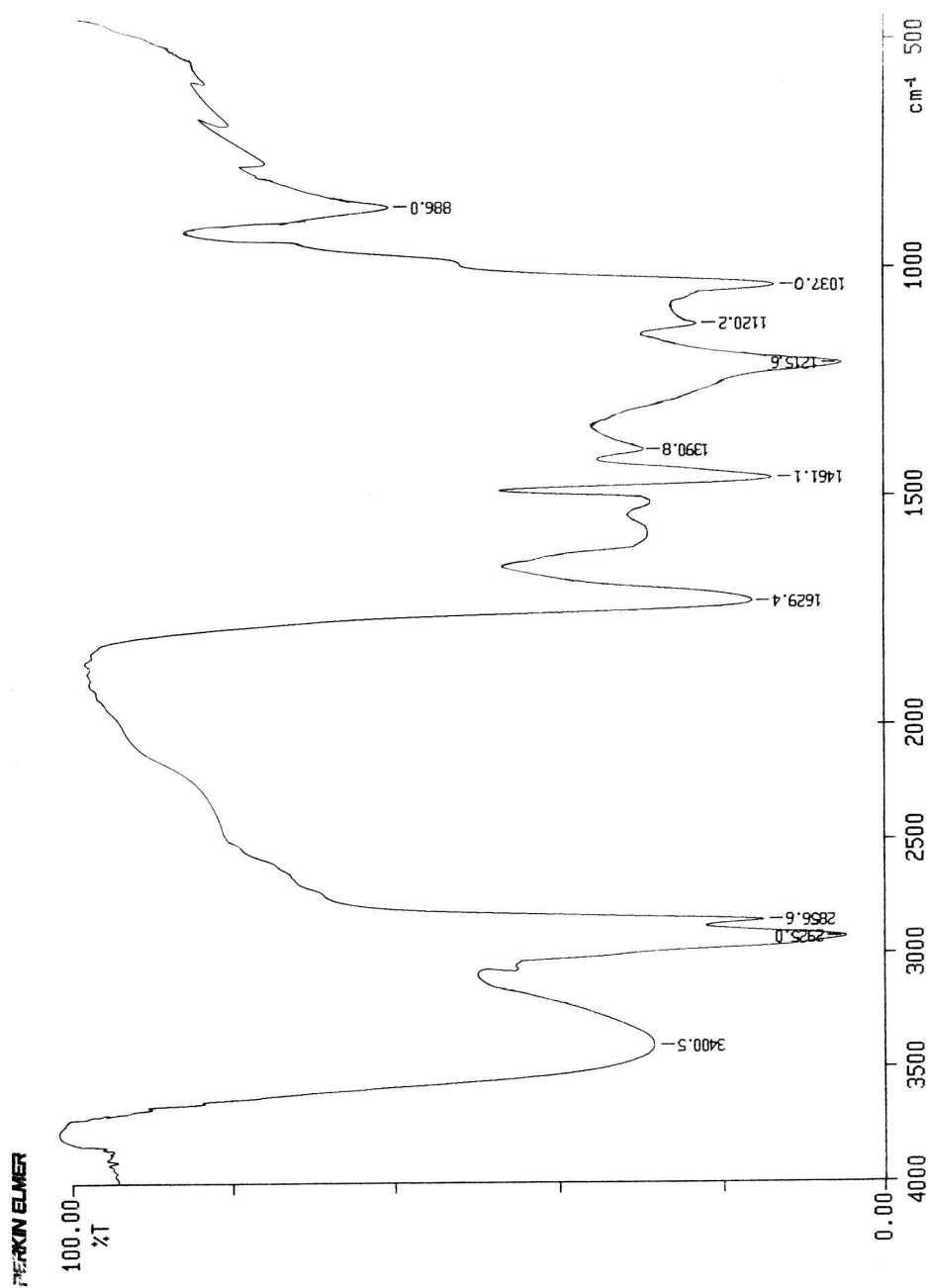
IR SPECTRUM OF THE SAPOGENIN

The significant bands which appeared in the IR spectrum of the sapogenin PS-III (A) [FIG.4.3] and structural units ascribed to the molecule with help of the available literature²²⁻²⁴ are recorded in table-4.2.

Table – 4.2
IR spectrum of the sapogenin PS-III (A)

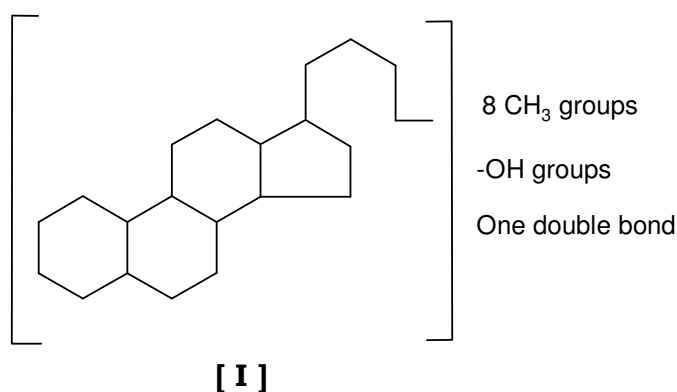
S.No.	Wave Number cm ⁻¹	Assignments
1.	3400.5	-OH group(s)
2.	2925.0	CH ₃ stretching vibration in CH ₃
3.	2856.7	C-H stretching in the cyclopentane nucleus
4.	1629.4, 886.0	>C = C< bond
5.	1461.1	C-H bending
6.	1215.6, 1120.2, 1037.0	C-H twisting and wagging of steroidal nucleus
7.	1390.8	Angular methyl group between five and six member ring

IR SPECTRUM OF THE SAPOGENIN



04/02/25 10:52 AM, CODE-PS-IIIA
X: 4 scans, 4.0cm-1, flat, smooth, abex

On the basis of above deliberations, the tentative structure assigned to the sapogenin PS-III (A) is shown in Fig. I



PRESENCE OF HYDROXYL GROUP(S) IN PS-III(A)

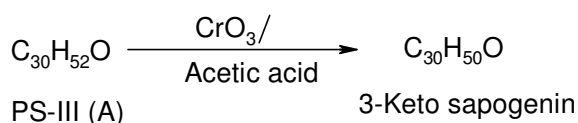
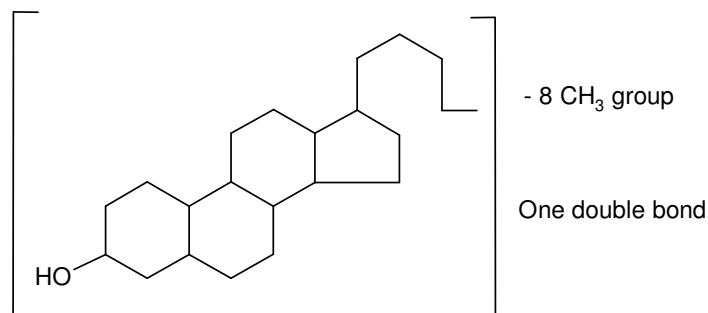
The significant IR band at 3400.5 cm⁻¹ in the IR spectrum of sapogenin PS-III (A) indicated the presence of –OH group(s) in it. On acetylation of PS-III (A) with Ac₂O/pyridine, it gave monoacetate of sapogenin. The acetylated product analysed for the molecular formula C₃₂H₅₄O₂, m.p. 104-105°C and [M⁺] 470 (FAB-MS).

The percentage of acetyl group was quantitatively estimated by Weisenberger¹⁸ process as described by Belcher and Godbert¹⁹, which was found to be 9.14%, and so revealed the presence of one hydroxyl group in the sapogenin PS-III (A).

POSITION OF HYDROXYL GROUP(S) IN THE SAPOGENIN PS-III (A)

The sapogenin PS-III (A) on oxidation with $\text{CrO}_3/\text{acetic acid}$ afforded a ketone, m.f. $\text{C}_{30}\text{H}_{50}\text{O}$, m.p. $177-119^\circ\text{C}$ and $[\text{M}^+]$ 426 (FAB-MS), which responded positively to the Zimmerman test specific for C-3 keto group thereby confirming the presence of $-\text{OH}$ group at position C-3. Further it indicated its nature as secondary in PS-III (A).²⁵⁻²⁷

On the basis of above facts, the following tentative structure was assigned to sapogenin PS-III (A).



The ^1H -NMR signal for the methylene proton at δ 3.25 (1H, dd, $J = 4.5, 11.5$ Hz) in the spogenin PS-III (A) further confirmed the secondary nature of a hydroxyl at C-3 position.²⁸

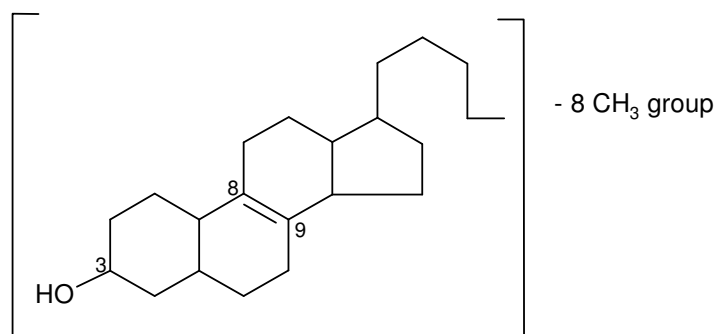
POSITION OF DOUBLE BOND IN SAPOGENIN PS-III (A)

A band at 1629.4 cm^{-1} in the IR spectrum of the sapogenin PS-III (A) indicated the presence of double bond in it. The presence of double bond was further assisted by positive TNM test in which solution of sapogenin in CCl_4 produced yellow colour with tetra nitro methane.

A band in the IR spectrum of PS-III (A) was appeared at 886.0 cm^{-1} , indicated the presence of a tetra substituted double bond which could be placed between C-8 and C-9 because this double bond was inert to hydrogenation, even under vigorous condition.²⁹

Further the presence of tetra substituted double bond between C-8 and C-9 was confirmed by the ^{13}C -NMR spectrum where the two carbon resonances appeared at 139.8 and 139.7.^{30-32, 35}

On the basis of above facts the following tentative structure was assigned to the sapogenin PS-III (A).



POSITION OF METHYL GROUP(S) IN PS-III (A)

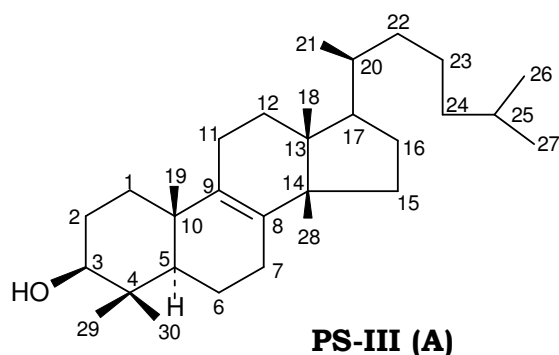
The IR spectrum of sapogenin PS-III (A) showed a band at $\nu_{\max}^{\text{KBr}}$ 2925.0 cm^{-1} which indicated the presence of methyl groups in it.

The quantitative estimation of methyl groups was done by Zeisel's²¹ method and was found to be 44.60%, which showed the presence of eight methyl groups in it.

Further the positions of methyl groups were established by the ^1H -NMR spectrum. The ^1H -NMR spectrum of PS-III (A) gave three proton intensity singlets each at δ 0.68, δ 0.99, δ 1.09, δ 1.03 and δ 0.89 and doublet at δ 0.90, δ 1.06 and δ 0.96 assigned for the position of methyl groups at C-18, C-19, C-21, C-26, C-27, C-28, C-29, C-30 in sapogenin PS-III (A).³³⁻³⁵

The ^{13}C -NMR spectrum of sapogenin PS-III (A) showed chemical shift at δ 15.2, δ 18.1, δ 18.5, δ 22.3, δ 22.2, δ 23.5, δ 27.4, δ 15.0 assigned for the methyl groups at C-18, C-19, C-21, C-26, C-27, C-28, C-29, C-30 respectively.³⁶⁻³⁸

On the basis of above points, the sapogenin PS-III (A) was dihydro euphol, which was identified by spectral analysis and with the support of available literature.³⁹⁻⁴⁹



¹H-NMR SPECTRUM OF SAPOGENIN PS-III (A)

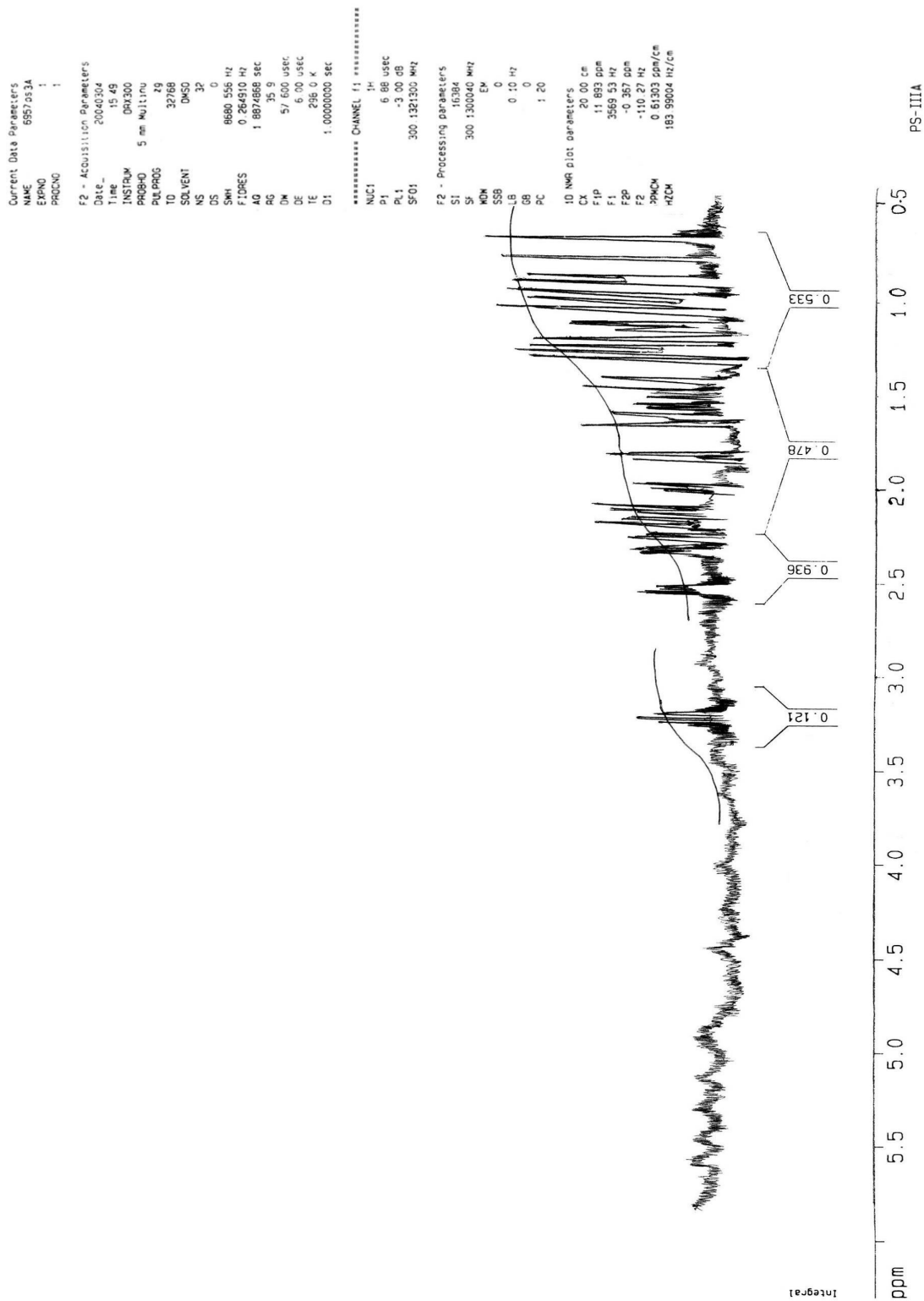
The significant signals obtained in the ¹H-NMR spectrum of the aglycone PS-III (A) [FIG. 4.4] and structural unit inferred on the basis of available literature³⁹⁻⁴⁵ are filed in table –4.3

Table – 4.3
¹H-NMR spectrum (300 MHz, C₆D₆) of sapogenin PS-III (A)

S.No.	δ-Value	Pattern	J-Value Hz	No. of Protons	Assignments
1.	1.01	m	—	2	H-1
2.	1.38	m	—	2	H-2
3.	3.23	dd	4.4 11.2	1	H-3
4.	2.18	dd	10.2 2.0	1	H-5

S.No.	δ -Value	Pattern	J-Value Hz	No. of Protons	Assignments
5.	1.92	m	—	2	H-6
6.	1.23	m	—	2	H-7
7.	1.31	m	—	2	H-11
8.	1.19	m	—	2	H-12
9.	1.34	m	—	2	H-15
10.	1.39	m	—	2	H-16
11.	2.50	m	—	1	H-17
12.	0.68	s	—	3	H-18
13.	0.99	s	—	3	H-19
14.	1.43	m	—	1	H-20
15.	0.90	d	7	3	H-21
16.	1.53	m	—	2	H-22
17.	2.12	m	—	2	H-23
18.	2.14	m	—	2	H-24
19.	2.20	m		1	H-25
20.	0.96	d	6.5	3	H-26
21.	1.06	d	7	3	H-27
22.	1.09	s	—	3	H-28
23.	0.89	s	—	3	H-29
24.	1.03	s	—	3	H-30

¹H-NMR SPECTRUM OF SAPOGENIN PS-III (A)



¹³C-NMR SPECTRUM OF THE SAPOGENIN

The significant signals observed in the ¹³C-NMR spectrum of spogenin PS-III (A) [FIG. 4.5] and structural units inferred on the basis of available literature⁴⁶⁻⁴⁹ are tabulated below in table-4.4

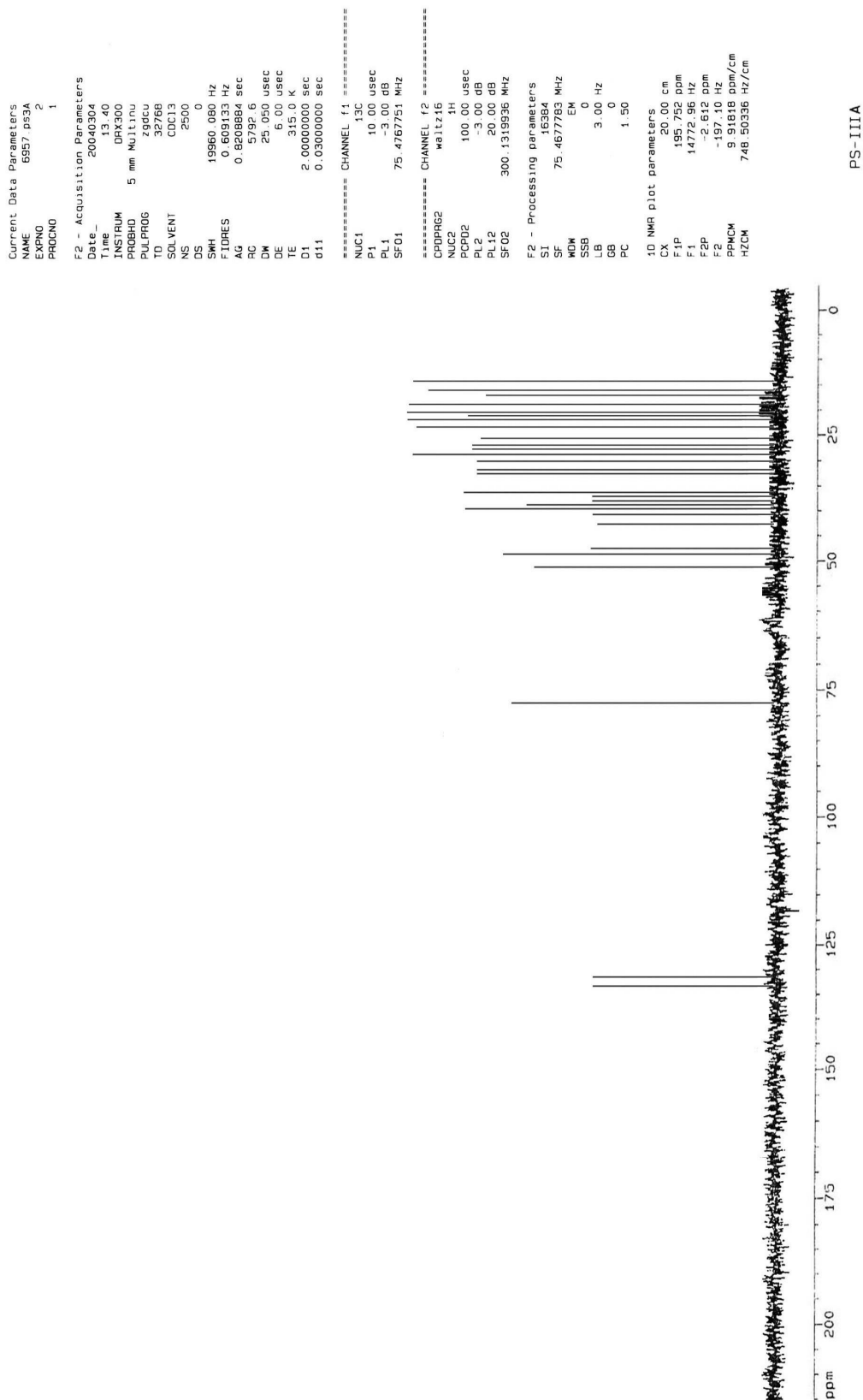
Table – 4.4

¹³C-NMR spectrum (300 MHz, DMSO) of sapogenin PS-III (A)

S.No.	δ-Value	Carbon no.	Carbon type
1.	35.1	C-1	CH ₂
2.	29.4	C-2	CH ₂
3.	78.3	C-3	CH
4.	38.2	C-4	C
5.	49.8	C-5	CH
6.	20.5	C-6	CH ₂
7.	27.7	C-7	CH ₂
8.	133.8	C-8	C
9.	133.9	C-9	C
10.	36.5	C-10	C
11.	17.6	C-11	CH ₂
12.	25.8	C-12	CH ₂

S.No.	δ -Value	Carbon no.	Carbon type
13.	43.8	C-13	C
14.	49.1	C-14	C
15.	30.4	C-15	CH ₂
16.	30.2	C-16	CH
17.	49.8	C-17	CH ₂
18.	15.2	C-18	CH ₃
19.	18.0	C-19	CH ₃
20.	35.8	C-20	CH
21.	18.5	C-21	CH ₃
22.	35.9	C-22	CH ₂
23.	23.4	C-23	CH ₂
24.	38.9	C-24	CH ₂
25.	27.6	C-25	CH
26.	22.3	C-26	CH ₃
27.	22.0	C-27	CH ₃
28.	23.6	C-28	CH ₃
29.	27.4	C-29	CH ₃
30.	15.0	C-30	CH ₃

¹³C-NMR SPECTRUM OF THE SAPOGENIN

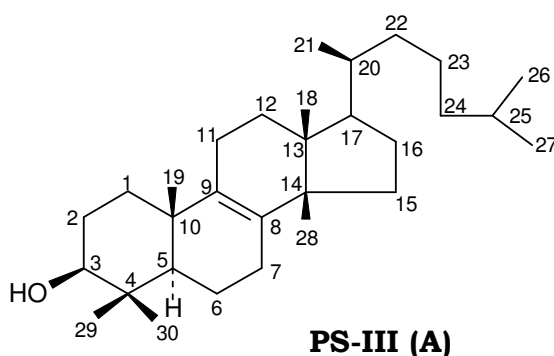


MASS SPECTRUM OF THE SAPOGENIN PS-III (A)

The important fragmentation species obtained in the FAB-MS of PS-III (A) are as follows:-

m/z 428, [M⁺] 413, 410, 395, 385, 357, 342, 310, 301 299, 296, 284, 281, 270, 207 and 203

Various species obtained during mass fragmentation are represented in the scheme-I and were in full agreement of the proposed structure of sapogenin ^{48, 50-52} and also confirmed its identity as; 24,25 dihydro euphol. ⁵³⁻⁵⁵



STUDY OF THE SAPONIN HYDROLYSATE

The saponin PS-III on acid hydrolysis yielded sapogenin PS-III (A) which was separated by filtration and sugar moiety remained in the solution. The aqueous hydrolysate was neutralised by adding BaCO₃ and white precipitate of BaSO₄ was filtered off. The filtrate was concentrated to syrupy mass, which was subjected to Co PC and CoTLC, indicating the presence of D-arabinose (R_f 0.22). ⁵⁶⁻⁶⁰

QUANTITATIVE ESTIMATION OF SUGAR

The sugar was quantitatively estimated by the procedure of Mishra and Rao⁶¹, which revealed the presence of sapogenin PS-III (A) and D-arabinose in equimolar ratio (1:1).

POSITION OF ATTACHMENT OF SUGAR TO THE SAPOGENIN PS-III (A)

Since there is only one hydroxyl group at C-3 position available for glycosidic formation, therefore the sugar moiety(ies) must be attached to the sapogenin PS-III (A) through the hydroxyl group at C-3.

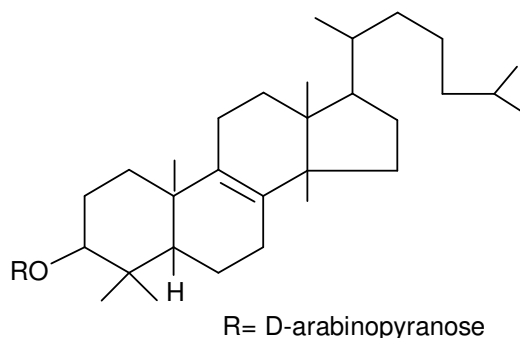
PERMETHYLATION OF THE SAPONIN PS-III

The saponin PS-III on permethylation by Kuhn⁶² method followed by acid hydrolysis yielded an aglycone PS-III (A) and 2', 3', 4'-tri-O-methyl-D-arabinose (CoPC and CoTLC), indicating that C₁' of D-arabinose was involved in the glycosylation.

PERIODATE OXIDATION OF THE GLYCOSIDE PS-III

On treatment with sodium meta periodate, one mole of glycoside PS-III consumed 2.04 moles of periodate and liberated 1.05 moles of formic acid, indicating that the sugar was in pyranose form in the glycoside and also suggested that one mole of D-arabino pyranose was attached to the aglycone.

Thus a tentative structure given below was assigned to the glycoside PS-III;



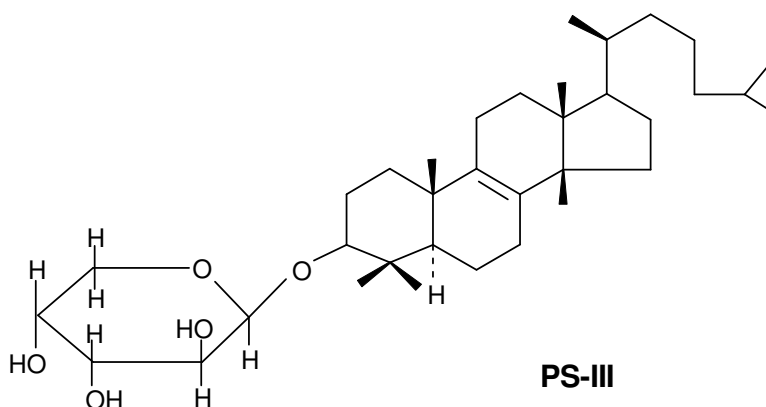
ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE PS-III

The steroidal saponin PS-III on enzymatic hydrolysis with enzyme almond emulsion⁶³ yielded aglycone PS-III (A) and D-arabinose confirming C-3 –OH of the aglycone was linked to C-1' of D-arabinose through β -linkage. Thus the glycoside PS-III was assigned the following structure and was identified as; 24, 25 dihydro-euphol-3-O- β -D-arabinopyranoside.

A doublet at δ 5.53 (1H, d, $J = 3\text{Hz}$, H-1') for the anomeric proton in the ^1H -NMR spectrum of the PS-III further confirmed the β -linkage between aglycone and D-arabinose

The all above facts when put together concluded that C-3 –OH group of sapogenin was linked to C-1' –OH of D-arabinose through β -linkage.

On the basis of all the above facts, the following structure of the glycoside PS-III was assigned as;



Further the proposed structure was confirmed by ^1H -NMR, ^{13}C -NMR and mass spectral studies.

^1H -NMR SPECTRUM OF THE SAPONIN PS-III

The ^1H -NMR spectrum of the saponin was found to be in complete conformity of the above structure.

The characteristic signals obtained in the ^1H -NMR spectrum of the saponin PS-III [FIG. 4.6] and structural units inferred with the help of available literature⁶⁴⁻⁶⁸ are recorded in table 4.5.

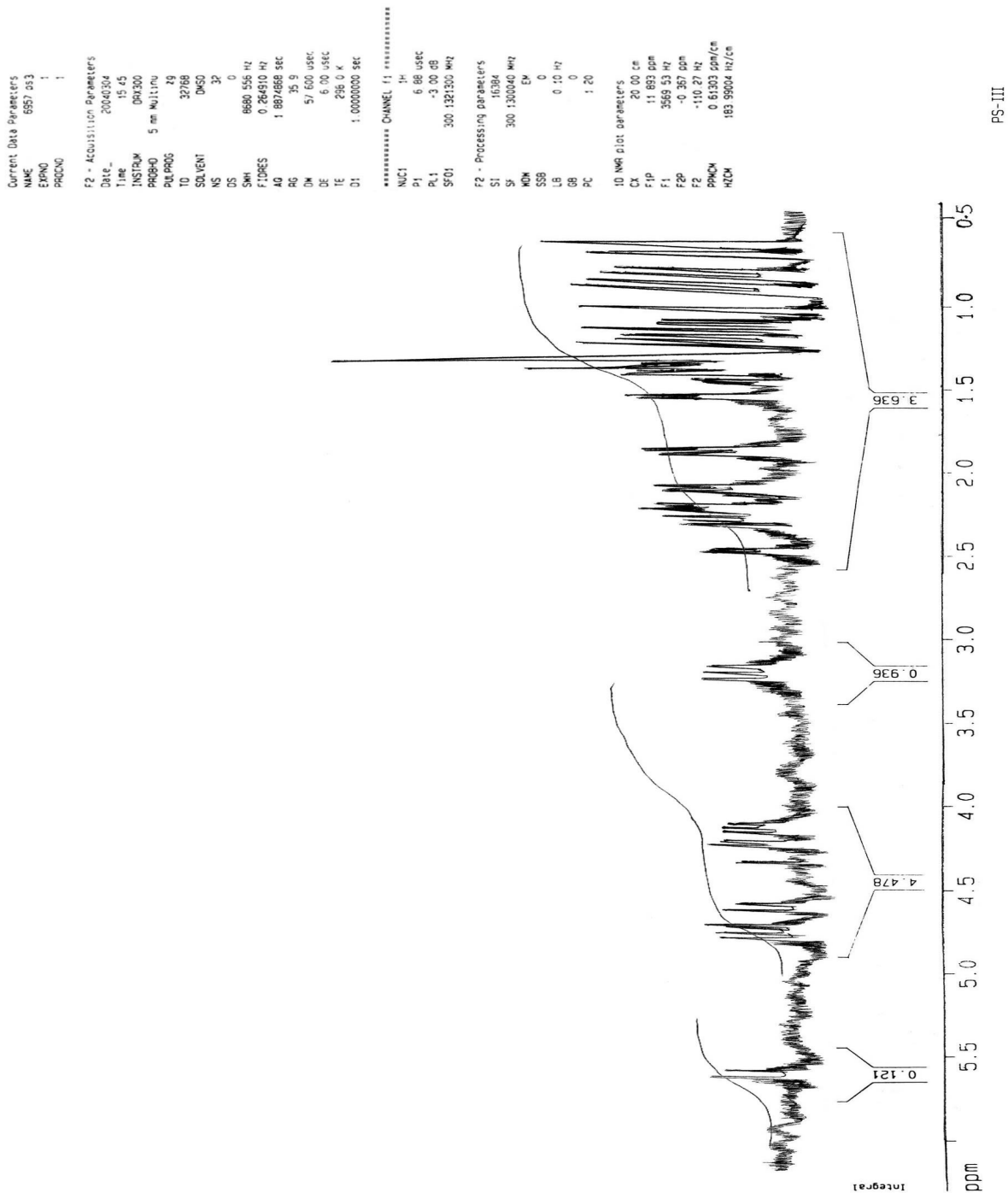
Table – 4.5

^1H -NMR spectrum (300 MHz, C_6D_6) of the saponin PS-III

S. No.	δ -Value	Pattern	J-Value Hz	No. of Protons	Assignments
1.	1.02	m	—	2	H-1
2.	1.18-1.40	m	—	12	H-2, 7, 11, 12, 15, 16
3.	3.25	dd	4.5 11.5	1	H-3
4.	2.20	dd	10.0 2.0	1	H-5
5.	1.90	m	—	2	H-6
6.	2.50	m	—	1	H-17
7.	0.67	s	—	3	H-18
8.	1.00	s	—	3	H-19
9.	1.42	m	—	1	H-20
10.	0.91	d	7	3	H-21
11.	1.53	m	—	2	H-22
12.	2.12	m	—	2	H-23
13.	2.15	m	—	2	H-24
14.	2.23	m	—	1	H-25

S. No.	δ -Value	Pattern	J-Value Hz	No. of Protons	Assignments
15.	0.95	d	7	3	H-26
16.	1.05	d	7	3	H-27
17.	1.08	s	—	3	H-28
18.	0.89	s	—	3	H-29
19.	1.04	s	—	3	H-30
20.	5.53	d	3	1	1' anomeric proton
21.	4.65	dd	10.0 3.0	1	H-2'
22.	4.55	dd	10.0 3.0	1	H-3'
23.	4.45	brs	—	1	H-4'
24.	4.27	d	12	1	H-5' α
25.	4.11	dd	12.0 2.0	1	H-5' β

¹H-NMR SPECTRUM OF THE SAPONIN PS-III



¹³C-NMR SPECTRUM OF SAPONIN PS-III

The various characteristic chemical shifts observed in the ¹³C-NMR spectrum PS-III [FIG. 4.7] and structural unit inferred with literature⁶⁹⁻⁷¹ are recorded below in the table-4.6.

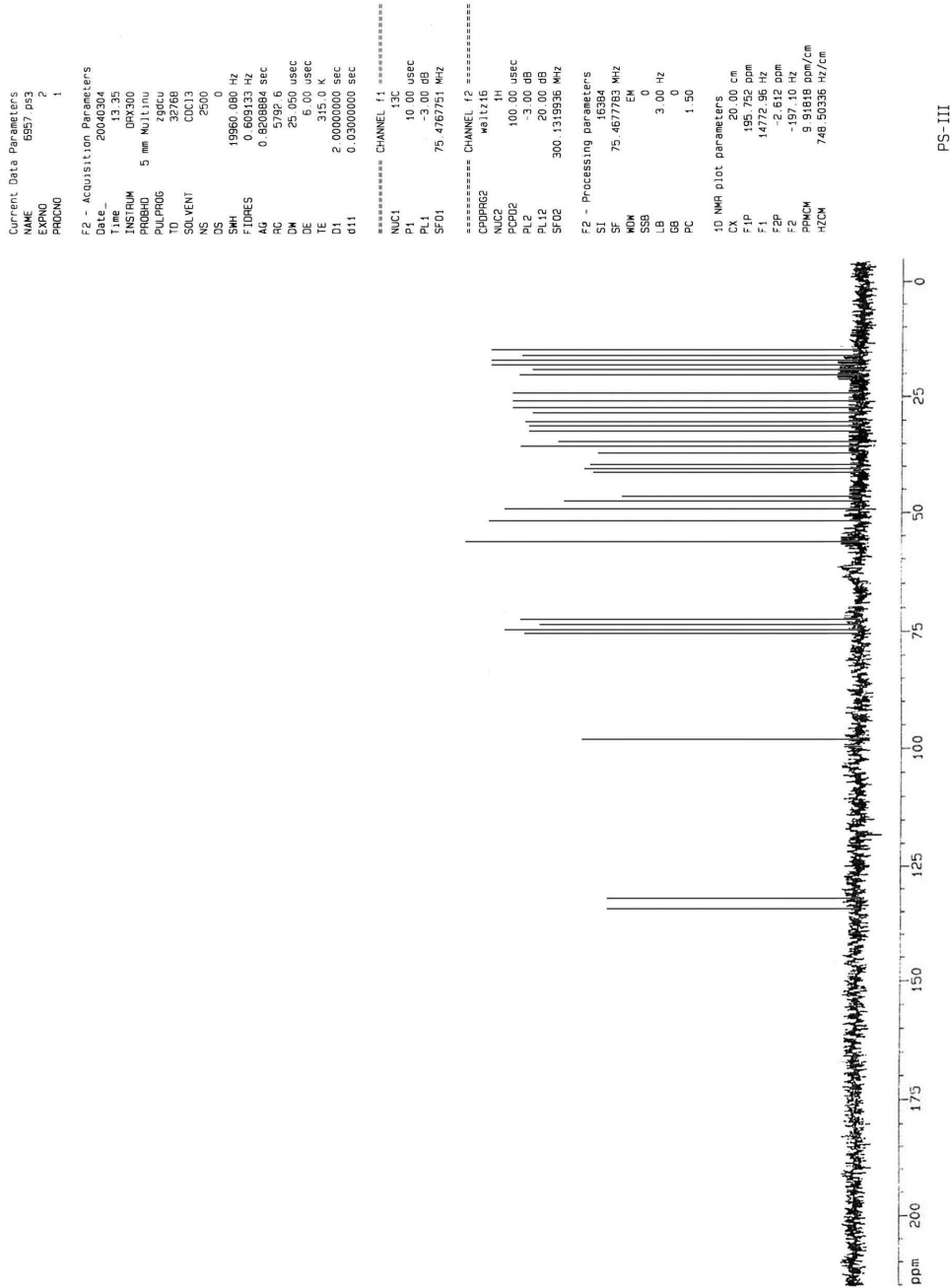
Table – 4.6

¹³C-NMR spectrum (300 MHz, CDCl₃) of the steroidal saponin PS-III

S.No.	δ-Value	Carbon No.	Carbon Type
1.	36.6	C-1	CH ₂
2.	30.0	C-2	CH ₂
3.	77.2	C-3	CH
4.	39.2	C-4	C
5.	50.8	C-5	CH
6.	22.5	C-6	CH ₂
7.	26.4	C-7	CH ₂
8.	139.8	C-8	C
9.	139.7	C-9	C
10.	35.5	C-10	C
11.	16.6	C-11	CH ₂
12.	26.8	C-12	CH ₂

S.No.	δ -Value	Carbon No.	Carbon Type
13.	42.7	C-13	C
14.	49.1	C-14	C
15.	30.3	C-15	CH ₂
16.	30.2	C-16	CH ₂
17.	48.8	C-17	CH
18.	15.0	C-18	CH ₃
19.	18.0	C-19	CH ₃
20.	35.7	C-20	CH
21.	18.4	C-21	CH ₃
22.	35.8	C-22	CH ₂
23.	23.6	C-23	CH ₂
24.	37.9	C-24	CH ₂
25.	27.5	C-25	CH
26.	22.2	C-26	CH ₃
27.	22.0	C-27	CH ₃
28.	23.3	C-28	CH ₃
29.	27.3	C-29	CH ₃
30.	15.2	C-30	CH ₃
31.	99.2	C-1'	CH
32.	70.3	C-2'	CH
33.	70.7	C-3'	CH
34.	71.7	C-4'	CH
35.	64.4	C-5'	CH ₂

¹³C-NMR SPECTRUM OF SAPONIN PS-III



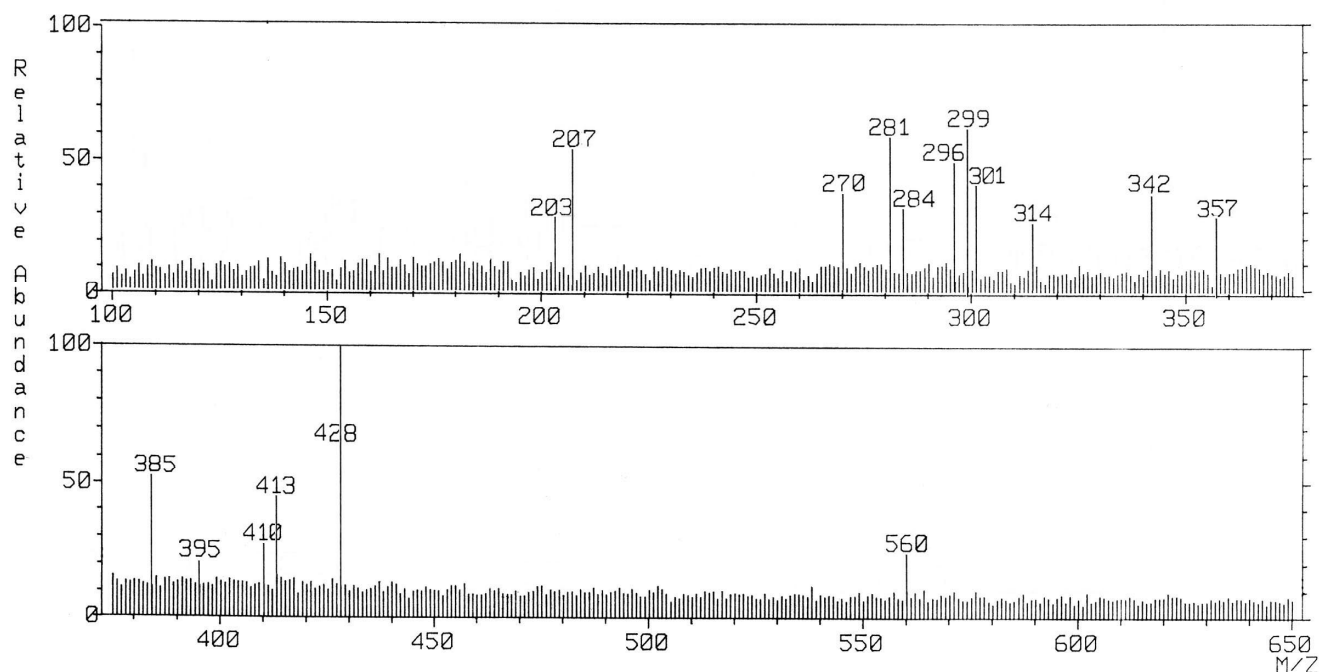
MASS SPECTRUM OF SAPONIN PS-III

The significant fragment peaks⁷² obtained in the FAB-MS of the saponin PS-III [FIG. 4.8] are follows as :-

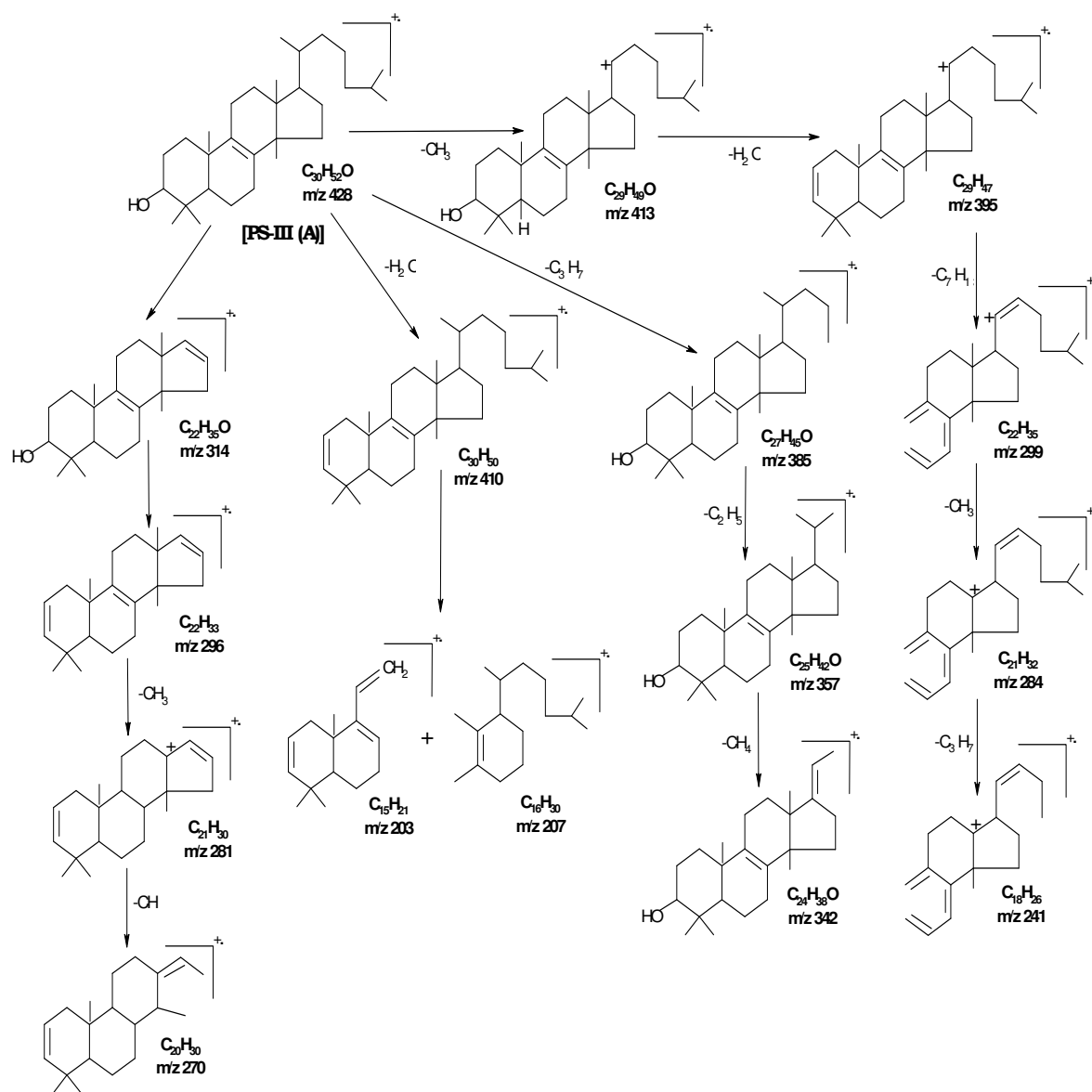
M/z 560, 428, 413, 410, 395, 385, 357, 342, 310, 301, 299, 296, 284, 281, 270, 207, 203 and 203.

The various species formed during fragmentation are shown in scheme-II, which further confirmed the identity of the saponin PS-III as 24,25-dihydro-euphol-3-O- β -D-arabinopyranoside.

MASS SPECTRUM Data File: 4EAP06K 6-APR- 4 11:56
Sample: PS-III PROF V K SAXENA SAGAR #6957
RT 0'24" FAB(Pos.) GC 1.4c BP: m/z 428.0000 Int. 59.7934 Lv 0.00
Scan# (1 to 5)



SCHEME-I



EXPERIMENTAL

PLANT MATERIAL

The roots of the *Euphorbia hirta* Linn.were collected locally in the month of September-October 2002 and were authenticated by taxonomist of Botany Department, Dr. H.S. Gour University, Sagar (M.P.). A voucher specimen No.XXXV has been submitted in the Chemistry Department of room no. 36.

ISOLATION OF STEROLDAL SAPONIN

The air-dried and powdered roots (3.0 kg) of *Euphorbia hirta* (Linn.) were extracted with 95% ethanol.The ethanolic extract was concentrated under reduced pressure to brown viscous dark coloured mass. It was successively extracted with petroleum ether (60-80°C), benzene, chloroform, acetone, ethyl acetate and methanol.

Except the methanol soluble fraction, all other fraction could not consider for the further study because they yielded very les amount, which was insufficient for any substantive study.

STUDY OF THE METHANOL SOLUBLE FRACTION

The methanol soluble portion was concentrated under reduced pressure to a dark brown colour mass. The concentrated mass was subjected to TLC examination using solvent system; n-butanol: acetic acid: water (4:1:5) and visualizing agent: I₂ vapours when depicted two spots, which indicated it to be the mixture of two compounds. It was therefore purified over silica gel G column by eluting with benzene: methanol in varying proportions. Elution from Benzene: methanol (19: 1) showed single spot of same R_F values and so were mixed. Removal of the solvent, afforded compound PS-III, which was crystallised from methanol.

The details of column chromatography are given below:-

S.No	Parameter	Amount
1.	Length of column	100 cm
2.	Diameter of column	3.0 cm
3.	Weight of crude extract	15.0 g
4.	Weight of the silica gel G	50 g

The results of the column chromatography are recorded below in table-4.7.

Table-4.7

S. No.	Fraction No.	Eluants	Spot On TLC	Remarks
1.	1-9	Benzene: MeOH (13:1)	Two	Mix. Rejected
2.	10-16	Benzene: MeOH (15:1)	Two	Mix. Rejected
3.	17-20	Benzene: MeOH (19:1)	One	Compound PS-III

STUDY OF FRACTIONS (17-20)

Eluates obtained from fraction Benzene: MeOH (19:1) were found to have same R_f values and therefore were combined together. Evaporation of the solvent afforded an amorphous mass (yield 0.062%). The mass gave single spot on TLC examination, using solvent system; n-butanol: acetic acid: water (4:1:5) and I_2 vapours as visualizing agent. The mass on crystallisation from methanol gave a compound PS-III.

STUDY OF THE STEROIDAL SAPONIN PS-III

The saponin PS-III was found to be soluble in methanol and ethanol. It analysed for the molecular formula $C_{35}H_{60}O_5$, m.p. 262-263°C, and $[M^+]$ 560 (FAB-MS).

It responded positively to all characteristic colour reactions of steroidal saponin and also responded to Molisch's test positively.

The following reaction showing its steroid nucleus.

(1) Salkowski Reaction:

A solution of compound in chloroform when mixed with concentrated sulphuric acid, produced a yellow colour changing to red.

(2) Liebermann-Burchard Reaction:

When few drops of concentrated sulphuric acid was added to the solution of the compound in acetic anhydride, produced red violet colour.

(3) Tschugaeff Reaction:

When a solution of the compound in chloroform treated with acetyl chloride and zinc chloride in small amount followed by heating, produced a red colour with greenish yellow fluorescence.

(4) Noller's Reactions:

When a small amount of the compound was treated with Noller's reagent (0.1% SnCl_2 in pure thionyl chloride), produced a light pink colour changing to violet.

The following reaction showed the compound to be saponin.

(1) Foam Test:

The compound PS-III (20mg) was dissolved in 20 ml of distilled water with vigorously shaking. The formation of a thick soapy layer, which persisted for a long time, indicated the presence of saponin.

(2) Haemolytic Test:

5.0 gm of gelatin and 80 ml of (0.1%) saline solution was taken in a 100 ml measuring flask. Blood (3.0 ml) was taken separately in a small conical flask (10 ml) to which small quantity of the gelatin suspension was added and stirred well.

The blood solution of conical flask was spread over a glass plate and a drop of the extract to be tested for the presence of saponin was applied with

the help of micropipette. After an hour the red blood gelatin become transparent.

Elemental Analysis:

Found	Calculated For $C_{35}H_{60}O_5$
C = 75.10%	C = 75.00%
H = 10.72%	H = 10.71%
Molecular weight 560 by FAB-MS	

ACETYLATION OF THE STEROIDAL SAPONIN PS-III

80 mg saponin PS-III was taken in a round bottomed flask containing 15 ml of pyridine, to which acetic anhydride (20ml) was added to the contents of the flask and refluxed for 5-6 hrs on water bath. After cooling, the mixture was poured into ice-cold water when resulted in a precipitate. The precipitate obtained was extracted with ethanol in a separating funnel. The ethanolic layer was separated, washed with sodium carbonate and on concentration afforded an acetylated derivative. It analysed for the molecular formula $C_{41}H_{66}O_8$, $[M^+]$ 686 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{41}H_{66}O_8$
C = 71.71%	C = 71.72%
H = 9.63%	H = 9.62%
% of acetyl group 18.79	% of acetyl group 18.80
Molecular weight 686 by FAB-MS	

ACID HYDROLYSIS OF SAPONIN PS-III

65 mg of the compound PS-III was refluxed with 7% conc. H_2SO_4 for 5-6 hrs. The contents of the flask were cooled and poured into ice-cold water. The compound obtained was crystallised from methanol to get colourless needles PS-III. The aqueous layer was then worked up separately for the identification of sugar.

STUDY OF THE SAPOGENIN

Sapogenin PS-III (A) analysed for m.f. $C_{30}H_{52}O$, m.p. 127-128°C and $[M^+]$ 428 (FAB-MS).

It responded positively to all the characteristic colour reactions of steroids as described on page 143 and also to responded Zimmerman colour test for C-3 keto group. It did not give Molisch's test for glycoside.

Zimmerman Colour Test:

The oxidised product was dissolved in 2N KOH (in absolute alcohol and kept for 15 minutes. After then the mixture was diluted with 10 ml absolute alcohol. Appearance of violet colour, which faded on dilution, confirmed the presence of keto group at C-3 position.

Elemental Analysis:

Found	Calculated For $C_{30}H_{52}O$
C = 84.10%	C = 84.11%
H = 12.12%	H = 12.14%
Molecular weight 428 by FAB-MS	

PREPARATION OF ACETYLATED PRODUCT OF AGLYCONE PS-III (A)

40 mg of the sapogenin PS-III (A) was mixed with 3ml of acetic anhydride and 200 mg of sodium acetate and taken in 100 ml round bottomed flask fitted with air condenser. The total contents of the flask were heated on an oil bath for 6 hrs. After then the mixture was allowed to cool and poured into cold water (50 ml), which resulted into a white precipitate. The precipitate obtained was extracted with solvent ether. The ethereal layer was then washed with water and sodium bicarbonate solution. The ethereal layer was then finally dried over anhydrous sodium sulphate. On evaporation of the solvent ether and after crystallisation from methanol gave an acetylated product, m.p 104-106°C, m.f. $C_{32}H_{54}O_2$ and $[M^+]$ 470 (FAB-MS).

Elemental Analysis:

Found	Calculated For $C_{32}H_{54}O_2$
C = 81.69%	C = 81.70%
H = 11.47%	H = 11.48%
% of acetyl group 9.13	% of acetyl group 9.14
Molecular weight 470 by FAB-MS	

IDENTIFICATION OF SUGAR IN THE HYDROLYSATE

After the acid hydrolysis of the saponin PS-III, sapogenin PS-III (A) was separated by filtration. The aqueous hydrolysate (60 ml) obtained after acid hydrolysis was neutralised with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated to syrupy mass under reduced pressure. The concentrated mass was subjected to paper chromatography on Whatmann filter paper No. 1 using aniline hydrogen phthalate as visualizing agent.

The results of the paper chromatography of the saponin are showed in table-4.8:-

Table-4.8
Identification of Sugars

S. No.	Solvent system	R _f Values		Sugar identified
		Reported	Found	
1.	n-butanol: acetic acid:water	0.21	0.22	D-arabinose

PERMETHYLATION OF SAPONIN PS-III FOLLOWED BY HYDROLYSIS

The saponin PS-III was dissolved in 3 ml of DMF and mixed with 7 ml of methyl iodide and 30 mg of Ag_2O in a 150 ml conical flask and allowed to stand for 72 hrs. The total contents of the flask were filtered. The filtrate obtained was concentrated under reduced pressure to afforded viscous mass.

This mass was again methylated with methyl iodide (6ml) and Ag_2O (60mg) and was filtered off. The precipitate obtained was dissolved in CHCl_3 . The CHCl_3 layer on removal of the solvent afforded a methylated product, which on acid hydrolysis with 7% concentrated H_2SO_4 gave sapogenin and methylated sugar. The sapogenin PS-III (A) was separated and the aqueous hydrolysate was neutralised with BaCO_3 and BaSO_4 was filtered off. The filtrate obtained was concentrated under reduced pressure. Sugar was identified by paper chromatography using Whatmann filter. paper No. 1, using solvent system (4:1:5) and visualising agent as aniline hydrogen phthalate, as; 2', 3',4'-tri-O-methyl-D-arabinose (CoPC and CoTLC).

ENZYMATIC HYDROLYSIS OF THE SAPONIN PS-III

60 mg of the compound PS-III was dissolved in 15 ml of MeOH and mixed with enzyme almond emulsion (15 ml) in a 100 ml conical flask fitted with a stopper. The total content of the flask were allowed to stand at room temperature and then it was filtered. The aqueous hydrolysate was concentrated and subjected to paper chromatography using Whatman filter paper No. 1 and solvent system: nBAW (4 : 1 : 5) . The sugar was identified as D-arabinose (R_f 0.22).

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70. Li,L.,Xue,H. Kangouri,K., Ikedo,A., Omura, S., ***Planta Medica.***, Vol. 55, p. 294-296, (1989).
71. Agarwal, P.K., ***Phytochemistry***, Vol. 31, p. 3307-3330, (1992).
72. Hamming, M. and Foster, N., “Interpretation of Mass Spectra of Organic Compounds”, Academic Press, New York, (1972).

- Research is to see what everybody
- else has been and thinking what
- nobody has thought.

A vertical strip of seven stylized, colorful letters: 'A', 'B', 'C', 'D', 'E', 'F', and 'G'. Each letter is filled with a vibrant, multi-colored pattern and has a thin black outline.

PART-A

ISOLATION AND STUDY OF THE TRITERPENOID PS-IV: TARAXEROL FROM THE STEM OF *EUPHORBIA HIRTA* LINN.*

*This work has published in Research *Journal of Chemical Sciences*.

E*uphorbia hirta* Linn.¹ belongs to natural order Euphorbiaceae. It is an annual herbaceous plant with a slender hairy stem. Its flowers are small and yellow in colour. The plant produces milky latex, which is irritating to the mucus membrane.

The leaves of this plant have relaxing action upon the smooth muscles of lungs. It possesses great therapy in bronchial asthma. The whole plant is used for the treatment of diseases in children especially in worms, bowels complain and cough. Its juice is used in bronchial asthma, affections and chronic coughs. The latex of this plant is used as an application for warts. The roots of this plant possess anti-emetic properties. Its juice is used in dysentery, colic and also in warm infection.²⁻⁶

ISOLATION OF THE TRITERPENOID PS-IV

The stems of the *Euphorbia hirta* Linn. were collected locally and identified by taxonomist of Botany Department of Dr. H.S. Gour University Sagar (M.P.). The plant material was collected in the month of September and October-2002 and herbarium specimen no. XXXV has been deposited in Chemistry Department, room no. 36 of this University.

The air-dried and powdered stem (4.0 kg) of the plant *Euphorbia hirta* Linn. was extracted with 95% ethanol. The total ethanolic extract was concentrated under reduced pressure to get a light brown viscous mass, which was successively and sequentially fractionated with various solvent

from non polar to polar as petroleum ether (60–80°C), benzene, chloroform, ethyl acetate, acetone and methanol.

Except the ethyl acetate other fractions were not considered for phytochemical investigation as they yielded very less amount of residue for any substantive study.

The ethyl acetate extract on concentration under reduced pressure yielded a brown viscous mass, which on addition of excess of solvent, ether gave a precipitate. The precipitate was then subjected to TLC examination, using n-butanol: acetic acid: water (4: 1: 5) as solvent system and I₂ vapour as visualising agent when depicted two spots. Therefore, it was subjected to column chromatography over silica gel G for purification and eluted with benzene, benzene: chloroform (1: 1), benzene: chloroform: ethyl acetate (1: 1: 1) and studied separately.

STUDY OF THE ELUATES FROM BENZENE: CHLOROFORM: ETHYL ACETATE (1: 1: 1)

Eluates collected from benzene: chloroform: ethyl acetate (1: 1: 1) were found to have same R_f values and hence were combined. Removal of the solvent, gave an amorphous mass, which was found to be homogeneous on TLC examination using solvent system [n-BAW (4:1:5)] and I₂ vapour as visualizing agent. The mass on crystallisation from methanol gave colourless needles compound PS-IV, whose yield was 0.055%.

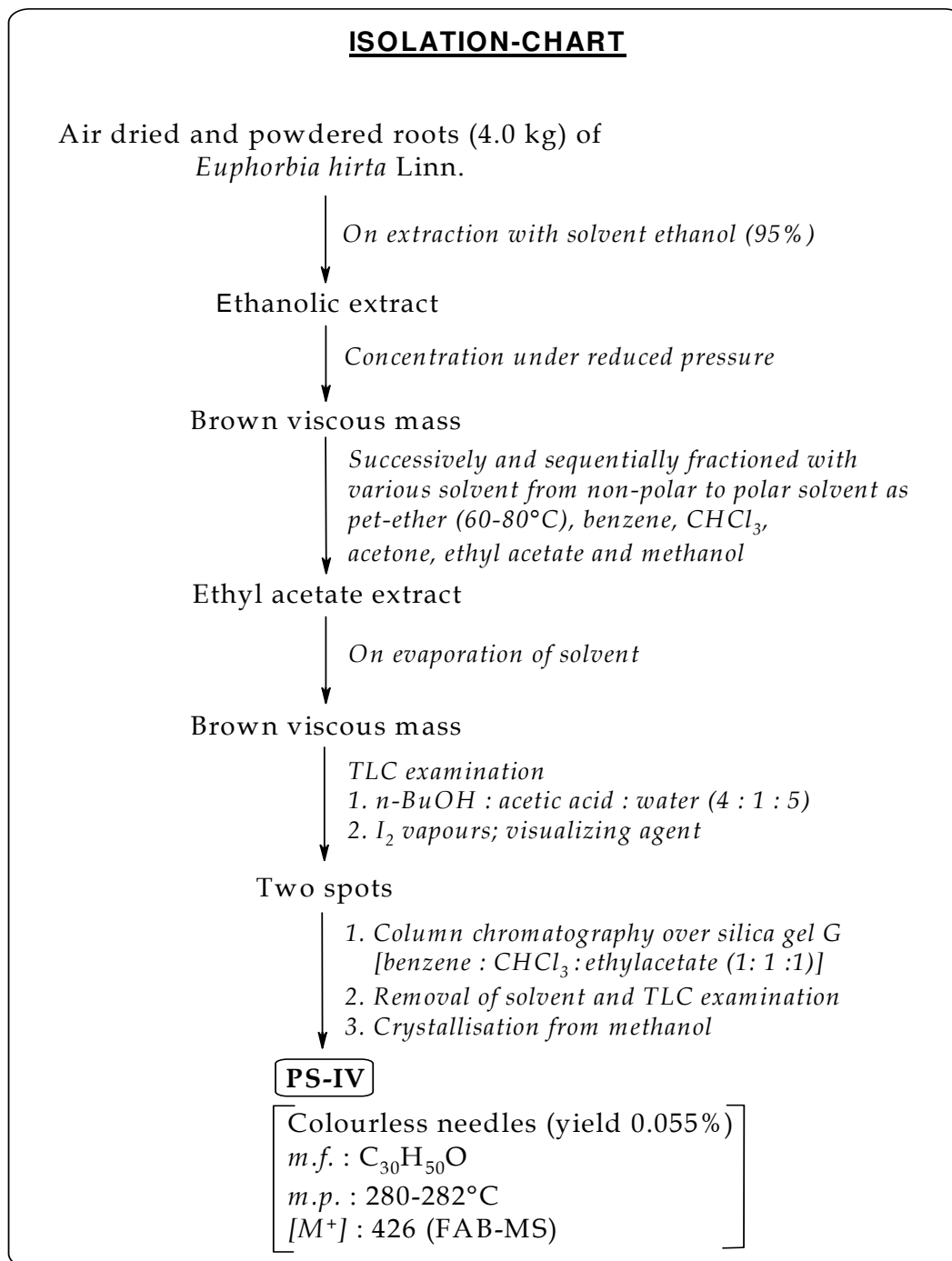


FIG. 5.1: Fractionation protocol for isolation of compound PS–IV from plant
Euphorbia hirta Linn.

STUDY OF TRITERPENOID PS-IV

The colourless needles were soluble in methanol and ethanol. It analysed for molecular formula, $C_{30}H_{50}O$, m.p. 280–282°C and $[M^+]$ 426 (FAB-MS). It responded to all colour reactions of triterpenoid.⁷⁻¹⁰

IR SPECTRUM OF THE TRITERPENOID PS-IV

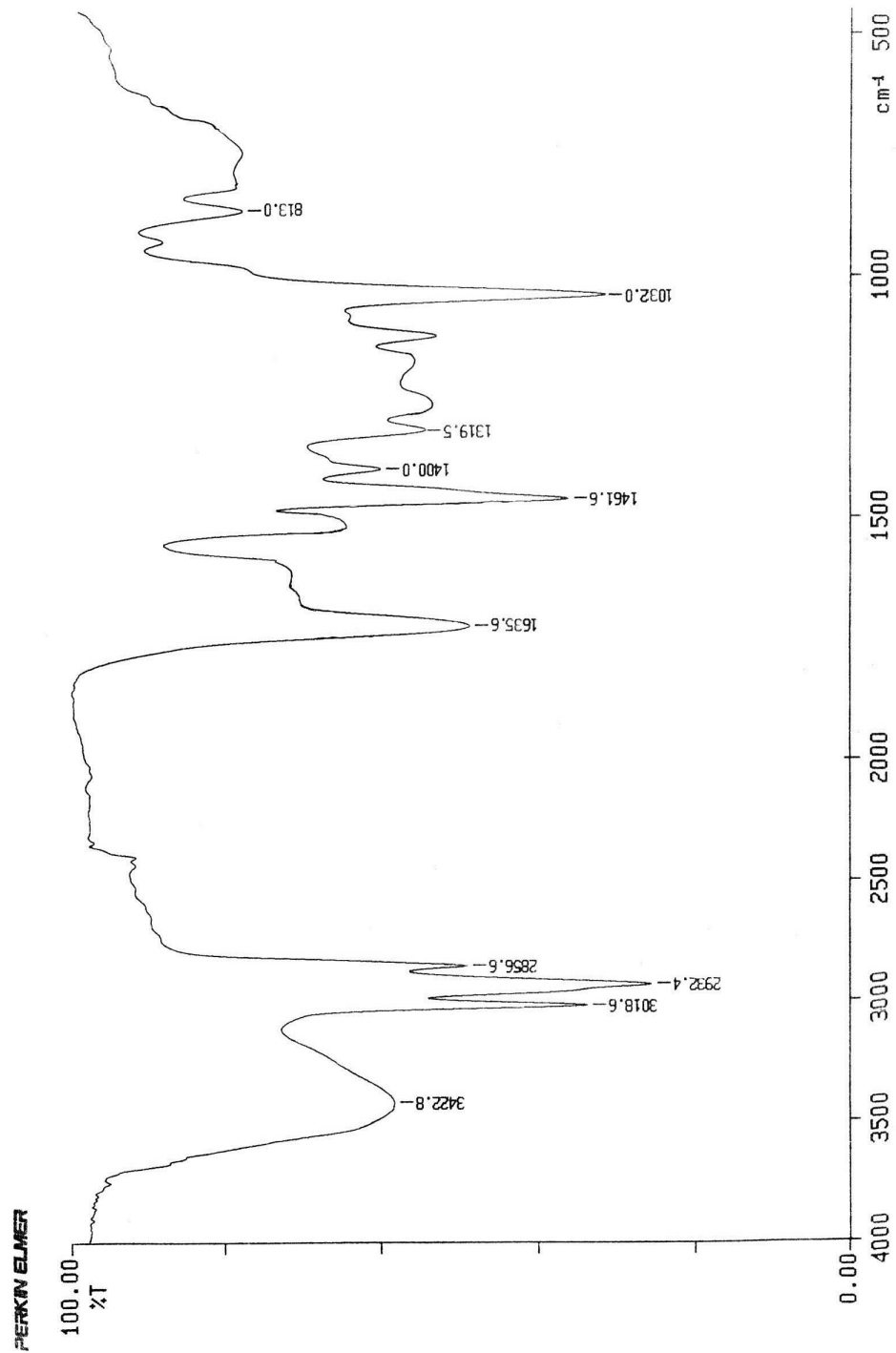
In the IR spectrum of triterpenoid PS-IV [FIG. 5.2] the prominent bands obtained and the structural units inferred with the help of available literature¹¹⁻¹⁶ are given below in Table-5.1.

Table-5.1

IR Spectrum of the triterpenoid PS-IV

S.No.	Wave Number cm^{-1}	Assignment
1.	3422.8	-OH group(s)
2.	3018.6	= C-H Stretching vibration
3.	2932.4	C-H Stretching vibration –CH ₃ group
4.	2856.6	C-H Stretching vibration
5.	1635.6, 813.0	> C=CH (trisubstituted)
6.	1461.6	C-H bending vibration
7.	1400.0, 1319.5, 1032.0	Triterpenoidal nature

IR SPECTRUM OF THE TRITERPENOID PS-IV



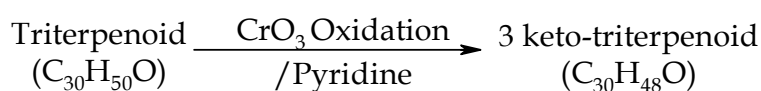
04/02/25 10:54 AM, CODE-PS-IV
X: 4 scans, 4.0cm-1, flat, smooth, abex

PRESENCE OF –OH GROUPS IN THE TRITERPENOID PS-IV

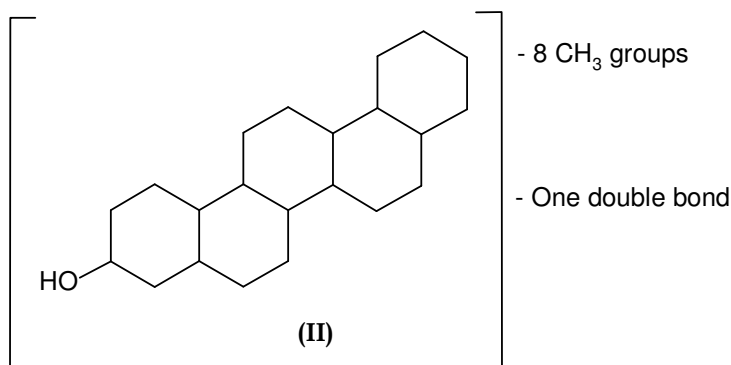
In the IR spectrum of the triterpenoid PS-IV, a band at $\nu_{\text{max}}^{\text{KBr}}$ 3422.8 cm^{-1} showed the presence of hydroxyl group in it. On acetylation with pyridine and acetic anhydride PS-IV furnished an acetylated product, which analysed for molecular formula $\text{C}_{32}\text{H}_{52}\text{O}_2$, m.p. 304–306°C and $[\text{M}^+]$ 468 [FAB-MS]. The percentage of an acetyl group was determined by Wiesenberger¹⁷ method as described by the Belcher and Godbert¹⁸ (9.18%), which indicated the presence of one hydroxyl groups in it.

POSITION OF THE –OH GROUP IN THE TRITERPENOID PS-IV

Chromic acid oxidation of PS-IV with pyridine at room temperature afforded an oxidised product having m.f. $\text{C}_{30}\text{H}_{48}\text{O}$, m.p. 242-244°C and $[\text{M}^+]$ 425, which responded to Zimmermann test positively for C-3 keto group. Thus indicating the presence of –OH group at C-3 position and further confirmed its nature as secondary in PS-IV¹⁹⁻²¹.

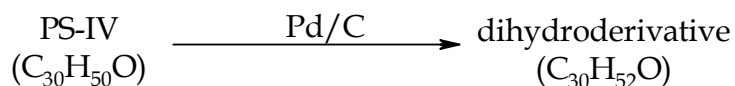


On the basis of above deliberations, the following tentative structure-II was assigned to the triterpenoid PS-IV as:-



PRESENCE OF DOUBLE BOND IN THE TRITERPENOID PS-IV

In the IR spectrum of the triterpenoid PS-IV the characteristic band at $\nu_{\text{max}}^{\text{kBr}}$ 1635.6 cm^{-1} indicated the presence of double bond in it. On catalytic hydrogenation with Pd/C, it gave dihydroderivative, molecular formula, $\text{C}_{30}\text{H}_{52}\text{O}$, m.p. 186-187°C, which indicated the presence of one double bond in it.



POSITION OF THE DOUBLE BOND IN THE TRITERPENOID PS-IV

The position of the double bond was ascribed by the following aspects :-

- (1) PS-IV gave yellow colour with tetranitromethane (RUZICKA'S REACTION)²² which showed the presence of double bond in the nucleus.
- (2) The position of trisubstituted olefinic proton in the compound was ascribed by double doublet which appeared in ^1H -NMR spectrum of PS-

IV at δ 5.53 for 1H, which indicated the presence of double bond at C-15 position.²³⁻²⁵

- (3) The band, which appeared at 813.0 cm⁻¹ in the IR spectrum of PS-IV, further, assisted the presence of trisubstituted double bond.^{24, 26}

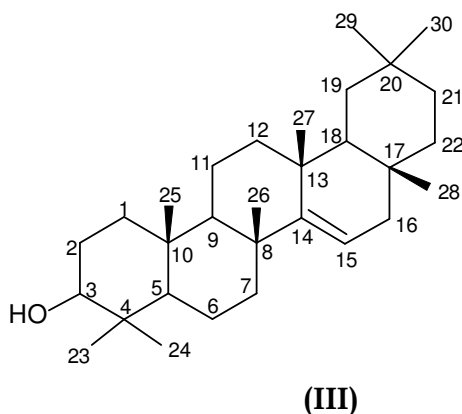
PRESENCE OF THE METHYL GROUP(S) IN THE PS-IV

Band at wave number 2932.4 cm⁻¹ in the IR spectrum of the PS-IV showed the presence of methyl group(s) in it. PS-IV when quantitatively estimated by Zeisel's²⁷ method (21.73%) revealed the presence of eight methyl groups in it.

The position of methyl groups were established by the ¹H-NMR spectrum of PS-IV which gave three proton intensity singlets each at δ 1.09, δ 0.93, δ 1.22, δ 1.10, δ 0.78, δ 0.85, δ 0.98 and δ 0.95 were assigned for the position of methyl groups at C-23, C-24, C-25, C- 26, C-27, C-28, C-29, C-30 in PS-IV.²⁸⁻³⁰

The ¹³C-NMR spectrum showed chemical shift at δ 21.5, δ 26.2, δ 16.2, δ 26.7, δ 19.5, δ 30.2, δ 33.7, δ 29.9 for C-23, C-24, C-25, C-26, C-27, C-28, C-29, C-30.^{31- 32}

The above facts indicated that the triterpene PS-IV was taraxerol, which was identified by m.m.p. and superimposable spectral analysis and with the support of available literature.



The structure-III was further confirmed by ^1H -NMR, ^{13}C -NMR, and mass spectral studies.

^1H -NMR SPECTRUM OF THE TRITERPENOID PS-IV

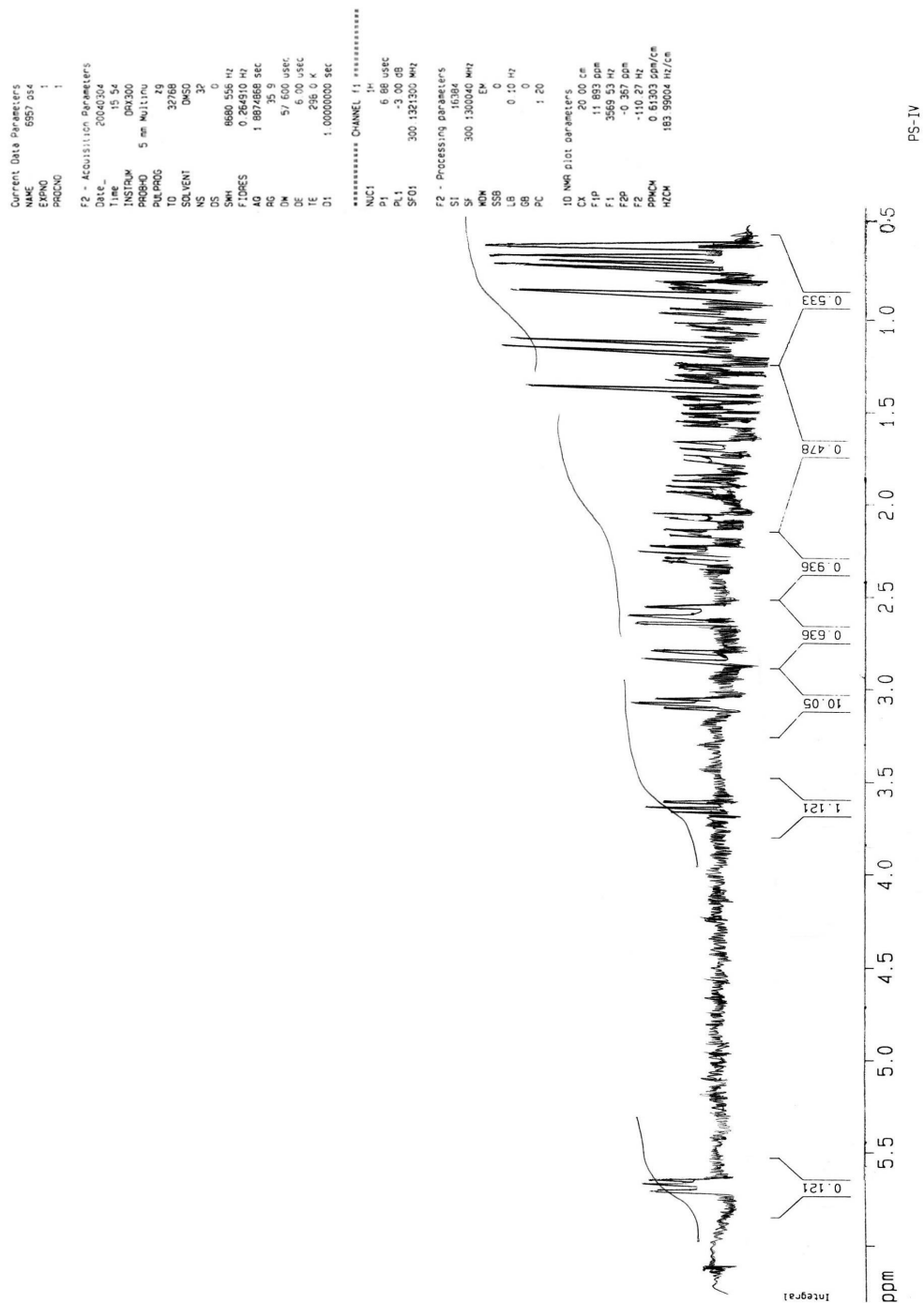
^1H -NMR spectrum of the triterpenoid was found to be in complete conformity to the above structure (III). The important signals, which appeared in the ^1H NMR spectrum of the triterpenoid PS-IV, [FIG. 5.3] and the structural units inferred with the help of the available literature³³⁻³⁵, are given below in table-5.2.

Table-5.2
¹H-NMR Spectrum of the Triterpenoid PS-IV

S. No.	δ -Value	Pattern	J-Value Hz	No. of Protons	Assignments
1.	2.03	m	-	1	H-1a
2.	2.10	m	-	1	H-1b
3.	2.38	ddd	15.2, 6.2, 2.4	1	H-2a
4.	2.63	ddd	16.4 11.0 6.6	1	H-2b
5.	3.62	t	-	1	H-3
6.	1.29	m	-	1	H-5
7.	1.71	m	-	1	H-6
8.	1.17	m	-	1	H-7a
9.	1.46	m	-	1	H-7b
10.	1.02	d	4.8	1	H-9
11.	3.14	t	4.8	1	H-11
12.	2.80	d	4.8	1	H-12
13.	5.53	dd	8.0, 3.5	1	H-15
14.	1.69	dd	14.6, 8.2	1	H-16a
15.	1.89	dd	14.6,	1	H-16b

S. No.	δ -Value	Pattern	J-Value Hz	No. of Protons	Assignments
			3.4		
16.	1.16	m	-	1	H-18
17.	1.55	m	-	1	H-19a
18.	1.42	m	-	1	H-19b
19.	0.98	m	-	1	H-22a
20.	1.07	m	-	1	H-22b
21.	1.09	s	-	3	H-23
22.	0.93	s	-	3	H-24
23.	1.22	s	-	3	H-25
24.	1.10	s	-	3	H-26
25.	0.78	s	-	3	H-27
26.	0.85	s	-	3	H-28
27.	0.99	s	-	3	H-29
28.	0.95	s	-	3	H-30

¹H-NMR SPECTRUM OF THE TRITERPENOID PS-IV



¹³C-NMR SPECTRUM OF THE TRITERPENOID PS-IV

The significant signals observed in the ¹³C-NMR spectrum of the triterpenoid PS-IV [FIG. 5.4] and structural units inferred on the basis of available literature³⁶⁻³⁹ are tabulated below in table-5.3: -

Table–5.3

¹³C-NMR spectrum of the Triterpenoid PS-IV

S.No.	δ Value	Carbon No.	Carbon type
1.	39.5	C-1	CH ₂
2.	33.8	C-2	CH ₂
3.	216.8	C-3	CH
4.	47.3	C-4	C
5.	54.6	C-5	CH
6.	38.6	C-6	CH ₂
7.	36.6	C-7	CH ₂
8.	36.5	C-8	C
9.	52.7	C-9	CH
10.	38.9	C-10	C
11.	51.6	C-11	CH ₂
12.	58.1	C-12	CH ₂
13.	37.6	C-13	C
14.	156.5	C-14	C

S.No.	δ Value	Carbon No.	Carbon type
15.	119.2	C-15	CH
16.	38.3	C-16	CH ₂
17.	35.5	C-17	C
18.	48.2	C-18	CH
19.	20.1	C-19	CH ₂
20.	28.7	C-20	CH ₂
21.	33.2	C-21	CH ₂
22.	35.2	C-22	CH ₂
23.	21.5	C-23	CH ₃
24.	26.2	C-24	CH ₃
25.	16.2	C-25	CH ₃
26.	26.7	C-26	CH ₃
27.	19.5	C-27	CH ₃
28.	30.2	C-28	CH ₃
29.	33.7	C-29	CH ₃
30.	29.9	C-30	CH ₃

¹³C-NMR SPECTRUM OF THE TRITERPENOID PS-IV

Current Data Parameters
NAME 6957 ps4
EXPNO 2
PROCNO 1

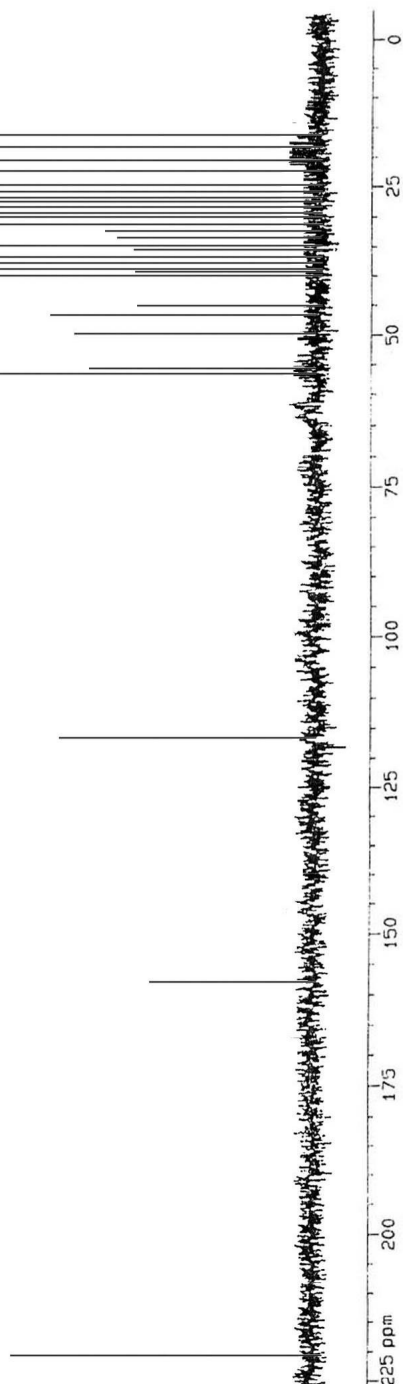
F2 - Acquisition Parameters
Date_ 20040304
Time 13.45
INSTRUM DRX300
PROBHD 5 mm Multinu
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 2500
DS 0
SWH 19960.080 Hz
FIDRES 0.609133 Hz
AQ 0.8208884 sec
RG 5792.6
DW 25.050 usec
DE 6.00 usec
TE 315.0 K
D1 2.00000000 sec
d11 0.03000000 sec

===== CHANNEL f1 =====
NUC1 13C
P1 10.00 usec
PL1 -3.00 dB
SF01 75.476751 MHz

===== CHANNEL f2 =====
CPOPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -3.00 dB
PL12 20.00 dB
SF02 300.1319936 MHz

F2 - Processing parameters
SI 16384
SF 75.4677783 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.50

1D NMR plot parameters
CX 20.00 cm
F1P 195.752 ppm
F1 14772.96 Hz
F2P -2.612 ppm
F2 -197.10 Hz
PPMCM 9.91818 ppm/cm
HZCM 748.50336 Hz/cm



PS-IV

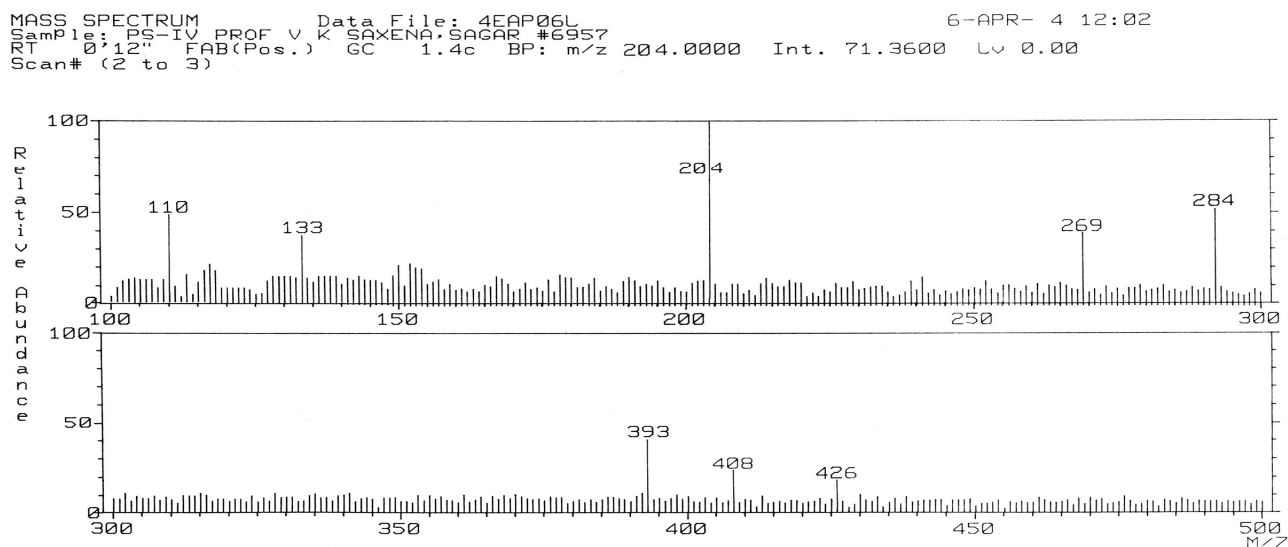
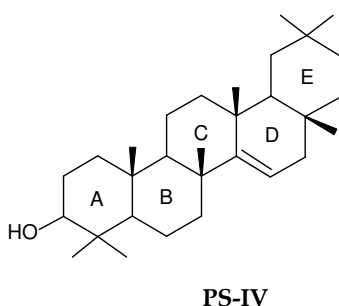
MASS SPECTRUM OF THE TRITERPENOID PS-IV

The significant mass fragmentation⁴⁰⁻⁴² pattern obtained in the FAB-MS of PS-IV [FIG. 5.5] is given below:-

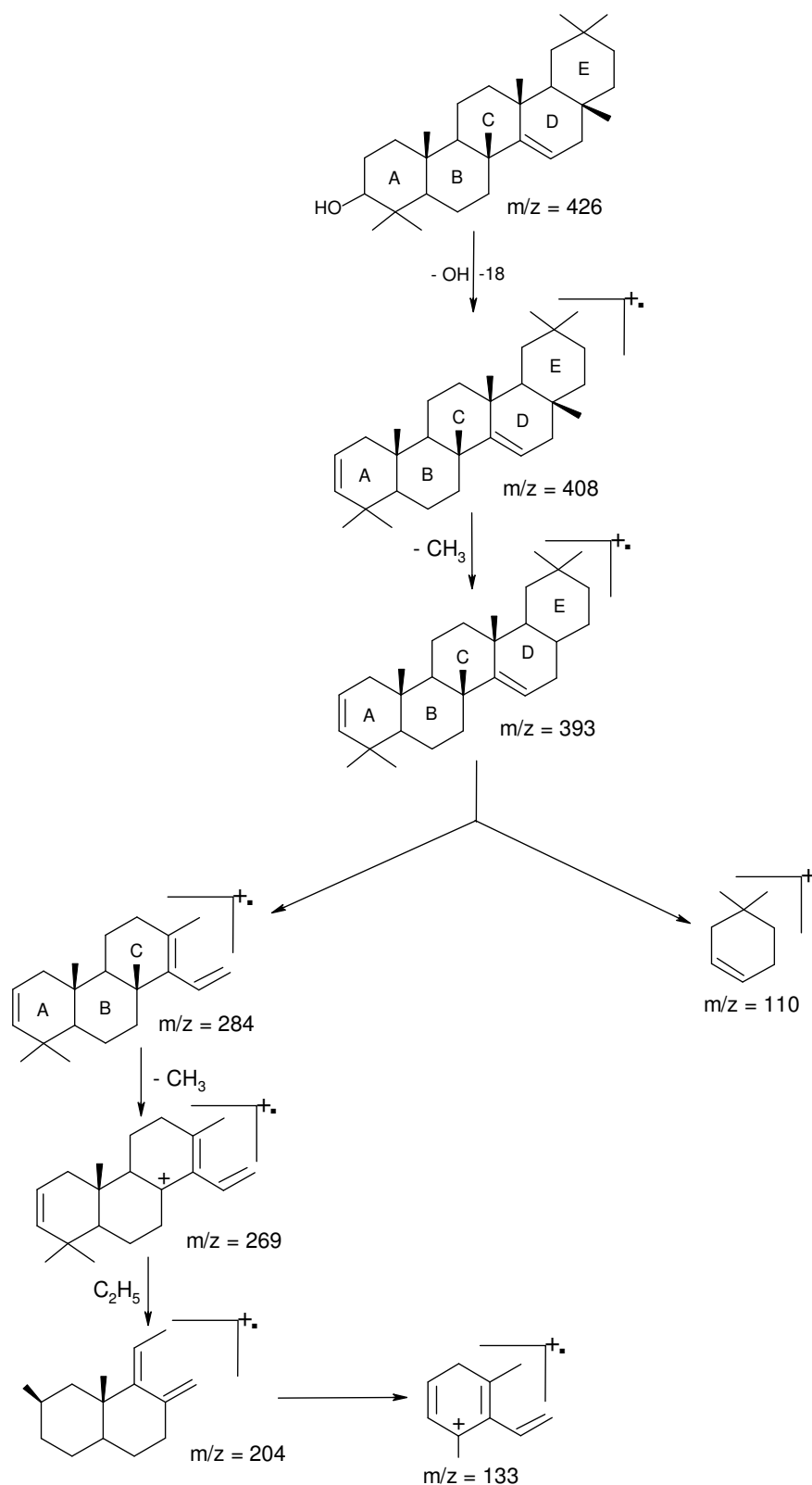
(FAB-MS) m/z 426 [M^+], 408, 393, 284, 269, 204 (base-peak) and 133.

Various species obtained are represented in the scheme-I below and were in full agreement with the proposed structure of triterpenoid and confirmed its identity as taraxerol.

After viewing all the above deliberations, the structure assigned to the triterpenoid PS-IV is given below.



SCHEME-I



EXPERIMENTAL

PLANT MATERIAL

The stem of the plant *Euphorbia hirta* Linn. was collected locally in the month of September and October-2002 and was authenticated by taxonomist of Botany Department, Dr. H.S. Gour University, Sagar (M.P.) A voucher specimen no. XXXV has been submitted in the room no. 36 of the Chemistry Department of this university.

ISOLATION OF THE TRITERPENOID

About 4.0 Kg stem of *Euphorbia hirta* Linn. was air dried and powdered and then extracted with 95% ethanol. The total ethanolic extract was concentrated under reduced pressure to give brown viscous mass, which was successively and sequentially fractionated with solvents from non- polar to polar as petroleum ether (60 –80° C), benzene, chloroform, acetone, ethyl acetate and methanol. Except the ethyl acetate fraction, other fractions were not considered for the phytochemical study as they yielded very less amount of residue.

The ethyl acetate soluble fraction on concentration under reduced pressure afforded a brown viscous mass, which on excess addition of solvent ether gave a precipitate. The precipitate was subjected to TLC examination over silica gel G, using n- butanol: acetic acid: water (4:1:5) as solvent system and I₂ vapours as visualizing agents when depicted two spots. Therefore, the mixture was subjected to the column chromatography over silica gel G and eluted with benzene, benzene: chloroform (1:1), benzene: chloroform: ethylacetate (1:1:1) and studied separately.

COLUMN CHROMATOGRAPHY

S.No	Parameter	Amount
1.	Length of column	150 cm
2.	Diameter of column	4.0 cm
3.	Weight of crude extract	7.0 g
4.	Weight of the silica gel G	100 g

The results obtained in column chromatography are recorded below in table-5.4.

Table- 5.4

S. No.	Fraction No.	Eluants	Spot On TLC	Remarks
1.	1-4	Benzene	Nil	Sticky Mass
2.	5-11	Benzene: chloroform(1:1)	Two	Mix. Rejected
3.	12-24	Benzene: chloroform: ethylacetate (1:1: 1)	One	Compound PS-IV

STUDY OF THE FRACTIONS (12-24)

Eluates collected from the fractions benzene: chloroform: ethyl acetate (1:1:1) were found to have same R_f values, and hence were combined together. On removal of the solvent, it gave a colourless needles compound PS-IV (0.055%). It was found to be homogeneous on TLC examination [n BAW (4:1:5), visualizing agent: I_2 vapours]. The compound was crystallised from methanol.

STUDY OF THE TRITERPENOID PS-IV

The compound PS-IV analysed for the molecular formula $C_{30}H_{50}O$, m.p. 280-282°C and $[M^+]$ 426 [FAB-MS].

It responded positive to the following characteristic colour reaction of triterpenoid given below which showing its steroidal nature.

(1) Salkowski Reaction:

The compound PS-IV was dissolved in chloroform and small amount of concentrated sulphuric acid was added to that solution. A yellow colour solution changing to red was obtained which confirmed the presence of triterpenoid.

(2) Liebermann-Burchard Reaction:

When few drops of concentrated sulphuric acid was added to the solution of compound PS-IV in acetic anhydride, red violet colour was obtained.

(3) Tschugaeff Reaction:

When a solution of compound PS-IV in chloroform was mixed with acetyl chloride and a small amount of zinc chloride followed by heating produced a red colour with greenish yellow fluorescence.

(4) Noller's Reaction:

The compound PS-IV when treated with Noller's reagent (0.1% $SnCl_2$ in thionyl chloride) gave a light pink colour changing to violet colour.

Elemental Analysis:

Found	Calculated For $C_{30}H_{50}O$
C= 85.70%	C=85.71%
H=11.74%	H=11.73%
Molecular weight = 426(FAB-MS)	

ACETYLATION OF THE TRITERPENOID PS-IV

70 mg of the compound PS-IV was taken in a 150ml of round-bottomed flask to which 20ml of acetic anhydride was added along with 10ml of pyridine. The reaction mixture was allowed to heat on a water bath for 3 hours. Pouring the ice-cold water quenched the mixture. The solid was obtained which was filtered and then washed with dilute hydrochloric acid followed by water. The product obtained was crystallised from benzene: chloroform: ethyl acetate (1: 1: 1) as white crystals, m.p. 302-304°C, m.f. $C_{32}H_{52}O_2$ and $[M^+]$ 468 (FAB-MS)

Elemental Analysis:

Found	Calculated For $C_{32}H_{52}O_2$
C= 82.06%	C = 82.05%
H=11.10%	H=11.11%
%of acetyl group 9.19%	%of acetyl group 9.18%
Molecular weight = 468 (FAB-MS)	

OXIDATION OF THE TRITERPENOID PS-IV WITH CHROMIC ACID

The compound PS-IV (25mg) was mixed with pyridine and 2ml of water and then heated for 2 hours with 15mg of chromic acid. The total reaction mixture of the flask was diluted with water and then extracted with solvent ether. The ethereal layer was obtained which on removal of the solvent afforded a ketonic product, m.f. $C_{30}H_{49}O$, m.p. 242-244°C and $[M^+]$ 425 (FAB-MS).

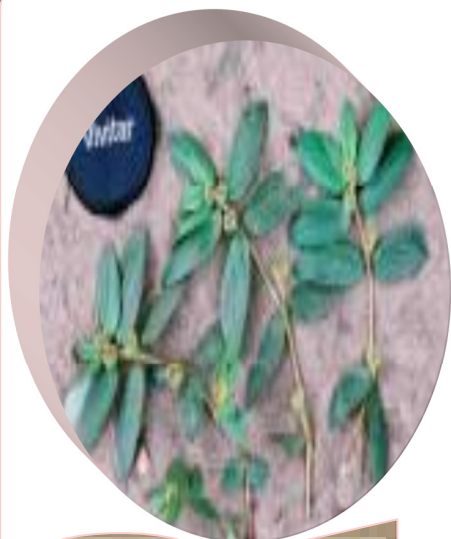
It responded to the Zimmermann test, for the presence of ketonic group.

Zimmermann Colour Test:

The oxidized product was dissolved in 2N KOH in absolute alcohol. After 15 min. the mixture was diluted with 15ml of absolute alcohol. Appearance of violet colour, which faded on dilution, confirmed the presence of keto group at C-3 position.

Elemental Analysis:

Found	Calculated For $C_{30}H_{49}O$
C= 84.71%	C=84.70%
H=11.53%	H=11.52%
Molecular weight = 425 (FAB-MS)	



- *Our mind is like a garden, which can*
- *either be intelligently cultivated or be*
- *allowed to run wild.*

PART-B

This work has been publish in Research Journal of Pharmeucitical Sciences (ISCA)

Asthma is a chronic respiratory disease. Chronic Obstructive Pulmonary Disease (COPD) is a lung disease that affects millions of people. COPD is a disease includes emphysema and chronic bronchitis. This may developed into asthma- A variety of inflammatory mediators and triggers may initiate and activate several target cells, in the airway, resulting in bronchoconstriction, microvascular leakage, oedema, mucus hypersecretion and stimulation of pleural reflexes thus worsen an asthma attack⁴³.

Asthma is a reactive airway disease, which causes the airways lining to swell and narrow, which in turns make the airways more sensitive. This further give rise to obstruction is caused by tightening of muscles surrounding the airways. This is also called bronchospasm^{44, 45}.

Experimental bronchospasm of guinea pigs provoked by inhalation of histamine has been widely employed to investigate the protective action of antihistaminic and bronchodilator agents⁴⁶.

It has been observed that the most of the anti-asthmatic agents have side effects. Therefore, there is a need to find the effective and safe drugs for asthma.

So the two plants; (1) *Acorus calamus*(Linn.) (2) *Euphorbia hirta* (Linn.) were choosen for their anti-asthmatic efficiency.

EXPERIMENTAL

EXTRACTION OF THE COMPOUNDS

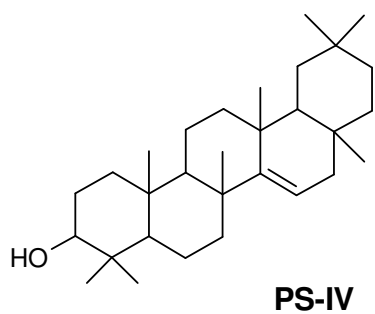
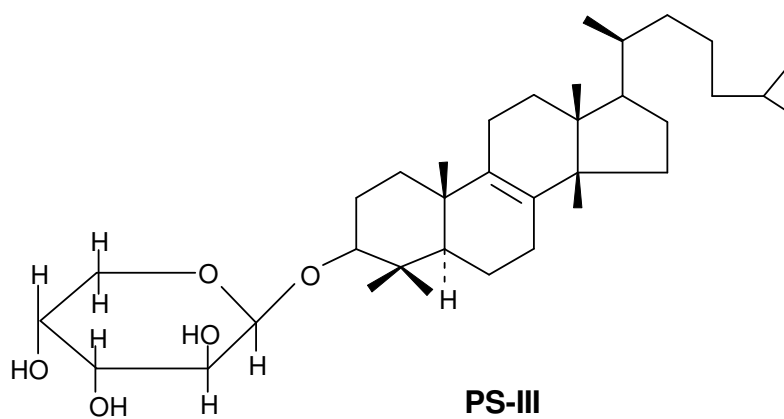
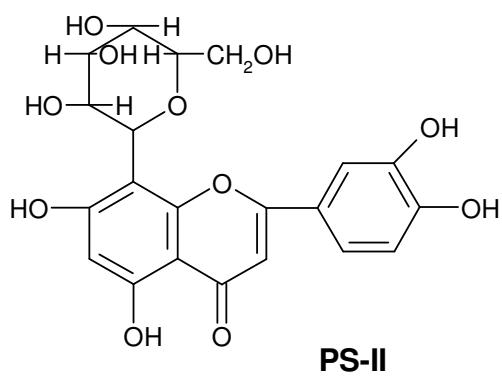
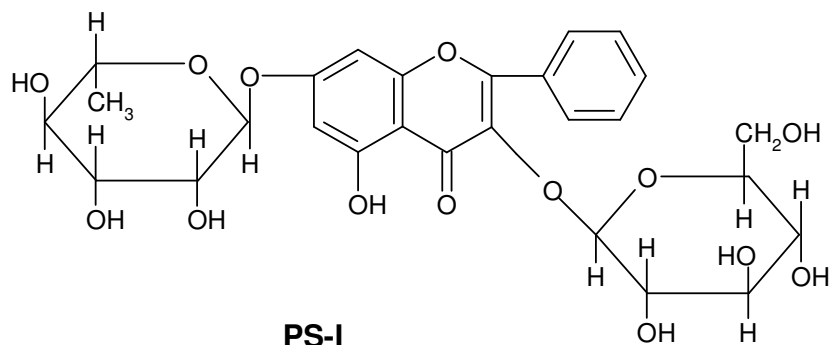
The air-dried and powdered roots of *Acorus calamus* Linn.were extracted with 95% ethanol. The ethanolic extract was concentrated under reduced pressure. The concentrated ethanolic extract was fractionated with various solvents as petroleum ether, benzene, chloroform, acetone, ethyl acetate and methanol. The chloroform soluble fraction on chromatographic resolution gave compound PS-I whereas ethyl acetate soluble fraction gave compound PS-II.

Air-dried, powdered roots and stem of *Euphorbia hirta* Linn.and were extracted with 95% ethanol separately. The ethanolic extract was concentrated under reduced pressure with various solvents according to polarity. The methanolic soluble fraction of concentrated ethanolic extract of the concentrated ethanolic extract of the roots afforded compound PS-III whereas the ethyl acetate soluble fraction of the concentrated ethanolic extract of the stem yielded compound PS-IV.

The brief accounts of the isolated compounds are tabulated in table– 5.5.

ANTI-ASTHMATIC SCREENING OF ISOLATED COMPOUNDS

Anti-asthmatic screening of the isolated compounds were carried out on histamine induced bronchostricted guinea pigs⁴⁷⁻⁵³. A detail method is elaborated below.



Test Animals:

Guinea pigs of either sex, weighing 250-500 gm of Pir Bright White strain were used for the biological study. The animals were housed under standard conditions of temperature and humidity for 24 hrs and fasted with fresh leafy vegetables, carrots and with water *ad libitum*.

Chemicals:

Histamine aerosol (histamine diphosphate (0.5%) in saline)(Sigma chemicals Co.), Salbutamol sulphate (Cipla Ltd., Mumbai), All the chemicals used was of analytical grade.

Equipments:

- 1) Histamine chamber
- 2) Glass sprayer.

Induction of Histamine⁵⁴⁻⁵⁷:

Animals were divided into four groups of three each i.e. A, B, C, and D. The guinea pigs of either sex were exposed to histamine aerosol (0.5% histamine diphosphate in saline) in a histamine chamber by using a glass sprayer, to induce the bronchospasm.

The guinea pigs after exposure to the histamine aerosol indicated the sign of progressive immobilization and bouts of coughing leading to convulsions when fell on its back.

The time taken by the guinea pigs to fall on its back after exposure to histamine aerosol was called as exposition time, which was noted for each animal. Exposition time also known as PCD (pre-convulsive dyspnoea).

After recording the exposition time, the animals were immediately removed from the chamber and placed in a fresh air, so that the animals came back to its normal state.

Drug Treatment Schedule⁵⁴⁻⁵⁷:

When the histamine induced the spasm, later after 1 hour, the first three groups of animals A, B and C were administered separately with each isolated compound, and the fourth groups animals (D) was given salbutamol (0.65 mg/kg) by the intraperitoneal route. After 1 hour of compounds (drugs) administration, the animals were again exposed to histamine aerosol and exposition times were noted. The fourth groups of animals were treated as a (control) standard group of animals.

The animals in a first three groups were treated with isolated compounds separately. The animals of first group (A) was administered intraperitoneally 50 mg/kg of PS-I, second group (B) was treated with 100 mg/kg of PS-I, and the third group (C) was administered with 200 mg/kg of PS-I. After 1 hour each of the animals of administration, each of the animal was exposed to histamine aerosol and their exposition time was recorded (Table 5.6).

After a washout period of seven days, the animals were used for the determination of anti-histaminic activity. Compound II was given in increasing doses of 50, 100 and 200 mg/kg. The exposition time for each animal was noted in table 5.7.

Similarly the other compounds PS-III and PS-IV were tested for the anti-asthmatic activity after the washout period of one week and their exposition time were noted in table 5.8 and 5.9.

The difference in the exposition time before and after extract administration was taken as a measure of the protective effect of the compound (drug).

The percentage protection offered by the isolated compounds was calculated by the formula given below.

$$\text{Percentage protection} = \frac{\text{Eta} - \text{Etb}}{\text{Etb}} \times 100$$

(% Protection offered by the extract)

Where:

1. Eta is the mean exposition time after administration of the compound.
2. Etb is the mean exposition time before administration of the compound.

The results are expressed as mean $\pm$ SD. The significance between post-treatment and pre-treatment exposition times was analysed by student test. The values are considered to be significantly different when the p value was less than 0.05.

RESULTS

The ethanolic extracts of the plants produced a dose dependent increase in respiratory capacity in the histamine-induced bronchospasm in guinea pigs. The results of the present investigation are summarized in table 5.6-5.9.

Table – 5.6

Effect of Compound PS-I on Histamine Aerosol induced Bronchospasm in Guinea Pig

Treatment	Dose mg/kg i.p.	Pre-treatment exposition time in seconds $\pm$ S.E	Post-treatment exposition time in seconds $\pm$ S.E	% Protection
PS-I	50	199.56 $\pm$ 3.55	126.35 $\pm$ 15.02	5.38% ^{NS}
PS-I	100	229.42 $\pm$ 10.15	299.38 $\pm$ 13.02	23.37%
PS-I	200	256.52 $\pm$ 8.62	370.20 $\pm$ 30.71	30.71 %
Salbutamol*	0.65	145.42 $\pm$ 5.10	385.45 $\pm$ 14.29	62.24 %

Values are $\pm$ SD, where n=3 in each case.

NS: Non-significance. $p < 0.05$ * Control Drug

Table – 5.7

Effect of PS-II Compound on Histamine Aerosol induced Bronchospasm in Guinea Pig

Treatment	Dose mg/kg i.p.	Pre-treatment exposition time in seconds $\pm$ S.E	Post-treatment exposition time in seconds $\pm$ S.E	% Protection
PS-II	50	117.4 $\pm$ 20.02	128.2 $\pm$ 24.15	8.42% NS
PS-II	100	118.2 $\pm$ 23.15	125.2 $\pm$ 22.03	5.59% NS
PS-II	200	192.75 $\pm$ 15.31	230.55 $\pm$ 30.11	16.30 %

Values are $\pm$ SD, where n=3 in each case.

NS: Non-significance.p< 0.05

Table – 5.8

Effect of Compound PS-III on Histamine Aerosol induced Bronchospasm in Guinea Pig

Treatment	Dose mg/kg i.p.	Pre-treatment exposition time in seconds $\pm$ S.E	Post-treatment exposition time in seconds $\pm$ S.E	% Protection
PS-III	50	115.26 $\pm$ 10.06	130.21 $\pm$ 10.95	11.48% NS
PS-III	100	116.23 $\pm$ 25.12	145.65 $\pm$ 12.57	20.86%
PS-III	200	122.23 $\pm$ 10.99	230.35 $\pm$ 46.93	46.93 %

Values are $\pm$ SD, where n=3 in each case.

NS: Non-significance.p < 0.05

Table – 5.9

Effect of Compounds PS-IV on Histamine Aerosol induced Bronchospasm in guinea Pig

Treatment	Dose mg/kg i.p.	Pre-treatment exposition time in seconds $\pm$ S.E	Post-treatment exposition time in seconds $\pm$ S.E	% Protection
PS-IV	50	117.56 $\pm$ 6.73	122.35 $\pm$ 12.52	4.08% ^{NS}
PS-IV	100	190.23 $\pm$ 9.86	230.32 $\pm$ 13.02	17.40%
PS-IV	200	256.65 $\pm$ 13.27	352.99 $\pm$ 13.04	27.33 %

Values are $\pm$ SD, where n=3 in each case.

NS: Non-significance. $p < 0.05$

DISCUSSION

The results obtained in the in vivo experiment showed existence of bronchodilator effects in guinea pigs. The results showed that the compounds PS-I, PS-II, PS-III and PS-IV did not significantly increases the post-treatment time. PS-I offered the dose-dependent protection to 23.37 % and 30.71 % at doses 100 and 200 mg/kg and PS-III offered the dose- dependent protection to 20.86 % and 46.93 % at doses 100 and 200 mg/kg, respectively.

The PS-II offered the significant protection at 16.39 % at dose 200 mg/kg. PS-IV did not show the significant increase in the post-treatment exposition time. It showed the percentage protection at 27.33% at 200mg/kg. Therefore PS-IV was found to be least potent.

Inhalation of histamine shown to induce bronchoconstriction by direct activation of H₁ receptor, this in turn activates the action potentials in intrapulmonary vagal afferents.⁵⁸⁻⁶⁰

Thus it can conclude that the plants drug PS-I isolated from the roots of *Acorus calamus* Linn and PS-III isolated from the roots of *Euphorbia hirta* (Linn.) showed a significant protection against histamine challenge. Thus they can have a very potent anti-asthmatic effect. They produce bronchoprotective effect due to its H₁-blocking effect.

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Research Papers

A specialist is one who knows everything about something and nothing about anything else.

• Ambrose Bierce

(II) LIST OF PAPERS PUBLISHED/ COMMUNICATED/ PRESENTED

- 1) **Prachi Saxena*** , **V K saxena** A Novel Steroidal Saponin 24, 25-dihydro-euphol-3-O- β -D-arabinopyranoside from the roots of the *Euphorbia hirta* (Linn.) **International Journal of Natural Product Science (IJNPS) Online** ,
ISSN 2249-6335 (Journal Impact Factor 1.29)
- 2) V.K. Saxena and **Prachi Saxena***, A Luteolin-8-C- β -D-glucopyranoside from the roots of the *Acorus calamus* (Linn.) **J. Inst. Chemists (India)**, Vol 81, 39-43, part-2, 2009 (**ISSN 0020-3254**)
- 3) V.K. Saxena and **Prachi Saxena***, Galangin-3-O- β –D-Glucopyranosyl-7-O- α –L-Rhamnopyranoside from the roots of *Acorus calamus* (linn) **J. Inst. Chemists (India)**, Vol77, 40-43 part-2, 2005 (**ISSN 0020-3254**)
- 4) V.K. Saxena and **Prachi Saxena***, India Medicinal: Storage of Potential Anti – Asthmatic –Agent, **Madhya Bharti, J. Physical. And Natural Sciences** , Vol. 50, 10-17, 2006 (**ISSN 0972-7434**)
- 5) V.K. Saxena and **Prachi Saxena***, Amino Acid Contents of Some Reputed Indigenous Anti –Asthmatic Plants (Linn.), **Madhya Bharti, J. Physical and Natural Sciences** , Vol.49, 72-74, 2005. (**ISSN 0972-7434**)

- 6) V.K. Saxena and **Prachi Saxena***Carbohydrate Contents of Some Well Known Indigenous Anti – Asthmatic Plants” **Madhya Bharti J.physical. and natural sciences** ,vol48,51-53,2004
(ISSN 0972-7434)

(II) PAPERS PRESENTED IN SEMINAR/CONFERENCES/WORKSHOP ATTENDED

- 1) Dr. Prachi Saxena, Participated in conference Organised by **Semi Solar con India 2013 held in Bangalore** 1 to 3 August 2013 in association with the **IEEE** Standard Association and the International Electrotechnical Commission (**IEC**) **and attended one day workshop.**
- 2) Contribution as Guide Teacher in **Children Science Congress** 2010 at District Level conducted by Rashtriya Vigyan Evam Prodyogiki Sanchar parishad(**DST**) Govt of India , New Delhi Held in Bhopal Madhya Pradesh
- 3) V.K. Saxena and Prachi Saxena, Indian Institute of Science of Bangalore, :90th Session of **Indian science congress**, 3-7 Jan 2003
- 4) V.K. Saxena and Prachi Saxena Punjab University and Institute of Microbiology Technology Chandigarh, Proc.91st**Indian science congress**partIII (Advance Abstract), 3-7 Jan 2004, 73-74 presented a

poster entitled “ Drugs Possessing Anti-Asthmatic Properties Isolated From Indian Medicinal Plant “

- 5) V.K. Saxena and Prachi Saxena **National Science Day** National Conference New Frontier of Science, Dr. H.S. Gour University Sagar (M.P.), Feb 28, 2003, 39. Presented a paper Indian Medicinal Plants: A Reservoir of Potential Antiasthmatic Agents

ABOUT AUTHOR

Prachi Saxena accomplishes her Ph.D. in Natural Product Chemistry and Organic Chemistry from *Dr. Harisingh Gour Vishwavidyalaya*, formerly University of Saugor (Madhya Pradesh) on “Phytochemical Investigation on Some Indigenous Anti-Asthmatic plants”. In her research role, she has elaborated the investigation of the plant’s extract in order to interpret their antiasthmatic properties. Her work involves a very defined and systematic approach, utilizing the scientific methodology and tools like extraction, chromatographic analysis, spectroscopic analysis (UV, FTIR, HNMR, ¹³ CNMR, FABMS), structural elucidation. In her stride of tasks, the antiasthmatic screening of compound has unveiled many complex chemical structures in these Natural resources.



Prachi has master’s degree in Organic Chemistry and Polymer chemistry from *Dr. Harisingh Gour Vishwavidyalaya Saugor Madhya Pradesh*. She joined the University as a researcher and teaching assistant where she gained a stimulating experience of developing the methodology for synthesis of polymer. She extended her capability in the field of Organic Chemistry, Chemistry of Natural Products, Isolation and Identification of Natural Products and Polymer Chemistry. She has assisted the seniors and attained the understanding in the research field of organic chemistry in collaboration of polymer chemistry by synthesizing a nano hydrogel polymer and learning its P^H and temperature behavior, highlighting various applications in the field of medical and food technology.

She has been involved in various activities conducted by university and has played a vital role in the National and International conferences, seminars, workshops held at university level as team leader.

Very recent, she represented in Solarcon International Conference organized by Semiconductor Equipment and Materials International (SEMI) at Bangalore ITPL. She has successfully accomplished the workshop on PV Standard-from Materials and Manufacturing to Systems conducted by SEMI, India in association with IEEE-SA (the Institute of Electrical and Electronics Engineers standards Association) and IEC (International Electrotechnical Commission) standards.

Prachi has publications in various international and national conferences. She has significantly contributed to the scientific community by publishing her work in the reputed journals such as Indian Science Congress, Advance Polymer Association, National Science Day, M. P. Council of Science and Technology, Bhopal (Madhya Pradesh). Her contribution as Peer review in International Journal of Chemical Research is highly valued. Her contribution to Children Science Congress satisfies her most as she values her time and efforts in igniting the young brains.

Prachi has joined as Lecturer in the Govt. H.M. Home Science & Science Women's College, Jabalpur, Madhya Pradesh and expanded her scope in the areas of Polymer Science, Spectroscopy, Organic Chemistry and Pharmacognosy to the Postgraduate scholars as well as for Undergraduate scholars. She has led the scholars in conducting Laboratory-scale practical.

She is also an active member of various professional bodies like Advance Polymer Association (Life member), Department of Science and Technology (-Annual Member), International Journal of Chemical Research (-Reviewer), Asia-Pacific Chemical, Biological & Environmental Engineering Society (APCBEES) (-Associate Editor) and recently she has been awarded with *Fellow membership of International Science Congress Association (ISCA)*.

Over all, Prachi has 10 years of experience in teaching whereby she found herself intrigued by the research activities.

Till date she has supported various programs in Polymer technologies and Natural Product Chemistry Program and has been invited for delivering lectures in many programs.

Out of Work, she enjoys kathak dancing, music and involved in various voluntary activities.