STUDIES ON SOME ORGANIC COMPOUNDS ISOLATED FROM LAURACEAE, SOLANACEAE & EUPHORBIACEAE FAMILIES

Thesis submitted for the Award of the
Degree of Doctor of Philosophy
in Chemistry

By

SANTOSH KUMAR GARG

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ROORKEE
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CERTIFICATE

Certified that the thesis entitled "Studies on some organic compounds isolated from Lauraceae, Solanaceae and Euphorbiaceae families" which is being submitted by Shri Santosh Kumar Garg for the award of the Degree of Doctor of Philosophy in Chemistry of the University of Roorkee, is a record of student's own work carried out by him under my supervision and guidance. The matter embodied in this thesis has not been submitted for the award of any other Degree.

This is further to certify that he has worked for a period of three years for the Ph.D. Degree at this University including one year at D. S. B. Govt. College, Naini Tal

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D. R. Gupta

(D.R. Gupta)
Reader in Chemistry
University of Roorkee
Roorkee (India).

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(Santosh Kumar Garg)

C O N T E N T S

GENERAL INTRODUCTION

The Indian system of medicine is considered to be the oldest system which is being fruitfully practised even to this day. To alleviate human suffering from diseases, peoples of all ages and countries have been using various drugs obtained both from plants and animals. The origin of materia medica of India dates back to the period of Rigveda. In this book, there are several hymns in the praise of "Soma" which has now been characterized as Ephedra pachyelade.

Ayurveda, the materia medica of the Hindus, was greatly enriched during the first few centuries of the christian era (200 A.D. to 600 A.D.). A number of famous "Acharyas" such as Charak, Sussuta, Narhari, Madanpal, Bagbhatta and Bhav Misra devoted most of their valuable time in evolving a system of curative medicines obtained from Indian medicinal plants. It was during this period that a number of standard books on medicine such as Nighantu and Bhav Prakash etc. (based on careful observations) were written. A substance known as "Sammohini" is also said to have been used as a general anaesthetic in the time of Budha, and a mention of some kind of surgical instruments had also been made.

Before the British rule, Ayurvedic system, supplemented to some extent by Unani system was the only system of medicine practised in India. The Britishers

brought with them a relatively more scientific system based on empirical knowledge of chemistry, known as allopathy. The indigenous system, although denounced as unscientific had its own metaphysical basis of diagnosis e.g. the acceptance of three vikras, Vata, Pitta and Kapha in diagnosing various diseases. However, in absence of much patronage, the indigenous system survived and even today it caters to the need of majority of the Indian population, particularly in rural areas through Ayurvedic dispensaries run by District Boards. Ayurvedic medicines have been found to be very specific for certain diseases such as Holorrhena antidysentrica for dysentery, Rauwolfia serpentina for insanity, Adhatoda vessica for respiratory diseases, Boerhavea diffusa for dropsy and Cassia alata for ringworm etc. Such information has undoubtedly been extremely useful in guiding plant chemists to examine different plants of proved medicinal value. In most of the cases, it has been found that the allopathic system uses only the active principle, while the Ayurvedic system uses the whole plant. It will not be out of place to mention that in some cases the whole plant is found to be more effective than its active principle. As such, there is no question of any sort of rivalry between the two systems. Rather, it is worthwhile to examine the vast available literature

on Ayurveda compiled by Votaries of the system and see if it can be of some service to the people.

In fact, the potentialities and possibilities of medicinal plants and vegetable drugs have not yet been properly and fully developed, but due to awakened interest in plants, some work has, however, been carried out in India and abroad. Americans are developing herbotherapy at a rapid pace. As a result of this, a large amount of knowledge has accumulated for the benefit and guidance of the indigenous practitioner. However, due to lack of coordination between the chemist, physiologist and practitioner, the investigations of the chemists do not always serve a useful purpose. The establishment of a number of drug research institutes and regional research laboratories (mainly C.S.I.R. sponsored) in the country augurs well for the future, and it is hoped that useful investigations carried out at these institutes shall go a long way in making the lives of suffering millions worthliving. This will be possible only when plants of proved efficacy are grown in gardens maintained by Governments to avoid adulteration. A number of emporia may also be opened at suitable places so that drugs and plants may be collected at a proper season and distributed to various research institutes for systematic investigations.

Historical development of plant chemistry:

The plant chemistry dates back to the middle of the seventeenth century A.D. If plant chemistry is regarded as the art of resolving plants into their chemical constituents, or at least as that of isolating the most important components in the form of pure chemical compounds, then C.W. Scheele may be regarded as the founder of the modern plant chemistry. The number of plant products known before his time was very small and perhaps not a single compound had been prepared in a perfectly chemically pure state. The two processes of isolation viz. dry distillation and steam distillation of plant products were frequently employed in his time. Extraction with solvents was not at all popular at that time. It was through dry distillation that George Agricola (1546) and Alexander Pedemontanus had obtained succinic and benzoic acids from amber and benzoin respectively. Essential oils were obtained by steam distillation of Valerius cordus and gave interesting results. In the seventeenth century, the solvent extraction method was also successfully employed by Angelus Sala who obtained sugar and salt of sorrel from plant extracts. The idea of acquiring the fundamental knowledge of plants by using force and decomposing them completely by fire, advanced by theoritical chemists, Duclos and Dudart of Academiedes

sciences (1666) proved useless after some time. Some fourteen hundered plants were dry distilled at this institute with the result that all the products obtained were more or less similar. In the eighteenth century, the extraction methods again came to the fore front when Boulduc, Boerhaave and Newmann employed them though without any noteworthy results. The best contribution during this period was that of Marggraf who demonstrated the preparation of cane sugar from indigenous plants in 1747.

It was Scheele who first raised phyto-chemistry out of this stagnation. He obtained tartaric, citric, malic, oxalic and benzoic acids in the pure form as a result of well planned and carefully conducted experimental investigations. In the last years of his life (1786), he discovered a new vegetable acid, gallic acid which was obtained by the spontaneous decomposition of gall nuts. These investigations of Scheele though not very significant greatly exceeded in value all the work carried out before his time in the field of plant chemistry.

The early years of nineteenth century are very important so far as the discovery of a number of important alkaloids is concerned. In 1804, Derosne and in 1806, Serturner discovered morphine though narcotine had been obtained by the former a year ago.

Many alkaloids were discovered during 1803-1837.

The enormous progress of phyto-chemistry which resulted from the discovery of Serturner, led also to the discovery of many non-alkaloidal vegetable principles during this period. The important investigations of Leibig and Wohler published in 1837 became the starting point for the discovery of a group of glucosides. They had established the fact that hydrolysis of glucosides gave glucose as one of the products. This work is also significant in the history of the vegetable enzymes.

The scent and essential oil industry of India dates back to the reign of Jehangir and his queen, Noor-i-Jehan who discovered the scent of rose. But the systematic investigations on essential oils and the separation of their individual constituents in a chemically pure state began first in the nineteenth century. This was made possible by the preparation and separation of derivatives of compounds by reacting them with different reagents. The first pure compounds prepared from this group were menthol, thymol and eugenol.

The occurrence of many active principles in minute quantities in plants posed a problem for the plant chemists. But now micro-analytical methods, tracer techniques, chromatography and a number of other methods

employed in the separation, purification and identification, have solved the problem to a great extent. A number of physical methods such as absorption spectra, X-Ray crystallography, infra red, ultra violet, mass spectrography and N.M.R. have proved of immense help in elucidating the basic structures of complex organic molecules. Thus the hithertotedious investigations are becoming relatively simpler and phyto-chemical investigations are receiving a fresh impetus.

Development of plant chemistry in India:

In the foregoing pages, it was mentioned that much useful information was collected by ancient Hindus about plants and drugs. There had been further useful additions to this branch upto the end of the Gupta dynasty. But for about a thousand years, before the establishment of British supremacy in India, no significant work had been carried out on plant chemistry. The Britishers brought with them the useful knowledge of modern Chemistry and Botany. The abundance of medicinal plants in the country awakened their interest in Indian indigenous drugs. Many informative books were written on these plants with their botanical names so that these plants could be properly classified according to their genera and families. Some of them also carried out preliminary investigations on the plants they came across. Ainslie was the first man who published his

book " materia medica of Hindustan" in 1828; Shangnessy produced " The Bengal Pharmacopia" in 1844 and Waring wrote " Indian Pharmacopia" in 1868. Besides these, William Jones (His botanical observations on select Indian plants), Roxburg (Flora indica), Wallach, Buchanan, Hamilton, Griffths Graham, John Fleming (catalogues of medicinal plants), Royle and Wright are some of the outstanding early personalities in the field of plant chemistry. F.J. Monat and F.N. Machamaria and other botanists did very useful work in classifying a large number of Indian medicinal plants. Besides these foreign workers, Indian scientists also carried out some good work. K.L. Dey wrote " The indigenous drugs of India" and while U.C. Dutta wrote the "materia medica of Hindus" in 1877. A gradual progress in the field was noticed with the development of commerce, medicine and science. Dymock's first book "materia medica of western India" in 1883 was a valuable contribution. The publication of "Pharmacographica India", embodying important researches of Dymock, Warden and Hooper, was a great event in the growth of plant chemistry in India, and the fact that it remains an indispensable book of reference even today adds to the credit of its authors.

For a long time Dymock's "Pharmacographica India" remained the main book of reference till 1924, the subject

received the attention of Col. R.N. Chopra who emphasised the importance of assessment of all the indigenous drugs from modern chemical and pharmacological stand point and a definite scheme of work was chalked out at the Indian School of Tropical Medicine, Calcutta. Since then, a lot of work in this direction has been done at various institutes in India, chiefly at Delhi, Calcutta, Allahabad, Agra and Andhra Universities; Bose institute, Calcutta; Unani and Tibbiya College, Delhi; Indian Institute of Science, Bangalore; Forest Research Institute, Dehra Dun, Central Drug Research Institute, Lucknow; National Chemical Laboratory, Poona; Regional Research Laboratory, Jammu; Presidency College, Madras and a number of other places. The opening of a separate centre for advanced studies in plant chemistry at the University of Delhi, Delhi under the able guidance of Prof. T.R. Seshadri, augurs well for the future of plant chemistry in India.

The natural orders of plants in which active principles have been found to occur are comparatively few in number. Out of more than two hundered families, only the following are quite important from medicinal point of view: Acanthaceae, Apocyanaceae, Anacardiaceae, Compositeae, Cucurbitaceae, Euphorbiaceae, Labiatae, Lauraceae, Leguminaceae, Oleaceae, Papaveraceae, Ranunculaceae, Solanaceae, Umbliferae, Convulvolaceae and Rubiaceae.

Phyto-chemical investigations carried out by the author:

The author has investigated four new Indian medicinal plants, viz., Litsea consimilis (Family: Lauraceae), Solanum ferox Linn. (Family: Solanaceae), Euphorbia hirta Linn. and Euphorbia thymifolia Linn. (Family: Euphorbiaceae), which have not been worked up so far for their active principles.

A number of lauraceae plants specially belonging to Litsea species, are a source of fatty oils, alkaloids, essential oils and triterpenoids. The barks, leaves and seeds of some Litsea species have found wide applications in medicine (1). The bark of Litsea chinensis which is demulcent, astringent and aphrodisiac, is used in diarrohea, dysentery and also as an antidote to bites of venomous animals. The seeds are aphrodisiac and the oil from its berries is used in rheumatism. The bark of Litsea polyantha is astringent and used in diarrohea, dysentery and as a stomach stimulant. The leaves of Litsea stocksii have been used for the irritation of bladder and urethra. The seed oil is used in the treatment of sprains, bruises and itch. The seed fats of Litsea plants, besides being medicinally important are of commercial interest as a source of excellent detergent, satisfactory lubricating agent for watches and other delicate machines and a wetting agent for insecticidal preparations (2-3).

Litsea consimilis, grows plentifully on the hilly tracks of Naini Tal. The oil from its berries is used by the local people in the treatment of rheumatism, various skin diseases and for healing wounds. This plant has not been investigated so far. The seeds, fruit coat and bark of this plant have been chemically analysed.

Narang and Puntambekar; Miyamictu and Nomura; Child and Nathanael; and Hata have chemically examined the seed fat of a number of Litsea species, i.e. Litsea chinensis, Litsea citrata, Litsea lanuginosa, Litsea zeylanica, Litsea japonica, Litsea longifolia and Litsea cubeba (4-7). The seed fat of Litsea consimilis was chemically examined by the author and was found to be slightly different from those investigated by earlier workers. The essential oil constituents of the seeds were found to be different from those of the essential oils of the seeds of other Litsea species (7). It was, however, interesting to observe that some of the essential oil constituents of the seeds of Litsea consimilis were identical to those found in the leaves of Litsea zeylanica and Litsea cubeba (8-10).

Fruit coat and bark fats of Litsea consimilis were also analysed. The major saturated acid component in the fruit coat fat was found to be lauric acid. Moreover, the glyceride of capric acid, which does not occur in

any fruit coat fat of lauraceae plants so far investigated (11-13), was found to be present in the fruit coat fat of this plant. The amino acid make-up of the fruit coat was determined and found to be similar to that of the seeds (14) except that the fruit coat did not indicate the presence of proline and cystine. The bark oils generally consist of oleic and palmitic acids as the major components along with minor amounts of stearic acid (15). The results of the author, show that the bark oil of Litsea consimilis contains lauric acid as the major component with a little capric acid instead of stearic acid. β - amyrin was obtained from the petroleum ether extract of the bark on chromatographing it over Brockmann alumina. The alkaloids of the bark and seeds were isolated and examined. The alkaloid isolated from the seeds appears to be quite different from alkaloids already reported in other Litsea species (16-20).

The seed fat of Litsea consimilis has also been examined for its possible use as a lubricant and in the preparation of alkyd resins and varnishes. The results were found to be quite encouraging.

A large number of plants belonging to solanaceae family have been used medicinally in various diseases (21) i.e., cough, asthma, diarrohea, dysentery, dropsy, chest pains, rheumatism, ulcers, fevers etc. Fats,

alkaloids and saponins have been isolated from a number of Solanum species and examined by earlier workers (22-46).

Solanum ferox Linn., grows wildly in Garhwal (U.P.), Madras, Assam, Bihar, Orissa, Ceylon and China. Various parts of this plant i.e. roots, leaves, stems, flowers, seeds and fruits have been used medicinally in sore throat, cough, asthma, chest pains, dropsy and rheumatism. This plant has not been worked up so far for its active principles. The seed fat and the alkaloidal content of the fruits of Solanum ferox Linn. have been examined.

Some plants belonging to Euphorbiaceae family grow wildly throughout India and have found wide applications in medicine (47). Different Euphorbia species have been found to contain terpenoids, steroids, higher hydrocarbons, higher aliphatic alcohols, lactones and alkaloids which have been examined by various workers (48-78).

Euphorbia hirta Linn. and Euphorbia thymifolia Linn., grow wildly throughout India in plains and lower hills. These plants have been medicinally used in various diseases i.e. bowel complaints, cough, dysentery, colic pains, bronchial affections, asthma, ringworm, snakebite and skin diseases. Although some varieties of Euphorbia hirta viz. procumbens, pedilantus calcaratus and pedilantus tehuacanus have been chemically examined

by earlier workers (79), no work seems to have been done on the active principles of Euphorbia hirta Linn. The petroleum ether and ethancl extracts of the stems and the ethanol extract of the flowers of this plant were chemically examined. It is interesting to observe that Euphorbia hirta Linn. contains myricyl alcohol, taraxerol and ellagic acid, besides the presence of friedelin, β- amyrin, hentriacontane and β-sitosterol already reported (79) in some varieties of Euphorbia hirta. A very little work seems to have been done on Euphorbia thymifolia Linn. The isolation of 5, 7-4-trihydroxy flavone-7-glucoside from the stems and leaves and the chemical examination of the essential oil constituents have been reported (80-81). On examining the petroleum ether and ethanol extracts of the whole plant, myricyl alcohol, taraxerol, tirucallol and hentriacontane were isolated and characterized. The free organic acid pattern of the stems and the leaves of both these plants was also determined.

The investigations carried out on the above four Indian medicinal plants form the subject matter of the subsequent chapters.

CHAPTER I

CHEMICAL EXAMINATION OF THE SEEDS OF LITSEA CONSIMILIS

The seeds of a number of tropical plants belonging to lauraceae family are reputed to contain large quantities of fatty oils (1) some of which, besides being medicinally important (2), are of commercial interest as a source of excellent detergent, satisfactory lubricating agent for watches and other delicate machines and a wetting agent for insecticidal preparations (3). The bark of Litsea chinensis which is demulcent, astringent and aphrodisiac, is used in diarrohea, dysentery and also as an antidote to bites of venomous animals. The seeds are aphrodisiac and the oil from its berries is used in rheumatism. The bark of Litsea polyantha is astringent and used in diarrohea, dysentery and as a stomach stimulant. The leaves of Litsea stocksii have been used against irritation of bladder and urethra. The oil from the seeds is used in the treatment of sprains, bruises and itch.

Litsea consimilis, grows plentifully on the hilly tracks of Naini Tal. The oil from it berries is used by the local people in the treatment of rheumatism, various skin diseases and for healing wounds. This plant has not been investigated so far. The seeds, fruit coat and bark of this plant have been chemically analysed and described in the foregoing chapters.

The botanical description of the plant is as follows (4):

Glaborous except the leaf buds and flowers. Leaves alternate, lower surface covered with white blooms, lanceolate 3-6 x 1-2" long pointed nerves lateral. Leaf buds softly pubescent 1/3" long. Flowers pale yellow or white, brackets 4 soon falling off. Umbles forming lateral clusters 1/3" diameter. Perianth four, parted; segments fringed, pubescent outside, stamens 6, much longer than the perianth, two inner ones each with a pair of sessile, kidney shaped glands near it base. Drupe globose 1/3" diameter. Seeds 0.8-1.2 cm. diameter, soft brownish black odorescent pericarp with two brown coloured cotyledons inside.

PHOTOGRAPH OF LITSEA CONSIMILIS (FLOWERS, SEEDS AND LEAVES ETC.).

CHEMICAL EXAMINATION OF THE SEED FAT

From the kernels of the seeds of Litsea consimilis a yellowish-brown oil is obtained in 50.6% yield, which consists of the glycerides of capric acid (1.7%), lauric acid (59.1%), myristic acid (11.5%) and oleic acid (27.7%).

EXPERIMENTAL

The oil from the seeds was extracted with petroleum ether (b.p. 60-80°C) which gave the maximum yield of the oil (50.6%).

The seeds were air-dried, powdered and extracted with petroleum ether (b.p. 60-80°C) in a soxhlet apparatus.

The oil after removal of the solvent was purified by treating it with Fuller's earth and activated charcoal. It was then freed from volatile essential oils as far as possible by heating in Vacuo. The pure oil thus obtained was of yellowish brown colour. It possessed the following characteristics: Yeild, 50.6% ; specific gravity at 35°C, 0.8946 ; refractive index at 40°C, 1.4509 ; acid value, 4.257 ; saponification value, 253.4 ; saponification equivalent, 221.39 ; ester value, 249.143 ; iodine value (Hanus), 28.62 ; acetyl value, 22.71 ; Hehner value, 92.8% ; Reichert-Meissl value, 1.43 ; Polenske value, 12.9 ; unsaponifiable matter, 2.1%.

(a) Determination of the percentage yield:

Weight of the seeds extracted = 100 gms.

Weight of the conical flask = 60.4216 gms.

Weight of the conical flask + Oil =111.0216 gms.

∴ Weight of the oil = 50.6 gms.

∴ % yield = $\frac{50.6 \times 100}{100}$

= 50.6%

(b) Specific gravity:

The specific gravity of the oil was determined at 35°C by means of a specific gravity bottle.

Weight of the specific gravity bottle = 6.8548 gms.

Weight of the specific gravity bottle + water =17.5769 gms.

Weight of the specific gravity bottle + oil =16.4468 gms.

∴ Weight of the water taken =10.7221 gms.

Weight of the oil taken = 9.5920 gms.

∴ Specific gravity of the oil = $\frac{\text{Weight of the oil taken}}{\text{Weight of the water taken}}$

= $\frac{9.5920}{10.7221}$

= 0.8946

(c) Refractive index:

The Abbe's refractometer was used for the determination of the refractive index. The reading was taken in ordinary day light. The instrument gave a direct reading.

Refractive index at 40°C = 1.4509

(d) Determination of the acid value:

The acid value may be defined as the number of

milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of the oil or fat. Though it is determined by titrating it against a standard solution of sodium hydroxide, calculations are made on the basis of potassium hydroxide.

2.6354 gms. of the oil were taken in a conical flask and were dissolved in methyl alcohol (50 ml). The solution was titrated against N/10 sodium hydroxide solution.

Weight of the oil taken = 2.6354 gms.

Strength of NaOH solution = N/10

Volume of NaOH solution used = 2.0 ml

$$\therefore \text{ Acid value} = \frac{2 \times 56.1}{10 \times 2.6354}$$

$$= 4.257$$

(e) Determination of the saponification value:

Saponification value is defined as the number of milligrams of potassium hydroxide required to saponify one gram of the fat or oil.

Procedure:

2.1906 gms. of the oil were taken in a conical flask 'A' 25 ml of approximately N/2 ethanolic potassium hydroxide were added to it. In another conical flask 'B' only 25 ml of ethanolic potassium hydroxide were taken. The contents of the flask were refluxed over water bath for 45 minutes. On cooling, 25 ml of water were added to each of the flasks. The solution of the flasks 'A' and 'B' were titrated separately

against N/1.935 hydrochloric acid.

Weight of the oil taken in conical flask 'A' = 2.1906 gms.

Volume of approximately N/2 ethanolic potassium hydroxide solution taken in each flask = 25 ml

Volume of N/1.935 hydrochloric acid used:

(i) for blank 'B' = 21.65 ml

(ii) for oil 'A' = 2.60 ml

∴ Volume of N/1.935 hydrochloric acid consumed = 21.65-2.60 ml

= 19.05 ml

∴ Saponification value = $\frac{19.05 \times 56.1 \times 1000}{1.935 \times 1000 \times 2.1906}$

= 253.4

(f) Determination of the saponification equivalent:

The saponification equivalent gives an idea about the approximate molecular weight of the material under examination. The material should be either fatty acids or esters of monohydric alcohols. In case of triglycerides, the saponification equivalent is multipled by 3 to give the approximate molecular weight. It is calculated from the saponification value as given below:

Saponification equivalent = $\frac{56100}{\text{Saponification value}}$

= $\frac{56100}{253.4}$

= 221.39

(g) Determination of the ester value:

The ester value corresponds to the number of milligrams of potassium hydroxide required to saponify the esters

present in one gram of the sample. It is computed from the saponification value and the acid value.

Ester value = Saponification value - Acid value

= 253.4 - 4.257

= 249.143

(h) Determination of the iodine value:

The oils and fats are the glycerides of saturated and unsaturated fatty acids. The degree of unsaturation of an oil or a fat can be determined by finding out the iodine value or iodine number of the oil or fat. The iodine value is expressed as the amount of iodine absorbed by 100 grams of the oil or fat.

A number of methods for the determination of the iodine value of fats and oils have been given by Hanus, Wij, Kaufmann, and Rosenmund and Kuhnhenns. The main reaction in all the methods is the addition of the halogen at the double bonds together with a small amount of substitution.

$$>C=C< + I_2 \longrightarrow >\underset{I}{C}-\underset{I}{C}<$$

Iodine values of oils and fats were, however, determined by employing Hanus method.

Preparation of Hanus solution:

13.615 gms. of iodine crystals were dissolved in 825 ml glacial acetic acid by warming and stirring. The solution was cooled. 25 ml of this solution were pipetted out and diluted with water to about 200 ml and titrated against 0.1 N sodium

thiosulphate solution.

3 ml of bromine solution were mixed well with 200 ml of glacial acetic acid. 5 ml of this solution were diluted with water to about 150 ml. 10 ml of 10% potassium iodide solution were added to above solution and then titrated against 0.1 N sodium thiosulphate solution. The bromine solution required to double the halogen content of the remaining 800 ml of iodine solution was calculated as follows:

$$A = \frac{B}{C}$$

where A, ml of bromine solution required; B, 800 x thiosulphate equivalent of 1 ml of iodine solution; and C, thiosulphate equivalent of 1 ml of bromine solution.

The required bromine solution was added to the remaining iodine solution. The solution was mixed well and diluted to one litre with glacial acetic acid and stored in an amber glass bottle.

Procedure:

25 ml of Hanus solution were added to 0.2040 gms. of the oil dissolved in 10 ml of chloroform in a stoppered iodine flask 'A'. In another iodine flask 'B' only 10 ml of chloroform and 25 ml of Hanus solution were taken. Both the flasks were shaken well, covered with a dark black paper and kept in a dark place for about one hour with occasional shaking. The flasks were taken out after one hour and 10 ml of a 10% aqueous solution of potassium iodide were added

and the excess of iodine unreacted was titrated against N/10 sodium thiosulphate solution.

Weight of the oil taken in iodine flask 'A' = 0.2040 gms.

Volume of the Hanus solution taken in each flask = 25 ml

Strength of sodium thiosulphate solution = N/10

Volume of sodium thiosulphate solution used:

(i) for blank 'B' = 53.7 ml

(ii) for oil 'A' = 49.1 ml

∴ Volume of thiosulphate used

(Volume of N/10 iodine reacted)

= 53.7 - 49.1

= 4.6 ml

∴ Iodine value = $\frac{127 \times 4.6 \times 100}{10 \times 1000 \times 0.2040}$

= 28.62

(i) Determination of the acetyl value:

Acetyl value is the number of milligrams of potassium hydroxide required for the neutralization of acetic acid obtained on saponifying one gram of an acetylated oil or fat. It is a meassure of the number of hydroxyl groups contained in the oil. The study is, therefore, based on the following reactions:

1. $2\ C_n H_{2n} (OH)\ COOH + \begin{matrix} CH_3CO \\ CH_3CO \end{matrix} > O \longrightarrow$

$2\ C_n H_{2n} (OCOCH_3)\ COOH$

2. $C_n H_{2n} (OCOCH_3)\ COOR \xrightarrow{KOH} C_n H_{2n} (OH)\ COOK + CH_3COOK + ROH$

3. $CH_3COOK \xrightarrow{H_2SO_4} CH_3COOH + K_2SO_4$

Procedure:

20 ml of freshly distilled acetic anhydride were refluxed with 20 gms. of the fat for two hours, the mixture was poured into a beaker containing 200 ml of water and boiled for 20 minutes. To prevent bumping, a slow current of carbon dioxide was passed into the solution. A clear layer of the acetylated fat separated, which was removed and dried. The saponification value of this acetylated oil was determined in the usual manner.

The acetyl value of the oil can be calculated from the following formula:

$$\text{Acetyl value} = \frac{\text{(Saponification value of the acetylated oil - saponification value of the oil)}}{1-(0.00075 \times \text{saponification value of the oil})}$$

$$= \frac{271.8-253.4}{1-(0.00075 \times 253.4)}$$

$$= 22.71$$

(j) Determination of Hehner value:

Hehner value is defined as the percentage of insoluble fatty acids present in the oil or fat.

Procedure:

5 gms. of the oil were saponified with ethanolic potassium hydroxide. Ethanol was evaporated off and 0.5 N hydrochloric acid was added equivalent to quantity of potassium hydroxide used for saponification of the oil and 1 ml more (quantity of 0.5N hydrochloric acid to be added = titration for the blank-titration for the oil+1ml).

The solution was heated over water bath until the fatty acids formed a clear layer on the upper surface of the liquid. The flask was filled to neck with hot water and the contents were cooled in ice water until the cake of fatty acids became solid. Water was now decanted through a filter paper into a large conical flask and the process was repeated.

The insoluble fatty acids were allowed to dry for 12 hours and then they were transferred to a weighed conical flask. The solid acids remaining on the filter paper were also taken in the same conical flask by thorough washing of the filter paper with hot absolute ethanol. The ethanol was now evaporated, and the acids were dried for two hours at 100°C, cooled in a desiccator and finally weighed.

Weight of the oil taken for saponification = 5 gms.

Weight of the insoluble acids obtained = 4.64 gms.

$$\text{Hehner value} = \frac{\text{Weight of the insoluble acids} \times 100}{\text{Weight of the oil taken for saponification}}$$

$$= \frac{4.64 \times 100}{5}$$

$$= 92.8\%$$

(k) Determination of Reichert-Meissl value:

Reichert-Meissl value is the number of ml of 0.1N sodium hydroxide required to neutralize the soluble volatile fatty acids distilled from 5 gms. of the fat or oil.

Procedure:

5 gms. of the oil were accurately weighed in a clean and dry 250 ml flask, 2 ml of aqueous sodium hydroxide and

18 ml of glycerol were added. The contents of the flask were heated with occassional shaking till the mixture became perfectly clear. Now 135 ml of hot water and 6 ml of sulphuric acid (1:4) were added along with a few pieces of pumice stone.

The distillation was conducted and the distillate collected in a flask calibrated at 100 ml and 110 ml. The distillation was done quite slowly by regulating the flame so as to collect 110 ml of the distillate in about 30 minutes. After the collection of 110 ml of distillate, the flask was replaced by a 25 ml graduated cylinder to catch any further drops. The 110 ml distillate was mixed well by violent shaking, cooled and filtered through Whatman filter paper No. 42. 100 ml of this filtrate were titrated against 0.1N sodium hydroxide solution using phenolphthalein as indicator. A blank determination was also done.

Weight of the Oil taken = 5 gms.

Strength of sodium hydroxide solution = N/10

Volume of sodium hydroxide solution used for oil = 2.3 ml

Volume of sodium hydroxide solution used for blank = 1.0 ml

Reichert-Meissl value = (ml of alkali solution used by oil - ml of alkali solution used by blank) x normality x 10 x 1.1

$$= (2.3-1.0) \times \frac{1}{10} \times \frac{10}{1} \times \frac{1.1}{1}$$

$$= 1.43$$

(1) Determination of Polenske value:

Polenske value is the number of ml of 0.1N sodium

hydroxide required to neutralize the insoluble fatty acids distilled from 5 gms. of the fat or oil.

Procedure:

The volatile acids which were insoluble in water in the determination of Reichert-Meissl value were litrated as the Polenske value. The condenser, the 25 ml graduated cylinder, the receiving flask and the filter paper were washed three times with 20 ml of distilled water. These rinsings were discarded. The condenser, the 25 ml graduated cylinder, the receiving flask and the filter paper were then washed with 20 ml portions of neutralized ethanol, until the fatty acid residues were completely dissolved. These combined ethanolic washings were titrated against 0.1N sodium hydroxide solution using phenolphthalein as indicator.

Volume of alkali used = 12.9 ml

Polenske value = ml of alkali used x normality x 10

$= 12.9 \times \frac{1}{10} \times 10$

$= 12.9$

(m) Determination of the unsaponifiable matter:

When an oil or fat is saponified, a very minor portion remains unsaponified, which usually consists of higher alcohols such as phytosterol or cholesterol or some higher monohydric alcohols such as myricyl and cetyl alcohols, and a small amount of hydrocarbons together with some waxes. By characterizing phytosterol or cholesterol present in the unsaponifiable matter, the origin of an oil can be known whether it is of animal or of vegetable origin.

Procedure:

5 gms. of the oil were saponified by ethanolic potassium hydroxide, the ethanol was distilled off and a little water was added. The saponified oil after cooling was extracted with ether in a separating funnel for three to four times. The ethereal extracts containing the unsaponifiable matter were combined and dried over anhydrous sodium sulphate. Ether was then evaporated to dryness in a weighed conical flask. Finally the conical flask containing the unsaponifiable matter was heated to a constant weight in an oven at 100 to 110°C.

Weight of the oil taken for saponification = 5 gms.

Weight of the unsaponifiable matter = 0.105 gms.

∴ % of unsaponifiable matter $= \frac{0.105 \times 100}{5}$

$= 2.1\%$

Liberation of Fatty Acids (5):

100 parts by weight of the fat were taken in a two litre flask with 30 parts by weight of potassium hydroxide in 500 parts of 98% ethanol. The contents of the flask were refluxed over a water bath for about 3-5 hours. After refluxing, water was added to keep the concentration of potassium hydroxide moderate. Ethanol was partly distilled off. After removal of atleast half of the ethanol, excess of water was added and the contents were cooled. The contents were then transferred to a separating funnel and thoroughly shaken with ether. After some time, two distinct layers were formed. The ethereal layer containing the unsaponifiable matter and the aqueous layer containing the potassium salts of fatty acids were separated.

Both the aqueous layer (containing potassium salts) and ethereal layer (containing the unsaponified matter) were respectively washed with ether and water. The washings of the first were added to the second while the washings of the second were added to the first.

The contents of the flask containing the potassium salts of the fatty acids were taken in a two litre flask, a little water and 10% excess of 40% sulphuric acid were added. Steam was now passed in the flask for about 45 minutes, and the flask was heated over a water bath. A clear layer containing the fatty acids appeared in the flask over the aqueous layer. The oily layer was then extracted with

ether. The ethereal layer was separated from the aqueous layer by means of a separating funnel. The process was repeated 3-4 times, till whole of the oily layer was completely extracted. The ethereal layer was then thoroughly washed with water to remove any trace of the mineral acid, and dried over anhydrous sodium sulphate. The acids were obtained after distilling off the ether from the ethereal layer. Last traces of ether were removed by distillation under reduced pressure. The mixed fatty acids thus obtained possessed the following characteristics: iodine value (Hanus) 20.0; mean molecular weight, 216.2.

Separation of Saturated from Unsaturated Acids(6):

70 grams of the fatty acids were taken and dissolved in 400 ml of 98% ethanol. The solution was heated to boiling, and 500 ml of a boiling solution of lead acetate ~~was~~ were added to it. Lead acetate solution was prepared by mixing 80 gms. of lead acetate (A.R.), acetic acid (5 ml) and ethanol (500 ml). No immediate precipitate was obtained. The contents were shaken and cooled at 15°C till a white precipitate was formed. The contents were kept overnight at room temperature. The saturated acids so called "solid" acids formed insoluble lead salts while the unsaturated acids - called "liquid" acids, formed soluble lead salts.

The contents were filtered and the precipitate was thoroughly washed with ethanol. The lead salts of the

saturated acids were separated as a white precipitate from the soluble lead salts of the unsaturated fatty acids.

Regeneration of saturated acids(7):

The insoluble lead salts were transferred to a large porcelain basin with successive additions of concentrated hydrochloric acid and boiling water. Excess of 50% hydrochloric acid was then added. The contents were warmed till a clear layer of the acids was formed over the aqueous layer. The acid layer was now taken in a separating funnel along with some aqueous portion. After cooling, ether was added to it, with thorough shaking. The ethereal layer was separated. The process was repeated several times till the saturated acids were completely extracted with ether from the aqueous layer. The ethereal solution was washed with water to remove any trace of mineral acids present. The ethereal solution was kept overnight over anhydrous sodium sulphate. After filtering, ether was removed and the acids were finally weighed.

Weight of the conical flask = 43.4850 gms.

Weight of the conical flask + saturated acids = 85.3450 gms.

$\therefore$ Weight of the saturated acids = 41.86 gms.

% of saturated acids $= \frac{41.86 \times 100}{70}$

$= 59.8\%$

The mean molecular weight and iodine value of the mixed saturated fatty acids were determined and were found to be 214.7 and 4.5 respectively.

Regeneration of unsaturated acids (7):

Ethanol was removed from the solution of the soluble lead salts of the unsaturated acids by distillation. The lead salts were automatically decomposed by acetic acid which was added during the preparation of lead acetate solution. The unsaturated acids were extracted with ether, and the ethereal layer was freed from soluble lead acetate by washing several times with water. The ethereal solution was further washed with dilute hydrochloric acid to ensure complete decomposition of the lead salts. It was further washed with water to free it from traces of hydrochloric acid. After drying the ethereal solution of unsaturated acids over anhydrous sodium sulphate, ether was removed and the unsaturated fatty acids were finally weighed.

Weight of the conical flask = 42.6832 gms.

Weight of the conical flask + unsaturated acids = 70.8232 gms.

$\therefore$ Weight of the unsaturated acids = 28.14 gms.

% of the unsaturated acids $= \frac{28.14 \times 100}{70}$

$= 40.2\%$

The mean molecular weight and iodine value of the mixed unsaturated fatty acids were determined and were found to be 220.0 and 42.2 respectively.

Preparation of methyl esters of saturated and unsaturated fatty acids (8):

The saturated and unsaturated fatty acids were separately taken and dissolved in five times of their weight

of methyl alcohol to which sulphuric acid (1 ml) had already been added. The contents were refluxed over water bath for about two hours. About half of the alcohol was distilled off. The esters were then extracted with ethyl ether and the ethereal extract was successively washed with distilled water and a 10% potassium hydroxide solution. It was finally washed with distilled water till the washings were neutral to litmus paper. Ether was distilled off and the traces of moisture were removed by distillation under reduced pressure and then by keeping it in a vacuum desiccator.

Fractionation of methyl esters:

The methyl esters of the saturated and unsaturated fatty acids were separately distilled under reduced pressure. The weight, iodine value, saponification equivalent and mean molecular weight of each of the fractions were determined. The fatty acid composition was then calculated from their iodine values and saponification equivalents. The results are summarised in table nos. 1 and 2.

TABLE No. 1

DISTILLATION OF METHYL ESTERS OF SOLID ACIDS AT 8mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.)		
						Laurate	Myristate	Oleate
S_1	80-90	4.69	2.7	214.6	213.1	4.55	-	0.14
S_2	90-100	6.01	3.7	215.0	215.2	5.75	-	0.26
S_3	100-110	11.01	5.4	217.4	216.5	10.32	-	0.69
S_4	110-120	8.28	16.9	222.7	221.4	4.43	2.22	1.63
S_5	120-135	4.38	62.8	237.9	244.4	0.16	1.02	3.20
Residue	-	1.00	13.1	237.7	244.9	0.12	0.73	0.15
Loss	-	0.02	-	-	-	-	-	-
Total	-	35.39	-	-	-	25.33	3.97	6.07
% as ester	-	-	-	-	-	71.62	11.22	17.16
% as acid	-	-	-	-	-	71.32	11.27	17.41

TABLE No. 2

DISTILLATION OF METHYL ESTERS OF LIQUID ACIDS AT 10mm

Fraction number	Temp. °C	Wt. gms.	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.)			
						Caprate	Laurate	Myristate	Oleate
L_1	130-140	6.21	3.8	210.5	213.6	0.67	5.27	-	0.27
L_2	140-150	4.36	12.7	211.6	215.4	0.29	3.42	-	0.65
L_3	150-160	3.15	30.2	233.8	240.5	-	0.55	1.49	1.11
L_4	160-175	5.71	75.3	243.9	250.4	-	-	0.70	5.01
Residue	-	2.08	80.2	244.4	250.6	-	-	0.14	1.94
Loss	-	0.09	-	-	-	-	-	-	-
Total	-	21.60	-	-	-	0.96	9.24	2.33	8.98
% as ester	-	-	-	-	-	4.46	42.95	10.83	41.76
% as acid	-	-	-	-	-	4.77	37.02	11.87	46.34

Method for the calculation of the fatty acid composition of different fractions (9):

For fraction S_1:

$$L.L^I + 0.0^I = W.W^I \quad (1)$$

$$L + 0 = 4.69 \quad (2)$$

Where L, amount of lauric ester; L^I, iodine value of lauric ester; 0, amount of oleic ester; 0^I, iodine value of oleic ester; W, weight of the fraction; W^I, iodine value of the fraction.

The equation (1) reduces to (3), since L^I is zero.

$$0.0^I = W.W^I \quad (3)$$

$$0 \times 85.81 = 4.69 \times 2.7$$

$$0 = 0.14$$

∴ Weight of the oleic ester = 0.14 gms.

On substituting the value of 0 in equation (2),

$$L + 0.14 = 4.69 \quad (4)$$

$$L = 4.55$$

∴ Weight of the lauric ester = 4.55 gms.

For fraction S_2:

$$L.L^I + 0.0^I = W.W^I \quad (1)$$

$$L + 0 = 6.01 \quad (2)$$

The equation (1) reduces to (3), since L^I is zero.

$$0.0^I = W.W^I \quad (3)$$

$$0 \times 85.81 = 6.01 \times 3.7$$

$$0 = 0.26$$

∴ Weight of the oleic ester = 0.26 gms.

Substituting the value of 0 in equation (2),

$L + 0.26 = 6.01$ (4)

$L = 5.75$

∴ Weight of the lauric ester = 5.75 gms.

For fraction S_3:

$L.L^I + 0.0^I = W.W^I$ (1)

$L + 0 = 11.01$ (2)

The equation (1) reduces to (3), since L^I is zero.

$0.0^I = W.W^I$ (3)

$0 \times 85.81 = 11.01 \times 5.4$

$0 = 0.69$

∴ Weight of the oleic ester = 0.69 gms.

Substituting the value of 0 in equation (2),

$L + 0.69 = 11.01$ (4)

$L = 10.32$

∴ Weight of the lauric ester = 10.32 gms.

For fraction S_4:

$L.L^I + M.M^I + 0.0^I = W.W^I$ (1)

$L + M + 0 = 8.28$ (2)

$\frac{L}{214} + \frac{M}{242} = \frac{6.65}{222.7}$ (3)

Where M, amount of myristic ester; M^I, iodine value of myristic ester; $\frac{L}{214}$, saponification equivalent of lauric ester; $\frac{M}{242}$, saponification equivalent of myristic ester; 222.7, saponification equivalent of the fraction.

The equation (1) reduces to (4), since L^I and M^I are zero.

$$O.O^{I} = W.W^{I} \quad (4)$$

$$O \times 85.81 = 8.28 \times 16.9$$

$$O = 1.63$$

∴ Weight of the oleic ester = 1.63 gms.

Substituting the value of O in equation (2),

$$L + M + 1.63 = 8.28$$

$$L + M = 6.65 \quad (5)$$

On multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$4.67\ L + 4.13\ M = 29.85 \quad (6)$$

Multiplying equation (5) by 4.13 and substracting it from equation (6), we get:

$$0.54\ L = 2.39 \quad (7)$$

$$L = 4.43$$

∴ Weight of the lauric ester = 4.43 gms.

Substituting the value of L in equation (5),

$$4.43 + M = 6.65 \quad (8)$$

$$M = 2.22$$

∴ Weight of the myristic ester = 2.22 gms.

For fraction S_5:

$$L.L^{I} + M.M^{I} + O.O^{I} = W.W^{I} \quad (1)$$

$$L + M + O = 4.38 \quad (2)$$

$$\frac{L}{214} + \frac{M}{242} = \frac{1.18}{237.9} \quad (3)$$

Where 237.9, saponification equivalent of the fraction.

The equation (1) reduces to (4), since L^{I} and M^{I} are zero.

$$0.0^{I} = W.W^{I} \quad (4)$$

$$0 \times 85.81 = 4.38 \times 62.8$$

$$0 = 3.20$$

∴ Weight of the oleic ester = 3.20 gms.

Substituting the value of O in equation (2),

$$L + M + 3.20 = 4.38$$

$$L + M = 1.18 \quad (5)$$

Multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$4.67\ L + 4.13\ M = 4.96 \quad (6)$$

Multiplying equation (5) by 4.13 and substracting it from equation (6), we get:

$$0.54\ L = 0.09 \quad (7)$$

$$L = 0.16$$

∴ Weight of the lauric ester = 0.16 gms.

Substituting the value of L in equation (5),

$$0.16 + M = 1.18 \quad (8)$$

$$M = 1.02$$

∴ Weight of the myristic ester = 1.02 gms.

<u>For the residue</u>:

$$L.L^{I} + M.M^{I} + 0.0^{I} = W.W^{I} \quad (1)$$

$$L + M + 0 = 1.00 \quad (2)$$

$$\frac{L}{214} + \frac{M}{242} = \frac{0.85}{237.7} \quad (3)$$

Where, 237.7, saponification equivalent of the fraction.

The equation (1) reduces to (4), since L^I and M^I are zero.

$$O.O^I = W.W^I \quad (4)$$

$$0 \times 85.81 = 1.00 \times 13.1$$

$$O = 0.15$$

∴ Weight of the oleic ester = 0.15 gms.

Substituting the value of O in equation (2),

$$L + M + 0.15 = 1.00$$

$$L + M = 0.85 \quad (5)$$

Multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$4.67\ L + 4.13\ M = 3.575 \quad (6)$$

Multiplying equation (5) by 4.13 and substracting from equation (6), we get:

$$0.54\ L = 0.065 \quad (7)$$

$$L = 0.12$$

∴ Weight of the lauric ester = 0.12 gms.

Substituting the value of L in equation (5),

$$0.12 + M = 0.85 \quad (8)$$

$$M = 0.73$$

∴ Weight of the myristic ester = 0.73 gms.

<u>For fraction</u> L_1:

$$C.C^I + L.L^I + O.O^I = W.W^I \quad (1)$$

$$C + L + O = 6.21 \quad (2)$$

$$\frac{C}{186} + \frac{L}{214} = \frac{5.94}{210.5} \quad (3)$$

Where C, amount of capric ester; C^I, iodine value of capric ester; 210.5, saponification equivalent of the fraction.

The quation (1) reduces to (4), since C^I and L^I are zero.

$$0.0^I = W.W^I \quad (4)$$

$$0 \times 85.81 = 6.21 \times 3.8$$

$$0 = 0.27$$

∴ Weight of the oleic ester = 0.27 gms.

Substituting the value of 0 in equation (2),

$$C + L + 0.27 = 6.21$$

$$C + L = 5.94 \quad (5)$$

Multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$5.37\ C + 4.67\ L = 28.21 \quad (6)$$

Multiplying equation (5) by 4.67 and substracting from equation (6), we get:

$$0.70\ C = 0.47 \quad (7)$$

$$C = 0.67$$

∴ Weight of the capric ester = 0.67 gms.

Substituting the value of C in equation (5),

$$0.67 + L = 5.94 \quad (8)$$

$$L = 5.27$$

∴ Weight of the lauric ester = 5.27 gms.

<u>For fraction</u> L_2:

$$C.C^I + L.L^I + 0.0^I = W.W^I \quad (1)$$

$$C + L + O = 4.36 \quad (2)$$

$$\frac{C}{186} + \frac{L}{214} = \frac{3.71}{211.6} \quad (3)$$

Where 211.6, saponification equivalent of the fraction.

The equation (1) reduces to (4), since C^I and L^I are zero.

$$O.O^I = W.W^I \quad (4)$$

$$O \times 85.81 = 4.36 \times 12.7$$

$$O = 0.65$$

∴ Weight of the oleic ester = 0.65 gms.

Substituting the value of O in equation (2),

$$C + L + 0.65 = 4.36$$

$$C + L = 3.71 \quad (5)$$

Multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$5.37\ C + 4.67\ L = 17.533 \quad (6)$$

Multiplying equation (5) by 4.67 and substracting from equation (6), we get:

$$0.70\ C = 0.207 \quad (7)$$

$$C = 0.29$$

∴ Weight of the capric ester = 0.29 gms.

Substituting the value of C in equation (5),

$$0.29 + L = 3.71 \quad (8)$$

$$L = 3.42$$

∴ Weight of the lauric ester = 3.42 gms.

<u>For fraction</u> L_3:

$$L.L^I + M.M^I + O.O^I = W.W^I \quad (1)$$

$$L + M + O = 3.15 \quad (2)$$

$$\frac{L}{214} + \frac{M}{242} = \frac{2.04}{233.8} \quad (3)$$

Where 233.8, saponification equivalent of the fraction.

The equation (1) reduces to (4), since L^I and M^I are zero.

$$O.O^I = W.W^I \quad (4)$$

$$O \times 85.81 = 3.15 \times 30.2$$

$$O = 1.11$$

∴ Weight of the oleic ester = 1.11 gms.

Substituting the value of O in equation (2),

$$L + M + 1.11 = 3.15$$

$$L + M = 2.04 \quad (5)$$

Multiplying both the sides of the equation (3) by 10^3, the equation (3) reduces to (6),

$$4.67\ L + 4.13\ M = 8.72 \quad (6)$$

Multiplying equation (5) by 4.13 and substracting from equation (6), we get:

$$0.54\ L = 0.30 \quad (7)$$

$$L = 0.55$$

∴ Weight of the lauric ester = 0.55 gms.

Substituting the value of L in equation (5),

$$0.55 + M = 2.04 \quad (8)$$

$$M = 1.49$$

∴ Weight of the myristic ester = 1.49 gms.

<u>For fraction</u> L_4:

$$M.M^I + O.O^I = W.W^I \quad (1)$$

$M + O = 5.71$ (2)

The equation (1) reduces to (3), since M^I is zero.

$O.O^I = W.W^I$ (3)

$O \times 85.81 = 5.71 \times 75.3$

$O = 5.01$

∴ Weight of the oleic ester = 5.01 gms.

Substituting the value of O in equation (2),

$M + 5.01 = 5.71$ (4)

$M = 0.70$

∴ Weight of the myristic ester = 0.70 gms.

For residue fraction:

$M.M^I + O.O^I = W.W^I$ (1)

$M + O = 2.08$ (2)

The equation (1) reduces to (3), since M^I is zero

$O.O^I = W.W^I$ (3)

$O \times 85.81 = 2.08 \times 80.2$

$O = 1.94$

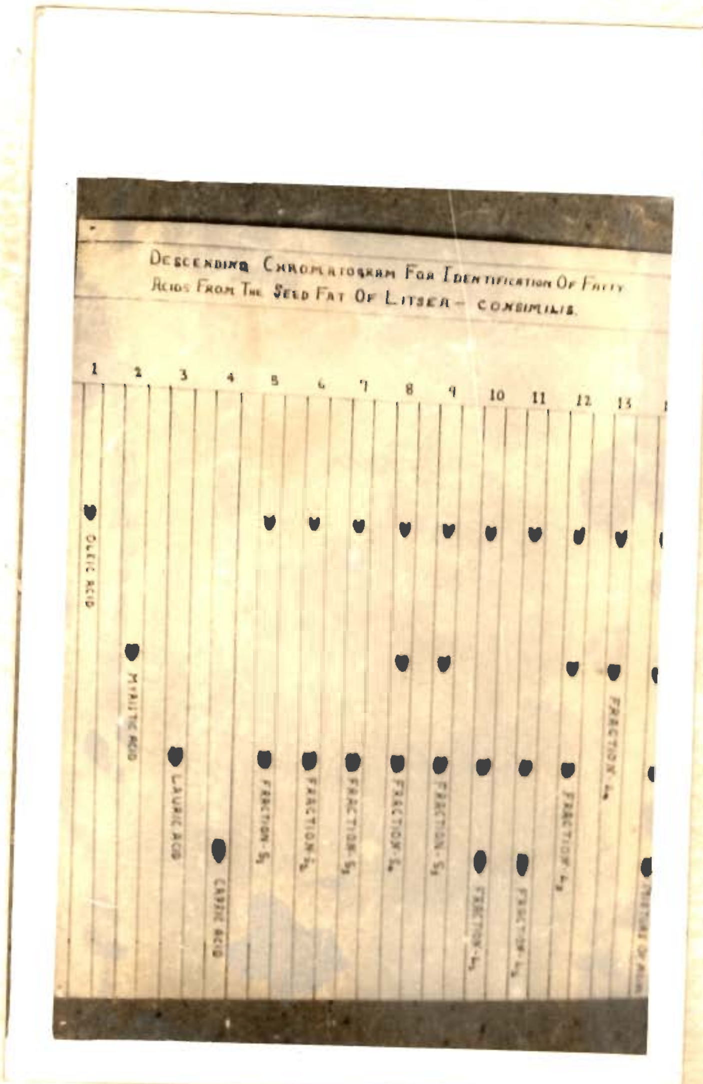
∴ Weight of the oleic ester = 1.94 gms.

Substituting the value of O in equation (2),

$M + 1.94 = 2.08$ (4)

$M = 0.14$

∴ Weight of the myristic ester = 0.14 gms.



CHARACTERIZATION OF FATTY ACIDS BY DESCENDING PAPER CHROMATOGRAPHY.

Characterization of the fatty acids:

The ester fractions were saponified with ethanolic potassium hydroxide solution, and the liberated fatty acids were characterized by reversed phase descending paper chromatographic technique and also by identifying the oxidation products of the acids. The following two methods were employed for the characterization of fatty acids by descending paper chromatographic technique.

First method:

Whatman filter paper No. 1 (sheets 24 x 12") was impregnated in a 12% solution of olive oil in toluene (10). The paper was dried in air for few hours, and 0.005 - 0.01 ml of 2% acetone solutions of saturated, unsaturated acids and 0.005 ml of 2% solutions of reference fatty acids in acetone were chromatographed together on the filter paper. The chromatograms were developed with 75% ethanol for 48 hours. The chromatograms were then taken out and dried in air overnight. The fatty acids were located by the following reagents:

(a) Aqueous lead acetate and sodium sulphide solutions (11):

The chromatograms were dipped in a 1% solution of lead acetate taken in a photographic tray, and were washed several times with water to remove excess of lead acetate from the chromatograms which were then dipped in a 10% solution of sodium sulphide. The fatty acids were located as brownish-black spots. A saturated solution of hydrogen

sulphide in water was, however, found to give better spots than a 10% solution of sodium sulphide.

(b) Aqueous copper sulphate and potassium ferrocyanide solutions (12):

The air dried chromatograms were dipped in copper sulphate solution (25 ml of a saturated solution of copper sulphate diluted with 300 ml of water) for about an hour in a photographic tray. The chromatograms were then washed in a current of water to free them from excess of copper sulphate and finally dipped in a 2.5% solution of potassium ferrocyanide. The fatty acids were located as chocolate coloured spots. Some of the fatty acids gave green spots before treating the chromatograms with potassium ferrocyanide solution.

Second method (12):

Whatman filter paper No. 1 (Sheets 24 x 12") was impregnated in a 15% solution of liquid paraffin in benzene or a 10% solution of liquid paraffin in petroleum ether, and the paper was dried in air for few hours. 0.005-0.01 ml of 2% acetone solutions of saturated and unsaturated acids were chromatographed on the paper along with 2% solutions of reference fatty acids in acetone. The chromatograms were developed with 90% acetic acid for about 30 hours. The fatty acids were located as described in the first method. The R_f values of different fatty acids were also

determined, and are recorded in table no. 3.

TABLE No. 3

R_f values of fatty acids.

Serial number	Fatty acid	R_f values
1.	Caproic acid	0.77
2.	Caprylic acid	0.69
3.	Capric acid	0.67
4.	Lauric acid	0.53
5.	Myristic acid	0.37
6.	Linoleic acid	0.26
7.	Oleic acid	0.22
8.	Palmitic acid	0.10
9.	Stearic acid	0.08

Characterization of fatty acids by oxidation method:

The liberated acids from the fractions L_1-L_4 and the residue on oxidation (13) with dilute ice-cold potassium permanganate solution, yielded a white crystalline solid identified as dihydroxystearic acid (m.p. 130°C, mol. wt., 315). This confirmed the presence of oleic acid in these fractions and the residue.

The unoxidised portion from L_1 and L_2 was extracted with ether. The residue left on removal of ether gave two products (m.p. 30°C and 43°C) on treatment with 50% and 70%

ethanol respectively. These were characterized as capric acid (m.p. and mixed m.p. 30°C) and lauric acid (m.p. and mixed m.p. 43°C).

The acids from fraction L_3 were characterised as lauric, myristic and oleic acids by reversed phase descending paper chromatography (12) as described previously.

The unoxidised acids liberated from the fractions L_4 and the residue on similar treatment gave with ethanol a solid which was identified as myristic acid (m.p. and mixed m.p. 53-54°C).

Similarly the acids liberated from ester fractions S_1-S_5 and the residue, on oxidation with cold dilute potassium permanganate solution gave dihydroxystearic acid. Acids obtained from S_1-S_3 which resisted oxidation gave a white crystalline solid with 70% ethanol, identified as lauric acid, while acids from S_4, S_5 and the residue were identified as lauric, myristic and oleic acids by paper chromatography (12).

The present studies on the component fatty acids of the seed fat of Litsea consimilis reveal that apart from lauric and oleic acids, which were stated to be the only components of other Litsea seed fats (Indian genera), about 1.7% of capric and 11.5% of myristic acids are also present.

CHEMICAL EXAMINATION OF THE UNSAPONIFIABLE MATTER:

β-sitosterol has been isolated from the unsaponifiable matter of the oil from the seeds of Litsea consimilis, and has been identified by its physical and chemical properties; and also by the preparation of its acetate, benzoate and digitonide derivatives. Further confirmation of its identity has been established by its mixed chromatogram with an authentic sample of β-sitosterol over a column of Brockmann alumina.

EXPERIMENTAL

Isolation and purification of the sterol:

Dry seeds of Litsea consimilis were powdered and soxhletted with petroleum ether (b.p. 60-80°C). The oil obtained after removal of petroleum ether was purified by treating it with Fuller's earth and activated charcoal, and freed from volatile essential oils as far as possible by heating in Vacuo. It was saponified by refluxing on water bath for 12 hours with ethanolic potassium hydroxide solution. After saponification, the product was diluted with water and the ethanol distilled off on water bath with the addition of corresponding amounts of water from time to time. The ethanol-free aqueous solution of soap was extracted several times with ether. The ethereal extract was washed with water, dried over fused calcium chloride and the solvent distilled off, when a crude brown semi-solid residue of the sterol was obtained.

The crude sterol was refluxed with methanol for 6 hours and kept for 20 hours in a refrigerator; the process was repeated twice when the precipitate of the crude sterol (2.0%; unsaponifiable matter) was obtained. The crude precipitate of the sterol was thoroughly dried in a vacuum desiccator; m.p. 130-135°C.

The crude sterol was dissolved in minimum quantity of benzene and was chromatographed over a column of Brockmann alumina (35 x 1.5 cm.) using petroleum ether, benzene, chloroform, methanol and their mixtures as eluants as shown in table no. 4. Eluants were collected in fractions of 30-35 ml each and were evaporated to dryness.

TABLE No. 4

Fractions	Eluants	Residue left on evaporation of solvent
1-6	Petroleum ether (b.p. 60-80°C)	Oily mass
7-10	Petroleum ether + Benzene (3:2 v/v)	Oily mass
11-14	Petroleum ether + Benzene (1:1 v/v)	Nil
15-18	Benzene	Nil
19-25	Benzene + Chloroform(3:2 v/v)	Shining flakes
26-30	Benzene + Chloroform(1:1 v/v)	Shining flakes
31-34	Chloroform	Nil
35-38	Chloroform + Methanol (99:1 v/v)	Nil

Fractions 19-30, containing the sterol in the form of shining flakes, were combined and on repeated crystallisations from a mixture of methanol and ethyl acetate (1:1 v/v) yielded colourless flakes with constant m.p. 136-137°C. The sterol was fairly soluble in benzene, methanol and ethanol and readily soluble in chloroform, ether and ethyl acetate. The compound developed a reddish purple colour in the Liebermann-Burchard test with chloroform and concentrated sulphuric acid; $[\alpha]_D^{25}$ - 36°.5 (chloroform). It did not depress the melting point of an authentic sample of β-sitosterol. (Found: C, 83.70; H, 12.21; $C_{29}H_{50}O$ requires C, 84.05; H, 12.08%). The sterol was further confirmed as β-sitosterol by preparing following derivatives:

(a) Preparation of the acetyl derivative (14):

The sterol (0.1 gms.) was treated with acetic anhydride (1 ml) and 2 drops of pyridine. The mixture was allowed to stand overnight at room temperature and then treated for about 6 hours on water bath. The reaction mixture was poured to crushed ice and kept in the refrigerator overnight. The solid was filtered off, washed with water and crystallised several times from a mixture of methanol and acetone (1:1 v/v) in colourless flakes, m.p. 126°C; yield, 0.051 gms.

(b) Preparation of the benzoate (14):

The sterol (0.1 gms.) was treated with benzoyl chloride (1 ml) and 2 drops of pyridine. The mixture was allowed to stand overnight at room temperature and then heated for about 6 hours on water bath. The mixture was poured into ice-cold water and kept in the refrigerator overnight. The solid was filtered off, washed with 2% aqueous potassium hydroxide (till free from benzoic acid) and then with water and finally crystallised from a mixture of methanol and acetone (1:1 v/v), m.p. 144°C; yield, 0.04 gms.

(c) Preparation of the digitonide (15):

A saturated solution of the sterol (0.1 gms.) in absolute ethanol at 60°C was heated with an equal volume of a saturated solution of digitonin in 95% ethanol. The mixture was refluxed on water bath for about 2 hours and then kept at 0°C for about 12 hours in a refrigerator, when the digitonide was obtained as a white powder, m.p. 230°C (decomp.); yield, 0.05 gms.

Mixed chromatogram of the sterol with an authentic sample of β-sitosterol (16):

The sterol (0.04 gms.) with an equal quantity of an authentic sample of β-sitosterol was dissolved in minimum quantity of benzene and chromatographed over a column of Brockmann alumina (15 x 0.5 cm.). Elution with benzene and a mixture of benzene and chloroform (1:1 v/v)

yielded from the first six fractions 0.07 gms. of a solid, which on crystallisation from a mixture of methanol and ethyl acetate (1:1 v/v) furnished colourless flakes with a constant m.p. 137°C.

CHEMICAL EXAMINATION OF THE ESSENTIAL OIL FROM THE SEEDS:

On steam distillation of the seeds, a yellowish brown coloured essential oil was obtained in 0.75% yield. The composition of the oil was determined and found to be α-d-pinene, 23.6%; cineole, 18.0%; ocimene, 13.9%; dipentene, 12.5%; linalool, 12.5%; citronellal, 7.0% and citral, 12.5%.

The leaves, seeds, fruits and barks of a number of lauraceae plants, particularly those of Litsea and Cinnamomum genera viz. Litsea cubeca, Litsea cubeba, Litsea zeylanica, Litsea guatemalensis, cinnamomum aromaticum Cinnamomum zeylanicum, Cinnamomum glanduliferum etc. have been reported to contain small amounts of essential oils, which have been studied by a number of workers (17-26). The essential oil content of the seeds of Litsea consimilis was chemically examined with a view to finding out whether the constituents were same or different from those examined by earlier workers.

EXPERIMENTAL

Extraction of the essential oil from the seeds:

The extraction of the essential oil was carried out by steam distillation of the seeds employing a copper still of capacity 15 litres. The seeds were crushed, powdered and placed on a perforated shelf resting about the middle of the still and were then heated by steam

generated below the perforated shelf. The still had an arrangement for adding water occasionally from outside without discontinuing the distillation. The hood was fixed into the circular groove of the still and was made air-tight by cementing with a paste of Plaster of Paris. The narrow end of the hood was connected with a copper condenser fixed in a copper cylinder, through which a continous flow of cold water was circulated.

It was found that eight litres of water could completely extract the essential oil from three kilograms of the seeds. The distillate was collected in winchester bottles and on allowing the distillate to stand, an oily layer separated and floated over the aqueous layer. The oily layer was removed by means of a pippette and the aqueous distillate was returned back to the still for the next distillation. The oil was allowed to stand for some time over anhydrous sodium sulphate and filtered. It was then extracted with chloroform. The chloroformic extract was allowed to stand overnight over anhydrous sodium sulphate. After drying, the solvent was distilled off on a water bath, first at atmospheric pressure and then under reduced pressure so as to remove the last traces of the solvent.

The oil which was obtained in 0.75% yield, possessed the following characteristics: specific gravity at 20°C,

0.8518; refractive index at 20°C, 1.4672; acid value, 1.12; ester value, 14.6.

The essential oil was fractionally distilled under reduced pressure (10 mm.), when the following fractions were collected as given in table no. 5:

TABLE No. 5

Fraction number	Temp. °C	Volume in ml
I	55-65	30
II	65-75	19
III	80-90	14
IV	105-112	9
Residue	-	1.5 (resinified)
Total	-	73.5

These fractions were then refractionated at atmospheric pressure. On refractionation, two fractions were collected from each of the fractions I, II and III and only one from IV. Their properties and identity are recorded in table no. 6.

TABLE No. 6

REFRACTIONATION OF FRACTIONS (I-IV) AT ATMOSPHERIC PRESSURE:

No.	^{o}C B.P. in	Volume in ml	d_{20}	n_{D}^{20}	Derivatives	Compounds identified
			FRACTION I			
A	155-156	17	0.8586	1.4659	Nitrosyl chloride, m.p. 103^{o}C. Nitrol piperide, m.p. 117^{o}C. Pinonic acid, m.p. 68^{o}C. Hydrochloride, m.p. 132^{o}C. Maleic anhydride adduct, m.p. 168^{o}C.	α-d-pinene
B	172-174	13	0.9026	1.4478	Addition compound with resorcinol, m.p. 85-87^{o}C. Addition compound with syrupy phosphoric acid.	Cineole
			FRACTION II			
A	176-177	10	0.8021	1.4851	Reduced to dihydromyrcene b.p. 66^{o}/12 mm; tetrabromide of the above, m.p. 87-88^{o}C. Ocimenol (phenyl urethane, m.p. 72^{o}C). Maleic anhydride adduct, m.p. 82-83^{o}C.	Ocimene
B	178-179	9	0.8402	1.4719	Tetrabromide, m.p. 124^{o}C. Nitrosyl chloride, m.p.78^{o}C. Maleic anhydride adduct, m.p. 148-149^{o}C.	Dipentene

TABLE No. 6 (Contd.)

No.	^{o}C B.P. in	Volume in ml	d_{20}	n_{D}^{20}	Derivatives	Compounds identified
			FRACTION III			
A	198-199	9	0.8627	1.4646	Phenylurethane, m.p. 65-66°C. α-naphthylurethane, m.p. 53°C.	Linalool
B	204-205	5	0.8508	1.4467	Semicarbazone, m.p. 90-91°C. Citronellyl-β-naphthocinchoninic acid, m.p. 225°C. Citronellylidenecyan-acetic acid, m.p. 137-138°C.	Citronellal
			FRACTION IV			
A	220-227	9	0.8879	1.4885	Semicarbazone, m.p. 164°C. Citrylidenecyanacetic acid, m.p. 122°C. Citryl-β-naphthocinchoninic acid, m.p. 200°C.	Citral

EXAMINATION OF FRACTION I:

I-A Characterization of α-d-pinene:

A little portion of the fraction was treated with 70% ethanol (27). The ethanolic layer on evaporation of ethanol left no residue indicating thereby the absence of β-d-pinene.

α-d-pinene was confirmed by the preparation and characterization of the following derivatives:

Preparation of the nitrosyl chloride (28):

To 2 ml of the fraction were added 2 ml of glacial acetic acid and the mixture was cooled to $0^{\circ}C$. Amyl nitrite (4 ml) was then added drop by drop, care being taken that the temperature remained constant. 0.4 ml of hydrochloric acid was gradually added and the mixture after being shaken well was kept in a freezing mixture. After some time, a mass of light crystals separated. The crystalline derivative was filtered off, recrystallised from ethyl acetate and dried, m.p. $103^{\circ}C$.

Preparation of the nitrol piperide:

The nitrol piperide was prepared by dissolving the nitrosyl chloride in the minimal amount of ethanol and adding an ethanolic solution of piperidine. The mixture was slightly warmed and poured to ice cold water, when a white solid precipitate separated. It was filtered and crystallised from ethanol, m.p. $117^{\circ}C$.

Preparation of pinonic acid (29):

Pinonic acid was obtained by treating a mixture of 2.4 ml of the fraction and 12 ml of water with 4 gms. of potassium permanganate dissolved in 40 ml of water. The mixture was vigorously stirred and kept cool by ice. After keeping it for about half an hour, manganese oxide was filtered off and the filtrate was evaporated to 20 ml in an atmosphere of carbon dioxide. After extracting the filtrate with ether, the aqueous layer was acidified and extracted with ether. After drying the solvent over anhydrous sodium sulphate, it was evaporated on water bath and the residue was recrystallised from ethanol and finally from petroleum ether, m.p. 68°C.

Preparation of the hydrochloride (30):

1 ml of the fraction was saturated with dry hydrochloric acid gas at 10-15°C. The mixture was then kept at -5°C for one hour. The separated solid was filtered with suction and crystallised from five times its weight of methyl alcohol, m.p. 132°C.

Preparation of the maleic anhydride adduct (31):

2.6 ml of the fraction were dissolved in 6 ml of dry benzene and the solution gradually added to a suspension of 1.9 gms. of maleic anhydride in 5 ml of benzene. The reaction was complete within five minutes. The mixture was kept for half an hour at room temperature and then heated in a well corked bottle (to exclude

moisture) on a water bath for four hours. The residue left on removal of the solvent was cooled in ice, when a solid separated, which was crystallised from ethanol, m.p. 168°C.

I-B Characterization of cineole:

Cineole was confirmed by the preparation and characterization of the following derivatives:

Preparation of the addition compound with resorcinol (32):

About 0.5 gms. of resorcinol was added to 1 ml of the fraction and the mixture warmed a little till the former dissolved. On allowing the contents to cool, fine crystals separated. These were filtered off and recrystallised from ethyl acetate-absolute alcohol mixture, m.p. 85-87°C.

Preparation of the addition compound with syrupy phosphoric acid (33):

2 ml of the fraction were cooled thoroughly in a freezing mixture and 1 ml of well cooled syrupy phosphoric acid (sp. gr. 1.75) was added slowly with constant shaking. A solid which separated became harder on cooling.

EXAMINATION OF FRACTION II:

II-A Characterization of Ocimene:

Ocimene was confirmed by the preparation and characterization of the following derivatives:

Preparation of dihydromyreene and its tetrabromo derivative (34):

2 ml of the fraction were treated with 1.0 gms.

of sodium and 10 ml of ethanol, and the resulting liquid distilled at 66°/12mm. On treatment with 1 ml bromine, a solid derivative was obtained which was crystallised from ethanol, m.p. 87-88°C.

Found: Br, 69.71; $C_{10} H_{18} Br_4$ requires Br, 69.82%.

Preparation of ocimenol and its phenylurethane (35):

2 ml of the fraction were refluxed with 4 ml of 50% solution of sulphuric acid in presence of glacial acetic acid. On distillation of the resulting liquid, a fraction passing at 96-100°C, was collected. On reacting it with phenyl isocyanate, a solid separated which was repeatedly crystallised from ethanol, m.p. 72°C.

Preparation of the maleic anhydride adduct (36):

The maleic anhydride adduct was prepared as described previously, (page, 60), m.p. 82-83°C.

II-B Characterization of dipentene:

Dipentene was confirmed by preparing the following derivatives:

Preparation of the tetrabromide (37):

A mixture of 2 ml of the fraction, 5 ml of amyl alcohol and 5 ml of ether was added drop by drop to an ice cold solution of bromine in ether. The tetrabromide derivative separated in a crystalline form, m.p. 124-125°C.

Preparation of the nitrosyl chloride (38):

The nitrosyl chloride was prepared as described previously (page, 59), m.p. 78°C. On further heating,

it solidified and melted again at 103-104°C.

Preparation of the maleic anhydride adduct (36):

2.5 ml of the fraction were refluxed for half an hour with 1 gms. of maleic anhydride in 10 ml of benzene. On cooling the contents, a solid separated, which was crystallised from ethanol, m.p. 148-149°C.

EXAMINATION OF FRACTION III:

III A Characterization of linalool:

Linalool was confirmed by the preparation and characterization of the following derivatives:

Preparation of the phenylurethane (39):

2 ml of the fraction were heated with 1.75 ml of phenyl isocyanate for 10 minutes on a steam bath. On cooling, a solid separated which was crystallised from petroleum ether, m.p. 65-66°C.

Preparation of the α-naphthylurethane (40):

2 ml of the fraction were heated with 1.75 ml of α-naphthyl isocyanate for 10 minutes on a steam bath. On cooling, a solid separated which was crystallised from carbon tetrachloride, m.p. 53°C.

III-B Characterization of citronellal:

Citronellal was confirmed by the preparation of the following derivatives:

Preparation of the semicarbazone (41):

A solution of 1 ml of the fraction in 5 ml of ethanol was shaken with a solution of semicarbazide

hydrochloride and sodium acetate. A crude solid separated, which was crystallised successively from chloroform and ligroine, m.p. 90-91°C.

Preparation of citronellyl β-naphthocinchoninic acid (42):

1.2 ml of the fraction and 0.6 gms. of pyruvic acid were dissolved in 5 ml of absolute alcohol. 1 gms. of β-naphthylamine, dissolved in 5 ml of absolute alcohol, was added to this solution. The mixture was then refluxed for 3 hours on a steam bath. On cooling, a crude product separated which was crystallised from ethanol containing hydrochloric acid, the hydrochloride thus obtained was dissolved in ammonia, the ammonium salt was decomposed with acetic acid, and recrystallised from dilute ethanol in colourless needles, m.p. 225°C. When heated above its melting point, the compound was converted into citronellyl-β-naphthoquinoline with cleavage of carbon dioxide. The base was crystallised from dilute ethanol in silky needles, m.p. 53°C.

Preparation of citronellylidenecyanacetic acid (43):

2 ml of 30% sodium hydroxide solution were added to a mixture of 1 ml of the fraction, 1 ml of cyanacetic acid and 5 ml of water. The citronellylidenecyanacetic acid was obtained by precipitating the mixture with hydrochloric acid. The precipitate was filtered and dissolved in benzene and then precipitated with petroleum

ether in yellow crystals, m.p. 137-138°C.

EXAMINATION OF FRACTION IV:

IV-A Characterization of citral:

Citral was confirmed by preparing its semicarbazone (44), m.p. 164°C; citrylidenecyanacetic acid (45), m.p. 122°C and citryl-β-naphthocinchoninic acid (46), m.p. 200°C as described previously (pages 63-64).

The foregoing investigations reveal that the constituents of the oil obtained from the seeds of Litsea consimilis are quite different from those of the seeds of other Litsea species studied so far (18). Besides the presence of the common constituents i.e. citral and cineole, it contains α-d-pinene, ocimene, dipentene, linalool and citronellal. It may, however, be interesting to observe that the majority of the constituents of this essential oil are identical with those occurring in the essential oils obtained from the leaves of Litsea cubeba (19) and Litsea zeylanica (21).

CHAPTER II

CHEMICAL EXAMINATION OF THE FRUIT COATS OF LITSEA CONSIMILIS

CHEMICAL EXAMINATION OF THE FRUIT COAT FAT

The fruit coat fat of the seeds of Litsea consimilis has been chemically analysed. The fatty acid composition was found to be capric acid, 4.51% ; lauric acid, 52.54% and Oleic acid, 42.95%.

EXPERIMENTAL

The fruit coat was removed mechanically from the seed kernels, powdered and soxhletted with petroleum ether (b.p. 60-80°C). A yellowish brown oil, which solidified completely at 15°C, was obtained in 16% yield. It was purified by treating it with Fuller's earth and activated charcoal. The purified oil was freed from volatile essential oils as far as possible by heating in Vacuo. The physical characteristics were determined as described earlier (chapter 1 ; pages 17-28)

(a) Determination of the percentage yield:

Weight of the fruit coat extracted = 250 gms.

Weight of the conical flask = 48.4640 gms.

Weight of the conical flask + Oil = 88.4640 gms.

$\therefore$ Weight of the Oil = 40 gms.

$\therefore$ % yield $= \frac{40 \times 100}{250}$

$= 16\%$

(b) Specific gravity:

Weight of the specific gravity bottle = 6.8548 gms.

Weight of the specific gravity bottle + water =17.5769 gms.

Weight of the specific gravity bottle + Oil =16.5326 gms.

∴ Weight of the water taken = 10.7221 gms.

Weight of the Oil taken = 9.6778 gms.

∴ Specific gravity of the Oil at 35°C

$$= \frac{\text{Weight of the oil taken}}{\text{Weight of the water taken}}$$

$$= \frac{9.6778}{10.7221}$$

$$= 0.9026$$

(c) Refractive index:

By Abbe's refractometer

Refractive index at 40°C = 1.4565

(d) Determination of the acid value:

Weight of the Oil taken = 2.5013 gms.

Strength of NaOH solution = N/10

Volume of NaOH solution used = 33.2 ml

∴ Acid value $= \frac{33.2 \times 56.1}{10 \times 2.5013}$

$$= 74.46$$

(e) Determination of the saponification value:

Weight of the oil taken in conical flask 'A'

= 1.7461 gms.

Volume of approximately N/2 alcoholic potassium hydroxide solution taken in each flask

= 25 ml

Volume of N/4 hydrochloric acid used

(i) for blank 'B' = 50.8 ml

(ii) for Oil 'A' = 20.8 ml

∴ Volume of N/4 hydrochloric acid consumed

= 50.8 - 20.8 ml

= 30 ml

∴ Saponification value = $\frac{30 \times 56.1 \times 1000}{4 \times 1000 \times 1.7461}$

= 240.96

(f) Determination of the saponification equivalent:

Saponification equivalent = $\frac{56100}{240.96}$

= 232.81

(g) Determination of the ester value:

Ester value = Saponification value - Acid value

= 240.96 - 74.46

= 166.50

(h) Determination of the iodine value:

Weight of the Oil taken in iodine flask 'A'

= 0.2490 gms.

Volume of Hanus solution taken in each flask

= 25 ml

Strength of sodium thiosulphate solution

= N/10

Volume of sodium thiosulphate solution used:

(i) for blank 'B' = 53.7 ml

(ii) for Oil 'A' = 44.2 ml

∴ Volume of thiosulphate used

(Volume of N/10 iodine reacted)

= 53.7 - 44.2 ml

= 9.5 ml

∴ Iodine value = $\frac{127 \times 9.5 \times 100}{10 \times 1000 \times 0.2490}$

= 48.45

(i) Determination of the acetyl value:

Weight of the acetylated Oil taken in flask 'A'

= 1.9991 gms.

Volume of alcoholic potassium hydroxide solution taken in each flask = 25 ml

Volume of N/4 hydrochloric acid used:

(i) for blank 'B' = 50.8 ml

(ii) for acetylated oil 'A' = 15.1 ml

Volume of N/4 hydrochloric acid consumed

= 50.8 - 15.1

= 35.7 ml

∴ Saponification value of the acetylated oil

$$= \frac{35.7 \times 66.1 \times 1000}{4 \times 1000 \times 1.9991}$$

= 250.45

$$\text{Acetyl value} = \frac{\text{Saponification value of the acetylated Oil - Saponification value of the oil}}{1-(0.00075 \times \text{saponification value of the oil})}$$

$$= \frac{250.45-240.96}{1-(0.00075 \times 240.96)}$$

= 11.58

(j) Determination of Hehner value:

Weight of the oil taken for saponification = 0.7774 gms.

Weight of the insoluble acids obtained = 0.7237 gms.

$$\text{Hehner value} = \frac{\text{Weight of the insoluble acids} \times 100}{\text{Weight of the oil taken for saponification.}}$$

$$= \frac{0.7237 \times 100}{0.7774}$$

= 93.10%

(k) Determination of Reichert-Meissl value:

Weight of the oil taken = 5.084 gms.

Strength of sodium hydroxide solution = N/10

Volume of sodium hydroxide solution used for the oil = 2.1 ml

Volume of sodium hydroxide solution used for the blank = 1.0 ml

Reichert-Meissl value = (ml of alkali solution used by the oil-ml of alkali used by the blank) x normality x 1.1 x 10

$$= (2.1-1.0) \times \frac{1}{10} \times \frac{1.1}{1} \times \frac{10}{1} \times \frac{5}{5.084}$$

$$= 1.19$$

(l) Determination of Polenske value:

Volume of alkali used = 10.2 ml

Polenske value = ml of alkali used x normality x 10

$$= 10.2 \times \frac{1}{10} \times 10$$

$$= 10.2$$

(m) Estimation of the unsaponifiable matter:

Weight of the oil taken for saponification = 1.5264 gms.

Weight of the unsaponifiable matter = 0.0303 gms.

$$\therefore \% \text{ of unsaponifiable matter} = \frac{0.0303 \times 100}{1.5264}$$

$$= 1.98\%$$

Liberation of the fatty acids:

A requisite amount of the oil was saponified with ethanolic potassium hydroxide. The ethanol-free aqueous solution of the

soap was thoroughly extracted with ether to remove unsaponifiable matter. The soap was treated with dilute sulphuric acid and the liberated fatty acids were extracted and examined in the usual manner. The mixed fatty acids possessed the following characteristics: iodine value, 41.79; mean molecular weight, 225.48.

The mixed fatty acids were separated into solid and liquid fatty acids using Hilditch's modification of Twitchell's lead salt method (1). The liberated acids possessed the following characteristics:

	Solid acids	Liquid acids
Percentage	43.26	56.74
Iodine value	13.80	50.02
Mean molecular weight	219.90	224.49

The solid and liquid acids were esterified with methanol (2) and the esters fractionally distilled under reduced pressure. The weight, iodine value, saponification equivalent and mean molecular weight of each of the fractions were determined in the usual manner. The fatty acid composition of each of the fractions was then calculated (Vide chapter I, page §36-44). The results have been summarised in table nos. 7 and 8.

TABLE No. 7

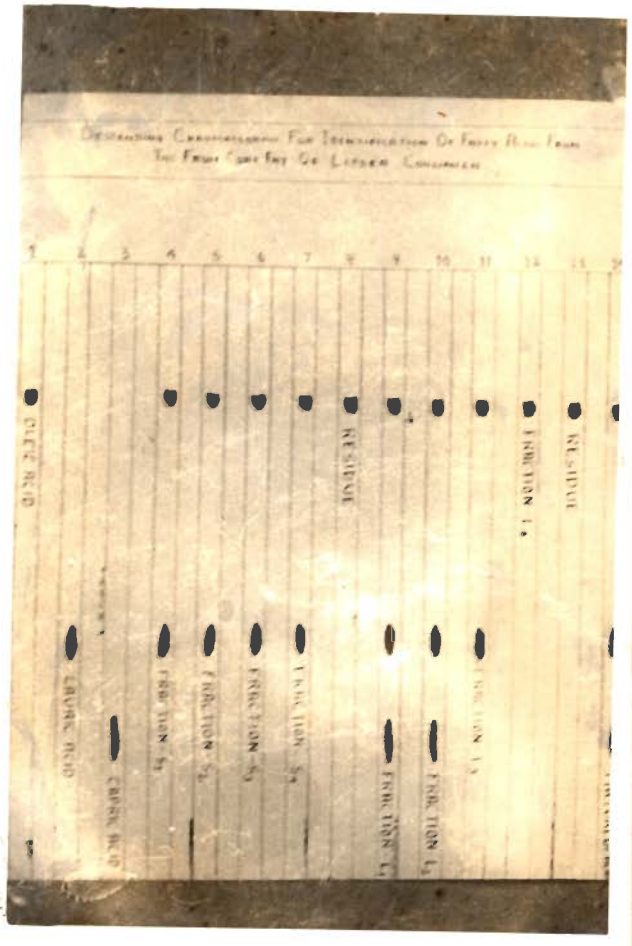
DISTILLATION OF METHYL ESTERS OF SOLID ACIDS AT 9mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.) Laurate	Oleate
S_1	90-100	3.8034	4.6468	214.25	214.196	3.5975	0.2059
S_2	100-110	2.9146	15.6035	228.40	229.728	2.3847	0.5299
S_3	110-120	2.3530	28.9830	259.30	260.604	1.5583	0.7947
S_4	120-130	1.7736	37.8400	266.69	270.070	0.9916	0.7820
Residue	-	1.0540	73.4680	276.53	275.714	-	1.0540
Loss	-	0.0514	-	-	-	-	-
Total	-	11.9500	-	-	-	8.5321	3.3665
% as ester	-	-	-	-	-	71.71	28.29
% as acid	-	-	-	-	-	71.29	28.71

TABLE No. 8

DISTILLATION OF METHYL ESTERS OF LIQUID ESTERS AT 10mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.)		
						Caprate	Laurate	Oleate
L_1	120-130	3.1558	10.128	205.12	209.60	0.7909	1.9924	0.3725
L_2	130-140	3.5746	13.394	209.46	211.20	0.4367	2.5802	0.5577
L_3	140-150	4.0780	55.107	229.82	235.00	-	1.4592	2.6188
L_4	150-160	2.8382	89.661	278.04	277.14	-	-	2.8382
Residue	-	1.9248	89.080	280.47	281.43	-	-	1.9248
Loss	-	0.0102	-	-	-	-	-	-
Total	-	15.5816	-	-	-	1.2276	6.0318	8.3120
% as ester	-	-	-	-	-	7.88	38.73	53.39
% as acid	-	-	-	-	-	7.81	38.34	53.85



CHARACTERIZATION OF FATTY ACIDS BY DESCENDING PAPER CHROMATOGRAPHY.

CHARACTERIZATION OF THE ACIDS

The liquid ester fractions L_1 - L_4 and the residue were saponified and the liberated acids on oxidation (3) with dilute ice cold potassium permanganate solution gave a white crystalline solid which was characterized as dihydroxystearic acid (m.p. 131°C ; mol. wt., 315), confirming the presence of oleic acid in these fractions and the residue. The unoxidized portion of the acids from these fractions and the residue were extracted with ethyl ether. The unoxidized portion of the acids from fractions L_1 -L_2 was resolved into two crystalline white solids with 50% and 70% alcohol. These were characterized as capric acid (m.p. and mixed m.p. 30°C) and lauric acid (m.p. and mixed m.p. 43.5°C). The unoxidized portion from the fraction L_3 on crystallisation with 70% alcohol gave only lauric acid (m.p. and mixed m.p. 43.5°C). The fraction L_4 and the residue left no solid on removal of ether.

Similarly the acids, liberated from ester fractions S_1 - S_4 and the residue, on oxidation with cold dilute potassium permanganate solution gave dihydroxystearic acid, thereby indicating the presence of oleic acid in these fractions and the residue. The unoxidized portion of the acids from S_1 - S_4 on crystallisation with 70% alcohol gave only lauric acid (m.p. and mixed m.p. 43.5°).

The fatty acids liberated from different ester

fractions were also characterized by reversed phase descending paper chromatography (4) (Vide chapter I, pages 45-47).

In almost all fruit coat fats of lauraceae plants, palmitic acid has been reported to be present as the major saturated acid component except one case i.e. the fruit coat fat of Actinodaphne hookeri (5), which contains lauric acid (33%) as the major saturated acid component. It is however, quite interesting to observe that the major saturated acid component in the fruit coat fat of Litsea consimilis is lauric acid (52.54%). Moreover, the glyceride of capric acid, which does not occur in any fruit coat fat of lauraceae plants so far studied, has been found to be present in the fruit coat fat of Litsea consimilis.

CHEMICAL EXAMINATION OF THE UNSAPONIFIABLE MATTER

β- sitosterol has been isolated from the unsaponifiable matter of the fruit coat fat of Litsea consimilis, and has been identified by its physical and chemical properties and by the preparation of its acetate, benzoate and digitonide derivatives. Further confirmation of its identity has been established by its mixed chromatogram with an authentic sample of β-sitosterol over a column of Brockmann alumina.

EXPERIMENTAL

Isolation and purification of the sterol:

Dry fruit coats of Litsea consimilis were powdered and soxhletted with petroleum ether (b.p. 60-80°C). The oil obtained after removal of petroleum ether was purified by treating with Fuller's earth and activated charcoal, and freed from volatile essential oils as far as possible by heating in Vacuo. It was saponified by refluxing on water bath for 8 hours with alcoholic potassium hydroxide solution. After saponification, the product was diluted with water and the ethanol distilled off on water bath with the addition of corresponding amount of water from time to time. The ethanol free aqueous solution of soap was extracted several times with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulphate and the solvent distilled off, when a crude brown semi-solid residue of the sterol was obtained in 1.98% yield.

The crude sterol was dissolved in minimum quantity of

benzene and was chromatographed over a column of Brockmann alumina (35 x 1.5 cm.) using petroleum ether, benzene, chloroform and their mixtures as eluants as shown in table no. 9. Eluants were collected in fractions of 30-35 ml each and evaporated to dryness.

TABLE No.9

Fractions	Eluants	Residue left on evaporation of solvent
1-5	Petroleum ether (b.p. 60-80°C)	Oily mass
6-12	Petroleum ether + Benzene(3:2 v/v)	Oily mass
13-17	Petroleum ether + Benzene(1:1 v/v)	Nil
18-23	Benzene	Nil
24-32	Benzene + Chloroform (3:2 v/v)	Shining flakes
33-41	Benzene + Chloroform (1:1 v/v)	Shining flakes
42-47	Chloroform	Nil

Fractions 24-41 on evaporation left a white solid with shining flakes which on repeated crystallisation from methanol-ethyl acetate mixture yielded (1.02%) colourless flakes with constant m.p. 137°C ; $[\alpha]_D^{35}$ - 36°5 (chloroform).
Found: C, 83.81 ; H, 12.02 ; $C_{29}H_{50}O$ requires C, 84.05 ; H, 12.08%.

The compound was fairly soluble in benzene, methanol and ethanol; and readily soluble in chloroform, ethyl ether and ethyl acetate. The compound gave a red colour in the

chloroform layer with sulphuric acid (Salkowski reaction) and a reddish purple colour in the chloroform solution of the compound with concentrated sulphuric acid and acetic anhydride (Liebermann-Burchard reaction). It formed an acetate (6), m.p. 126°C ; $[\alpha]_D^{25}$ - 42.0 (chloroform), (Found: C, 81.49 ; H, 11.46 ; $C_{31}H_{52}O_2$ requires C, 81.57 ; H, 11.40%), a benzoate (6), m.p. 144°C ; $[\alpha]_D^{25}$ - 14.5 (chloroform), (Found: C, 83.28 ; H, 10.48 ; $C_{36}H_{54}O_2$ requires C, 83.39 ; H, 10.42%) and a digitonide (7), m.p. 230°C (decomp.), (Found: C, 61.88 ; H, 8.58 ; $C_{29}H_{50}O.C_{55}H_{90}O_{29}$ requires C, 61.91 ; H, 8.60%).

The identity of the compound with β-sitosterol was further confirmed by mixed melting point and by the formation of a mixed chromatogram with an authentic sample of β-sitosterol.

The details of the preparation of the acetate, benzoate, digitonide derivatives and the formation of a mixed chromatogram of the compound with an authentic sample of β-sitosterol have been described previously (Vide chapter 1, pages 51-53).

AMINO ACID CONTENT OF THE FRUIT COAT:

The proteins from the fruit coat of Litsea consimilis were isolated by extracting with water, 10% aqueous sodium chloride, 0.2% aqueous sodium hydroxide and 80% ethanol. The extracted protein matter was dialysed separately, and the dialysates were hydrolysed with 6N hydrochloric acid. The hydrolysates were then examined by descending paper chromatographic technique for the characterisation of different amino acids present in them. The proteins were found to be made up of histidine, glutamic acid, alanine, tyrosine, valine, phenyl alanine and leucine.

EXPERIMENTAL

The most common methods, generally employed to isolate proteins from plants and seeds, make use of water, neutral saline solution, 70-80% ethanol and very dilute solutions of acids and alkalies (8). No single solvent was, however, found suitable to isolate completely the protein matter from the fruit coat of Litsea consimilis.

Isolation of the protein matter from the fruit coat:

The fruit coat was mechanically removed, powdered and soxhletted with petroleum ether (b.p. 60-80°C). The defatted fruit coat powder was treated successively with water, 10% aqueous sodium chloride, 0.2% aqueous sodium hydroxide and 80% ethanol.

50 gms. of the finely powdered material were mixed with 500 ml of distilled water and stirred for about four

hours by an electrical shaker and then filtered. The residue (No.1) was shaken with 400 ml of 10% aqueous sodium chloride solution, stirred for three hours and filtered. The residue (No.2) was freed from sodium chloride by washing it with distilled water and then stirred with 350 ml of 0.2% aqueous sodium hydroxide solution for three hours and filtered. The residue (No.3) after freeing it from sodium hydroxide was then shaken with 300 ml of 80% ethanol for three hours and filtered. The final residue (No.4) did not give a positive test for nitrogen. Thus the protein matter of the fruit coat was completely extracted by water, 10% aqueous sodium chloride, 0.2% aqueous sodium hydroxide and 80% ethanol.

The water, 10% aqueous sodium chloride, 0.2% aqueous sodium hydroxide and 80% ethanol soluble protein matter was dialysed separately for 4-5 days, and the dialysates were evaporated to dryness over water bath. The protein matter extracted with water, 10% aqueous sodium chloride, 0.2% aqueous sodium hydroxide and 80% ethanol weighed 2.10, 2.00, 1.70 and 1.20 gms. respectively.

Hydrolysis of the protein matter:

0.2 - 0.5 gms. of the protein matter from each extract was separately refluxed with 25 ml of 6N hydrochloric acid for 10-12 hours on a sand bath. The hydrolysate was, then, taken in a porcelain dish and most of the acid was removed by evaporation over a water bath. Evaporation was continued

repeatedly by adding distilled water from time to time till the vapours did not give white fumes with ammonia. The last traces of the acid were removed by keeping the hydrolysate in a vacuum desiccator containing a small amount of solid potassium hydroxide. Each of the hydrolysates was then extracted with absolute ethanol.

Characterization of amino acidsin protein hydrolysates:

Amino acids were characterized by employing descending paper chromatographic technique (9). 0.004-0.008 ml of protein hydrolysates and 0.002 ml of 0.1% solutions of reference amino acids were chromatographed together on Whatman filter paper No. 1 (sheets 48 x 24 cm.). The chromatogram was equilibrated with the lower layer of n-butanol-acetic acid-water (10) (4:1:5 v/v) for 24 hours and was developed by the upper layer (solvent phase) of the above mixture for 20 hours at the room temperature. The chromatogram was then taken out of the chamber, and the boundary of the solvent front was marked. After drying it in the air overnight, it was developed again by the same solvent phase till the solvent front reached the marked boundary line. Thus the multiple-development process was repeated three times in order to achieve a better and distinct separation of amino acids. After developing and drying, the chromatogram was sprayed with 0.1% solution of ninhydrin in acetone. Finally it was kept in an electric oven at 55°C for 5-10 minutes, and the amino acids were

located as pink spots.

The R_f values of a number of reference amino acids were also determined and are recorded in table no. 10

TABLE No. 10

R_f values of amino acids at 30°C

Amino acids	R_f value
Cystine	0.050
Arginine	0.066
Lysine	0.071
Histidine	0.085
Aspartic acid	0.120
Serine	0.140
Glutamic acid	0.180
Proline	0.240
Alanine	0.260
Tyrosine	0.330
Valine	0.480
Tryptophan	0.500
Phenyl alanine	0.560
Leucine	0.700

The total number of amino acids present in the above four protein fractions were also confirmed by analysing chromatographically a mixture of four hydrolysates taken together.

The amino acids present in the protein fractions

isolated from the fruit coat of Litsea consimilis have been recorded in table no. 11.

TABLE No. 11

Amino acids present in the protein hydrolysates of the fruit coat of Litsea consimilis

Serial number	Protein fractions	Amino acids identified
1.	Soluble in water	Histidine, Glutamic acid, Alanine, Tyrosine, valine and Leucine.
2.	Soluble in 10% aqueous sodium chloride solution	Histidine, Glutamic acid, Alanine, Tyrosine, Valine and Leucine.
3.	Soluble in 0.2% aqueous sodium hydroxide solution	Histidine, Glutamic acid, Alanine, Tyrosine, Valine, Phenyl alanine and Leucine.
4.	Soluble in 80% ethanol	Histidine, Glutamic acid and Alanine.
5.	Mixture of the above four protein fractions	Histidine, Glutamic acid, Alanine, Tyrosine, Valine, Phenyl alanine and Leucine.

The proteins of the fruit coat of Litsea consimilis are, therefore, made up of histidine, glutamic acid, alanine, tyrosine, valine, phenyl alanine and leucine.

It is interesting to note that the seeds and the fruit coat of this plant contain the same amino acids. The seeds (11), however, contain cystine and proline in addition.

C H A P T E R III

CHEMICAL EXAMINATION OF THE BARK OF LITSEA CONSIMILIS

CHEMICAL EXAMINATION OF THE BARK OIL

From the bark of Litsea consimilis, a yellow coloured oil has been obtained in 2.2% yield. On chemical examination, it has been found to contain the glycerides of capric, lauric and oleic acids and the percentage compesition of these acids has been determined as capric, 2.15%; lauric, 61.04% and oleic acids 36.81%.

EXPERIMENTAL

The bark was dried, powdered and extracted with petroleum ether (b.p. 60-80°C), when 2.2% of oil of yellowish colour was obtained. The oil was freed of essential oils as far as possible by heating in Vacuo and possessed the following characteristics: yeild, 2.2%; specific gravity at 35°C, 0.9071; refractive index at 40°C, 1.5768; acid value, 6.3; saponification value, 242.4; saponification equivalent, 231.43; ester value, 236.1; iodine value (Hanus), 33.1; acetyl value, 21.26; Hehner value, 96.91; Reichert-Meissl value, 1.76; Polenske value, 10.6; unsaponifiable matter, 3.01%.

The above given physical constants were determined as (Vide chapter 1, pages 17-28).

(a) Determination of percentage yield:

Weight of the bark extracted	= 1800 gms.
Weight of the conical flask	= 54.6424 gms.
Weight of the conical flask + oil	= 94.2424 gms.

∴ Weight of the oil = 39.6 gms.

∴ % yield = $\frac{39.6 \times 100}{1800}$

= 2.2%

(b) Specific gravity:

Weight of the specific gravity bottle = 6.8548 gms.

Weight of the specific gravity bottle + water =17.5769 gms.

Weight of the specific gravity bottle + oil =16.5818 gms.

∴ Weight of the water taken =10.7221 gms.

Weight of the oil taken = 9.7270 gms.

∴ Specific gravity of the oil at 35°C = $\frac{\text{Weight of the oil taken}}{\text{Weight of the water taken}}$

= $\frac{9.7270}{10.7221}$

= 0.9071

(c) Refractive index:

By Abbe's refractometer

Refractive index at 40°C = 1.5768

(d) Determination of the acid value:

Weight of the oil taken = 2.5823 gms.

Strength of Na OH solution = N/10

Volume of Na OH solution used = 2.9 ml

∴ Acid value = $\frac{2.9 \times 56.1}{10 \times 2.5823}$

= 6.3

(e) Determination of the saponification value:

Weight of the oil taken in conical flask 'A' = 1.9777 gms.

Volume of approximately N/2 ethanolic potassium hydroxide solution taken in each flask = 25 ml

Volume of N/4 hydrochloric acid used:

(i) for blank 'B' = 51.4 ml

(ii) for oil 'A' = 17.2 ml

∴ Volume of N/4 hydrochloric acid required for the saponification of oil = 51.4-17.2 ml

= 34.2 ml

∴ Saponification value $= \frac{34.2 \times 56.1 \times 1000}{4 \times 1000 \times 1.9777}$

= 242.4

(f) Determination of the saponification equivalent:

Saponification equivalent $= \frac{56100}{\text{saponification value}}$

$= \frac{56100}{242.4}$

= 231.43

(g) Determination of the ester value:

Ester value = Saponification value-Acid value

= 242.4-6.3

= 236.1

(h) Determination of the iodine value:

Weight of the oil taken in iodine flask 'A' = 0.3837 gms.

Volume of the Hanus solution taken in each flask = 25 ml

Strength of sodium thiosulphate solution = N/10

Volume of sodium thiosulphate solution used:

(i) for blank 'B' = 53.7 ml

(ii) for oil 'A' = 43.7 ml

∴ Volume of thiosulphate used:

(Volume of N/10 iodine reacted)

= 53.7 - 43.7 ml

= 10 ml

∴ Iodine value = $\frac{127 \times 10 \times 100}{10 \times 1000 \times 0.3837}$

= 33.1

(i) Determination of the acetyl value:

Weight of the acetylated oil in flask 'A' = 1.9419 gms.

Volume of approximately N/2 ethanolic potassium hydroxide solution taken in each flask = 25 ml

Volume of N/4 hydrochloric acid used:

(i) for blank 'B' = 51.4 ml

(ii) for oil 'A' = 15.4 ml

Volume of N/4 hydrochloric acid consumed = 51.4 - 15.4 ml

= 36 ml

∴ Saponification value of the acetylated oil = $\frac{36 \times 56.1 \times 1000}{4 \times 1000 \times 1.9419}$

= 260.0

Acetyl value = $\frac{\text{(Saponification value of the acetylated oil - saponification value of the oil)}}{1-(0.00075 \times \text{saponification value of the oil}}$

= $\frac{260.0 - 242.4}{1-(0.00075 \times 242.4)}$

= 21.26

(j) Determination of Hehner value:

Weight of the oil taken for saponification = 0.6190 gms.

Weight of the insoluble acids present = 0.600 gms.

Hehner value = $\frac{\text{Weight of the insoluble acids} \times 100}{\text{Weight of the oil taken for saponification}}$

= $\frac{0.600 \times 100}{0.6190}$

= 96.91%

(k) Determination of Reichert-Meissl value:

Weight of the oil taken = 5 gms.

Strength of sodium hydroxide solution = N/10

Volume of sodium hydroxide solution used for the oil = 2.6 ml

Volume of sodium hydroxide solution used for the blank = 1.0 ml

Reichert-Meissl value = (ml of alkali solution used by oil-ml of alkali solution used by blank) x normality x 1.1 x 10

= $(2.6-1.0) \times \frac{1}{10} \times \frac{1.1}{1} \times \frac{10}{1}$

= 1.76

(l) Determination of Polenske value:

Volume of alkali used = 10.6 ml

Polenske value = ml of alkali used x normality x 10

= $10.6 \times \frac{1}{10} \times 10$

= 10.6

(m) Determination of the unsaponifiable matter:

Weight of the oil taken for saponification = 0.3380 gms.

Weight of the unsaponifiable matter = 0.0102 gms.

$\therefore$ % of unsaponiable matter $= \frac{0.0102 \times 100}{0.3380}$

$= 3.01\%$

Liberation of the fatty acids:

A requisite amount of the oil was saponified with ethanolic potassium hydroxide. The ethanol-free aqueous solution of the soap was thoroughly extracted with ethyl ether to remove the unsaponifiable matter. The soap was treated with dilute sulphuric acid and the liberated fatty acids were extracted and examined in the usual manner. They possessed the following characteristics: iodine value (Hanus), 34.3; mean molecular weight, 230.7.

The mixed fatty acids were separated into solid (62.4%; iodine value, 26.1; mean molecular weight, 220.2) and liquid acids (37.6%; iodine value, 42.4; mean molecular weight, 237.5) using Hilditch's modification of Twitchell's lead salt method (1) (Vide chapter 1; pages, 29-32). The solid and liquid acids were separately converted into methyl esters (2) and then fractionally distilled under reduced pressure. The weight, iodine value, saponification equivalent, mean molecular weights of different fractions (S_1 - S_4, L_1 - L_4 and the residues) were determined, and fatty acid compositions of each of the fraction were calculated in the usual manner (Vide chapter 1; pages, 36-44). The results are given in table nos. 12 and 13.

TABLE No. 12

DISTILLATION OF METHYL ESTERS OF SOLID ACIDS AT 8mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.) Laurate	Oleate
S_1	70-80	2.56	14.4	228.1	228.2	2.13	0.43
S_2	80-90	3.32	16.6	229.4	229.8	2.68	0.64
S_3	90-100	2.23	24.6	237.2	237.2	1.59	0.64
S_4	100-110	2.42	36.5	246.7	247.1	1.39	1.03
Residue	-	1.23	40.8	250.2	250.4	0.65	0.58
Loss	-	0.04	-	-	-	-	-
Total	-	11.80	-	-	-	8.44	3.32
% as ester	-	-	-	-	-	71.77	28.23
% as acid	-	-	-	-	-	71.40	28.60

TABLE No. 13

DISTILLATION OF METHYL ESTERS OF LIQUID ACIDS AT 8mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.) Caprate	Laurate	Oleate
L_1	100-110	1.71	1.6	209.4	210.1	0.25	1.43	0.03
L_2	110-120	1.56	1.8	211.5	212.6	0.20	1.33	0.03
L_3	120-130	1.23	56.7	263.6	264.2	-	0.42	0.81
L_4	130-140	2.42	70.8	278.4	279.1	-	0.43	1.99
Residue	-	1.12	78.6	284.1	284.8	-	0.10	1.02
Loss	-	0.16	-	-	-	-	-	-
Total	-	8.20	-	-	-	0.45	3.71	3.88
% as ester	-	-	-	-	-	5.60	46.14	48.26
% as acid	-	-	-	-	-	5.29	45.90	48.81

TABLE No.20.

PROPERTIES OF THE VARNISH:

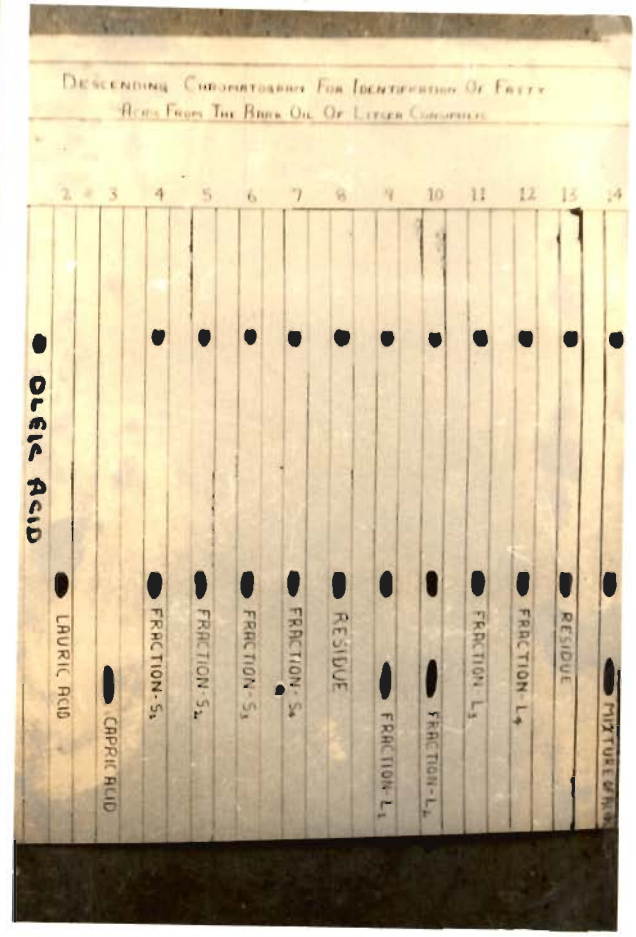
Alkyd resin varnishes	Gloss % (60°)	Scratch hardness	Mandrel Bend ¼"	Drying time	Water immersion test after 24 hours.	Scratch just after water immersion	Effect of 2% H_2SO_4 solution in 5 minutes	Resistance against 5% aqueous NaOH solution at 25°C	Xylene action test in 5 minutes
A	100	1000 gms.	Pass	(i) 9.30 hrs. (ii) 12.00 hrs. (iii) 17.00 hrs.	Slight whitening	400 gms.	7 minutes	Very slight whitening	No effect
B	100	1000 gms.	Pass	(i) 1.50 hrs. (ii) 3.00 hrs. (iii) 4.00 hrs.	Very slight whitening	800 gms.	7 minutes	Slight whitening	No effect
C	100	1000 gms.	Pass	(i) 6.30 hrs. (ii) 10.00 hrs. (iii) 16.00 hrs.	Very slight whitening	400 gms.	5 minutes	Very slight whitening	No effect
D	90	1000 gms.	Pass	30 minutes at 120°C	No effect	600 gms.	10 minutes	Pronounced whitening	No effect
E	80	1000 gms.	Pass	30 minutes at 120°C	No effect	400 gms.	17 minutes	Pronounced whitening	No effect

where: (i) set to touch (ii) surface dry and (iii) Hard dry.

TABLE No. 13

DISTILLATION OF METHYL ESTERS OF LIQUID ACIDS AT 8mm

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.) Caprate	Laurate	Oleate
L_1	100-110	1.71	1.6	209.4	210.1	0.25	1.43	0.03
L_2	110-120	1.56	1.8	211.5	212.6	0.20	1.33	0.03
L_3	120-130	1.23	56.7	263.6	264.2	-	0.42	0.81
L_4	130-140	2.42	70.8	278.4	279.1	-	0.43	1.99
Residue	-	1.12	78.6	284.1	284.8	-	0.10	1.02
Loss	-	0.16	-	-	-	-	-	-
Total	-	8.20	-	-	-	0.45	3.71	3.88
% as ester	-	-	-	-	-	5.60	46.14	48.26
% as acid	-	-	-	-	-	5.29	45.90	48.81



CHARACTERIZATION OF FATTY ACIDS BY DESCENDING PAPER CHROMATOGRAPHY.

Characterization of acids

The ester fractions S_1 - S_4 and the residue were saponified and the liberated acids on oxidation (3) with dilute ice cold potassium permanganate solution, yielded a white crystalline solid identified as dihydroxystearic acid (m.p. 130°C; mol. wt., 315). This confirmed the presence of oleic acid in these fractions and the residue. The unoxidized portion of the acids from these fractions and the residue were extracted with ethyl ether, and the residue left on removal of ether gave a white solid, recrystallised from 70% ethanol and identified as lauric acid (m.p. and mixed m.p. 43.5°C).

Similarly the acids, liberated from ester fractions L_1 - L_4 and the residue on oxidation with cold dilute potassium permanganate solution gave dihydroxystearic acid, thereby confirming the presence of oleic acid in these fractions and the residue. The unoxidized portion of the acids from L_1 - L_2 was resolved into two white crystalline solids with 50% and 70% ethanol. These were characterized as capric acid (m.p. and mixed m.p. 30°C) and lauric acid (m.p. and mixed m.p. 43.5°C). The unoxidized portion of the acids from L_3, L_4 and the residue gave a solid which was crystallised from 70% ethanol and was characterized as lauric acid (m.p. and mixed m.p. 43.5°C). The fatty acids liberated from different ester fractions were also confirmed by reversed phase descending paper chromatography (4) (Vide chapter 1;

pages, 454, 7).

The bark oils generally consist of oleic and palmitic acids as the major component along with smaller amounts of stearic acid (5). My results, on the other hand, go to show that the bark oil of Litsea consimilis contains lauric acid as the major component with a little capric acid instead of the often found stearic acid.

CHEMICAL EXAMINATION OF THE BARK

β-amyrin and β-sitosterol have been isolated from the bark of Litsea consimilis by extracting the bark with petroleum ether (b.p. 60-80°C) followed by chromatography of the extract over Brockmann alumina. These compounds have been identified by their physical and chemical properties and also by preparing their derivatives.

EXPERIMENTAL

The powdered bark (sun dried) (200 gms.) was soxhletted with petroleum ether (b.p. 60-80°C). The oily petroleum ether concentrate was saponified with ethanolic potassium hydroxide solution. The unsaponifiable matter (6 gms.) was separated and subjected to chromatography over Brockmann alumina using petroleum ether, benzene, chloroform and their mixtures as eluants as shown in table no. 14. Eluants were collected in fractions of 30-35 ml each and evaporated to dryness.

TABLE No. 14

Fractions	Eluants	Residue left on evaporation of solvent
1-8	Petroleum ether (b.p. 60-80°C)	Oily mass
9-15	Petroleum ether + Benzene (3:2 v/v)	White solid
16-26	Petroleum ether + Benzene (1:1 v/v)	White solid
27-32	Benzene	Nil
33-40	Benzene + chloroform (3:2 v/v)	Shining flakes
41-48	Benzene + chloroform (1:1 v/v)	Shining flakes
49-54	Chloroform	Nil

Fractions 9-26 on evaporation left a white solid which crystallised from chloroform-methanol mixture in needles, yield (2.2 gms.); m.p. 199-200°C; $[\alpha]_D^{25}$ + 87.0° (chloroform).

Found: C, 84.38; H, 11.85; $C_{30}H_{50}O$ requires C, 84.51; H, 11.74%.

The substance gave a positive Liebermann-Burchard test with chloroform and concentrated sulphuric acid for a triterpene. The acetate, benzoate and p-nitrobenzoate derivatives of the compound were prepared.

Preparation of the acetate (6):

To a solution of the compound (0.4 gms.) in 1 ml of dry pyridine, was added 4 ml of acetic anhydride. After refluxing over water bath for about one hour, the solution was cooled and kept in a refrigerator overnight. A white solid separated, which on recrystallisation from methanol-ethyl acetate mixture afforded colourless crystals, m.p. 237-238°C; $[\alpha]_D^{25}$ + 80.0° (chloroform). It produced a yellow colouration with tetranitromethane.

Found: C, 81.84; H, 11.24; $C_{32}H_{52}O_2$ requires C, 82.06; H, 11.11%.

Preparation of the benzoate (6):

A solution of the compound (0.4 gms.) in dry pyridine (4 ml) was treated with benzoyl chloride (4 ml). The reaction mixture was refluxed for three hours over water bath. On cooling and then keeping the contents overnight in a refrigerator, gave a product which was crystallised from

chloroform-methanol mixture, m.p. 231-232°C; $[\alpha]_D^{25}$ + 101.°1 (chloroform).

Found: C, 83.58; H, 10.30; $C_{37}H_{54}O_2$ requires C, 83.77; H, 10.18%.

Preparation of the p-nitrobenzoate (6):

To a solution of the compound (0.3 gms.) in dry pyridine (3 ml) was added p-nitrobenzoyl chloride (0.2 gms.). After heating on a steam bath for 3 hours, the product was recovered and crystallised from 95% ethanol, m.p. 257-258°C; $[\alpha]_D^{25}$ + 95.°6 (chloroform).

Found: C, 76.84; H, 9.36; N, 2.51; $C_{37}H_{53}NO_4$ requires C, 77.22; H, 9.22; N, 2.43%.

The identity of the triterpene with β-amyrin has been further confirmed by mixed melting point and by the formation of a mixed chromatogram (Vide chapter I, pages 52-53) of the white solid with an authentic sample of β-amyrin.

Fractions 33-48 on evaporation left a white solid with shining flakes which on repeated crystallisation from methanol-ethyl acetate mixture yielded (2.7 gms.) colourless flakes with constant m.p. 136-137°C; $[\alpha]_D^{25}$ - 36.°5 (chloroform).

Found: C, 83.70; H, 12.21; $C_{29}H_{50}O$ requires C, 84.05; H, 12.08%.

The compound was fairly soluble in benzene, methanol and ethanol; and readily soluble in chloroform, ethyl ether and ethyl acetate. It produced a red colour in the chloroform layer with sulphuric acid (Salkowski reaction); and a reddish

purple colour in the chloroform solution of the compound with concentrated sulphuric acid and acetic anhydride (Liebermann-Burchard reaction). It formed an acetate (7), m.p. 126°C, $[\alpha]_D^{25}$ - 42.0° (chloroform), (Found: C, 81.46; H, 11.51; $C_{31}H_{52}O_2$ requires C, 81.57; H, 11.40%), a benzoate (7), m.p. 144°C, $[\alpha]_D^{25}$ - 14.5°. (chloroform), (Found: C, 83.18; H, 10.46; $C_{36}H_{54}O_2$ requires C, 83.39; H, 10.42%) and a digitonide (8), m.p. 230°C, (Found: C, 61.85; H, 8.61; $C_{29}H_{50}O.C_{55}H_{90}O_{29}$ requires C, 61.91; H, 8.60%).

The identity of the compound with β-sitosterol was further confirmed by mixed melting point and by the formation of a mixed chromatogram (9) of the compound with an authentic sample of β-sitosterol.

The details of the preparation of acetate, benzoate, digitonide derivatives and the formation of a mixed chromatogram of the compound with an authentic sample of β-sitosterol have been described previously (Vide chapter 1; pages 51-53).

CHAPTER IV

CHEMICAL EXAMINATION OF THE ALKALOIDAL CONTENT OF THE BARK AND SEEDS OF LITSEA CONSIMILIS

EXAMINATION OF THE ALKALOIDAL CONTENT OF THE BARK:

Laurotetanine was isolated in 0.085% yield from the bark of Litsea consimilis. The alkaloid was characterized by its physical and chemical properties and by preparing its different derivatives. The seeds leaves, roots and barks of a number of lauraceae plants, particularly those of Litsea, Neolitsea and Actinodaphne genera, viz., Litsea chrysocoma Blume, Litsea citrata, Litsea cubeda, Litsea japonica, Litsea chinensis, Litsea stocksii, Neolitsea sericea and Actinodaphne hookeri etc. have been reported to contain small amounts of alkaloids, which have been investigated by a number of workers (1-14). The alkaloidal content of the bark and seeds of Litsea consimilis was isolated and chemically examined in order to find out whether the alkaloidal contents were same or different from those examined by earlier workers.

EXPERIMENTAL

The powdered, air dried bark (5 kg.) was soxhletted with petroleum ether (b.p. 60-80°C) for 48 hours. The petroleum ether extract was concentrated to a small bulk (10 ml) and chromatographed over Brockmann alumina. The column was subsequently eluted with petroleum ether, benzene, chloroform and their mixtures. β-sitosterol and β-amyrin were isolated and characterized (chapter 3, page 95-98). The defatted bark left after extraction with petroleum ether was soxhletted with 25% ammonium hydroxide

and chloroform (6 litres) for 7 days. The dark brown chloroform extract was concentrated to 200 ml, washed with water (4 x 100 ml) and extracted with 4 N hydrochloric acid (4 x 100 ml). The acidic layer was basified with ammonium hydroxide solution. The precipitate separated was taken up in chloroform (5 x 50 ml) and the brown chloroform solution was washed with water, dried over anhydrous sodium sulphate and distilled till the volume was reduced to 10 ml. The residue obtained from chloroform solution upon evaporation did not crystallise from any organic solvent , but gave excellent tests for alkaloids with Dragendorff's and Mayer's reagents proving that this residue contained alkaloids. It was dissolved in minimum quantity of chloroform (5 ml) and chromatographed over acid washed Brockmann alumina using benzene, chlorform, methanol and their mixtures as eluants as shown in the table no. 14. The eluants were collected in fractions of 25-30 ml each and evaporated to dryness.

TABLE No. 14

Fractions	Eluants	Residue left on evaporation of solvents.
1- 6	Benzene	Oily mass
7-12	Benzene + Chloroform(5:1 v/v)	Nil
13-18	Benzene + Chloroform(3:2 v/v)	Nil
19-24	Benzene + Chloroform(1:1 v/v)	Nil
25-30	Chloroform	Nil
31-40	Chloroform+Methanol(99:1 v/v)	Colourless solid
41-50	Chloroform+Methanol(98:2 v/v)	Colourless solid
51-60	Chloroform+Methanol(95:5 v/v)	Colourless solid
61-70	Chloroform+Methanol(90:10 v/v)	Nil

The fractions 31-60 on evaporation of the solvent gave a colourless solid. The solids obtained from these fractions were combined, and on repeated crystallisation from aqueous acetone gave colourless needles, m.p. 125°C; yield (4.25 gms.).

Found: C, 69.62; H, 6.49; N, 4.12; $C_{19} H_{21} O_4 N$ requires C, 69.72; H, 6.42; N, 4.20%.

The homogeneity of the base was confirmed by descending paper chromatography using Whatman filter paper No. 1, upper layer of Butanol-Formic acid-Water (12:1:7 v/v) as the developer and Dragendorff's reagent as the spray reagent. The alkaloid gave a single spot.

The molecular weight of the base was determined by chloroplatinate method and was found to be 327. The molecular formula of the alkaloid is, therefore, $C_{19} H_{21} O_4 N$.

The alkaloid was fairly soluble in chloroform, acetone and ethanol and relatively less soluble in benzene; while sparingly soluble in ether. It gave a dark brown colour with ferric chloride solution, indicating the phenolic nature of the alkaloid. It gave all the general colour and precipitation tests for alkaloids as shown in table nos. 15 and 16.

TABLE No. 15

COLOUR REACTIONS OF THE BASE:

Serial number	Reagent	Colour with the base
1.	Sulphomolybdic acid (Frohde's reagent)	Indigo → brown
2.	Sulphovanadic acid (Mandelin's reagent)	Indigo → yellow
3.	Nitric acid and sulphuric acid (Erdmann's reagent)	Blue → brown → violet
4.	Concentrated sulphuric acid	Rose red
5.	Concentrated nitric acid	Brown

TABLE No. 16

PRECIPITATION TESTS OF THE BASE:

Serial number	Reagent	Colour of the precipitate obtained with an aqueous solution of the hydrochloride of the base
1.	Potassium bismuth iodide (Dragendorff's reagent)	Orange brown
2.	Potassium tri-iodide (Wagner's reagent)	Brown
3.	Potassium mercuric iodide (Mayer's reagent)	Light yellow
4.	Auric chloride	Light yellow
5.	Phosphomolybdic acid (Sonnenschein's reagent)	Yellow
6.	Phosphotungstic acid (Scheibler's reagent)	White
7.	Picric acid (Hager's reagent)	Yellow
8.	Ammonia reineckate	Light brown
9.	Sodium carbonate	Light yellow

The alkaloid was identified as laurotetanine by preparing the following derivatives:

Preparation of the hydrochloride:

The hydrochloride of the base was prepared by passing dry hydrochloric acid gas into an ethanolic solution of the alkaloid. Ethanol was allowed to evaporate off first on water bath and then in a vacuum desiccator to give white needles, m.p. 245°C

Found: Cl, 9.67; $C_{19} H_{21} O_4 N.HCl$ requires Cl, 9.76%.

Preparation of the oxalate:

5 ml of ethanolic solution of oxalic acid (0.1 gms.) were added to 5 ml of ethanolic solution of the alkaloid (0.1 gms.). The mixture was warmed and stirred on a water bath for few minutes. On cooling, a white solid separated which was crystallised from ethanol, m.p. 233°C.

Preparation of the picrate:

0.05 gms. of the hydrochloride of the base was dissolved in hot water and an aqueous saturated solution of picric acid was added. A light yellow precipitate separated which was washed with water, dissolved in ethanol and kept overnight when small yellow needles separated, m.p. 147°C.

Preparation of the dibenzoyl derivative:

Benzoyl chloride (2 ml) was added to a solution of the base (1 gms.) in a 5% aqueous potassium hydroxide (10 ml). The above solution was taken in a stoppered flask. The contents were vigorously shaken for about 15 minutes, a colourless solid separated which was filtered, washed with distilled water and crystallised from acetone, m.p. 169°C.

Preparation of the thiocarbamide:

1 gms. of the alkaloid, 1.5 ml carbon disulphide and 10 ml of absolute alcohol were taken in a 100 ml round bottomed quickfit flask fitted with a condenser.

The contents were refluxed for about ten hours on water bath and then solidified on cooling. Excess of dilute hydrochloric acid was then added. The contents were shaken vigorously and filtered to remove any unreacted alkaloid. The residue was dried in a vacuum desiccator and then crystallised from hot ethanol, m.p. 154°C.

EXHAUSTIVE METHYLATION OF THE ALKALOID:

Preparation of dimethyl laurotetanine methiodide:

0.5 gms. of the alkaloid was dissolved in 5 ml of methyl alcohol, to this an ethereal solution of diazomethane was added. The contents were kept overnight in a corked flask. Next day ether and the excess of methyl alcohol were evaporated off. To the residue, sodium hydroxide solution and excess of methyl iodide in methyl alcohol were added, the contents were warmed and stirred. On concentrating the solution, fine colourless needles of dimethyl laurotetanine methiodide separated, m.p. 209°C.

Oxidation of the base with alkaline potassium permanganate solution:

1 gms. of the alkaloid and 0.1 gms. of potassium hydroxide were taken in a 250 ml round bottomed flask fitted with a long air condenser. A 10% potassium permanganate solution was added in portions of 10 ml, till there was no more decolouration of potassium permanganate solution by the contents. The contents

were heated over a sand bath for half an hour, 5 ml more of 10% potassium permanganate solution was then added and heated further for half an hour.

After the reaction was over, concentrated hydrochloric acid was added to destroy excess of potassium permanganate. The colourless solution thus obtained was concentrated on water bath and then dried in a vacuum desiccator. The dry solid mass obtained was dissolved in hot ethanol. After evaporating off ethanol slowly, colourless needles separated, m.p. 165°C. Found: C, 48.92; H, 3.68; $C_{11} H_{10} O_8$ requires C, 48.88; H, 3.70%.

The molecular weight of the acid was determined by titrating its solution against a standard solution of sodium hydroxide, and was found to be 270. Thus the molecular formula of the acid was determined as $C_{11} H_{10} O_8$. The acid was characterized as 1:2 dimethoxy benzene 3:4:5 tri-carboxylic acid.

EXAMINATION OF THE ALKALOIDAL CONTENT OF THE SEEDS:

A phenolic alkaloid (m.p. 158°C) has been isolated from defatted seeds of Litsea consimilis.

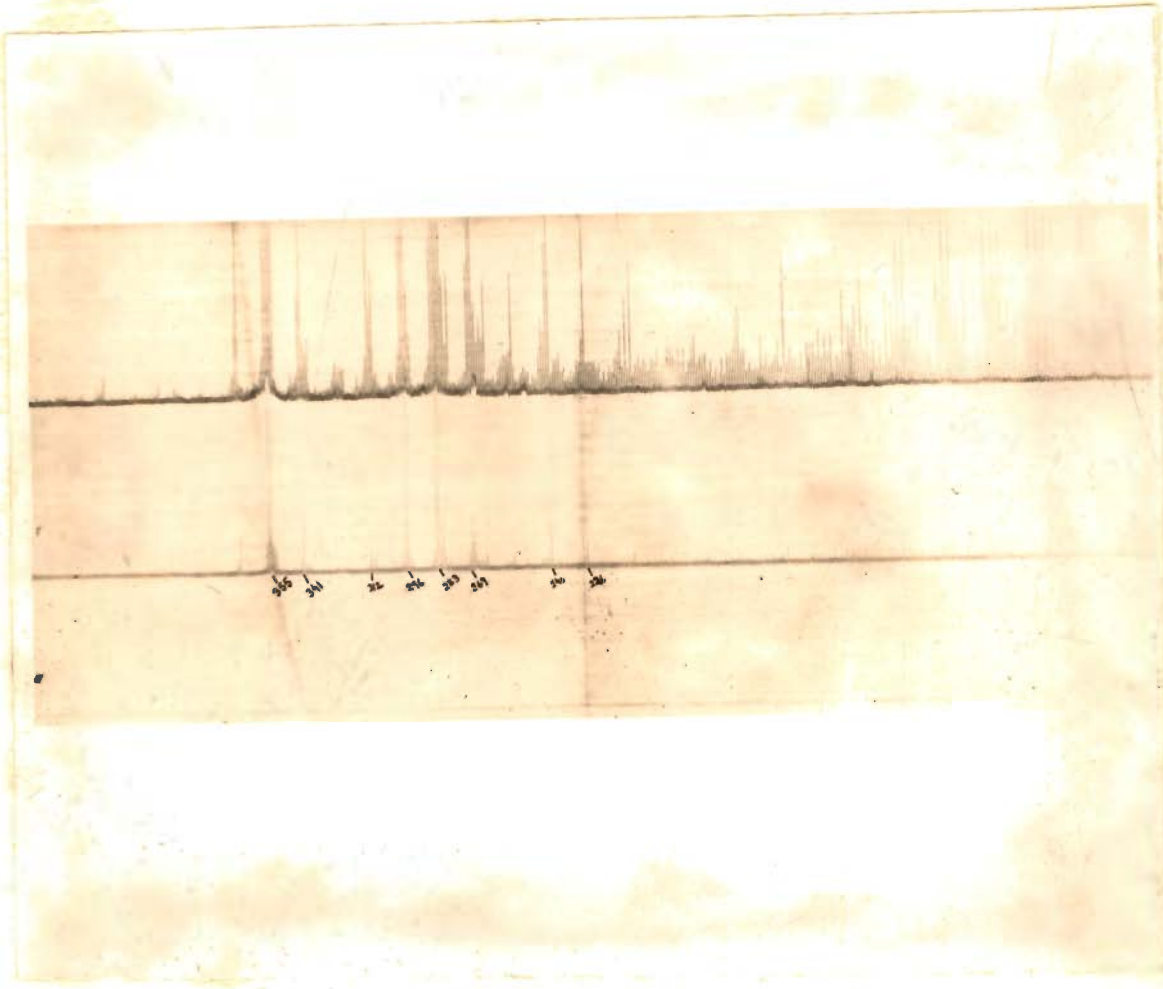
EXPERIMENTAL

The powdered, air dried seeds (2 kg.) were soxhletted with petroleum ether (b.p. 60-80°C) for 48 hours.

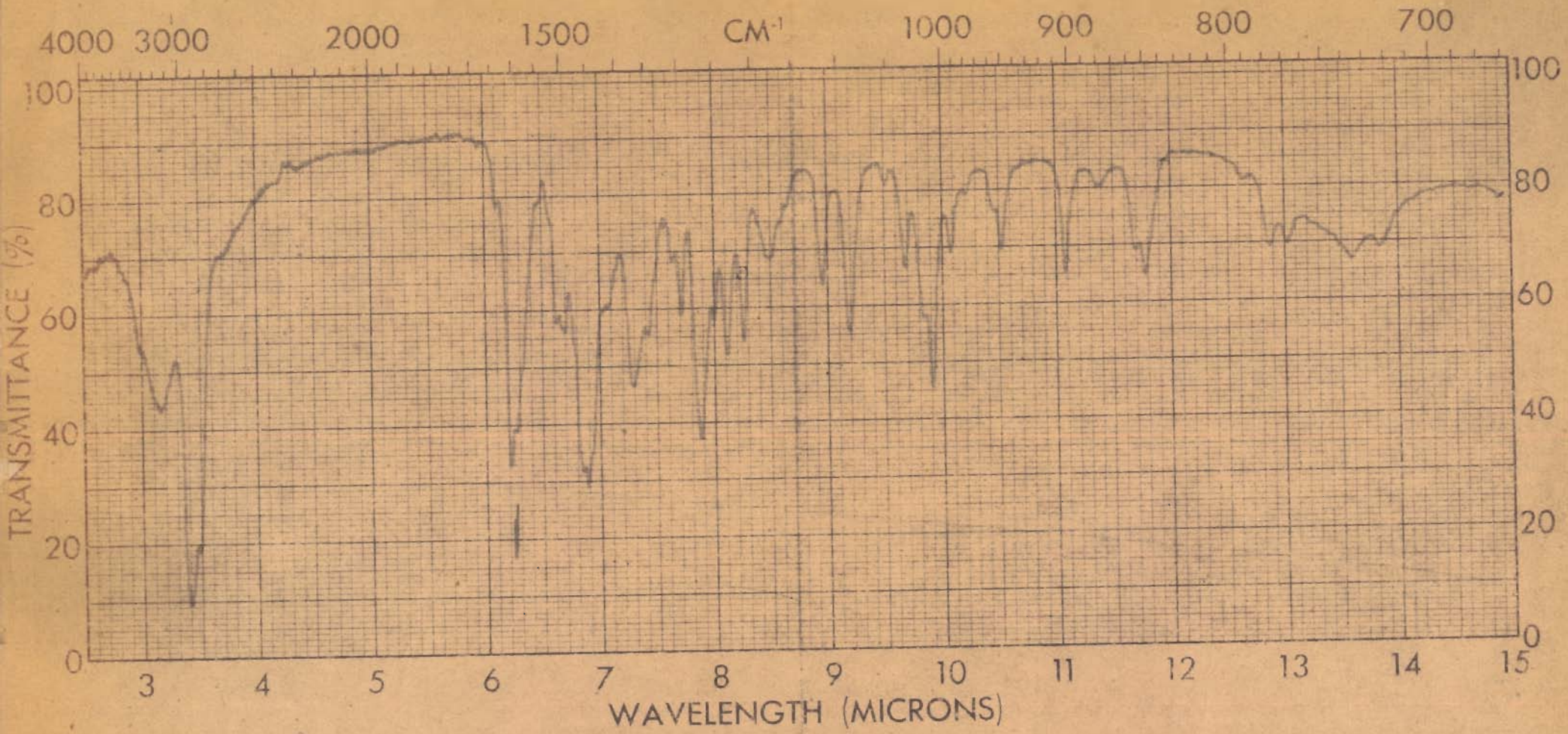
The defatted seeds were treated with 4 litres of rectified spirit containing 40 ml of glacial acetic acid. After keeping the powder for 12 days with occasional shaking, it was filtered and the filtrate gave a positive test for alkaloids with Dragendorff's and Mayer's reagents. The powdered seeds were again treated with 2.5 litres of rectified spirit containing 20 ml of glacial acetic acid, kept for about 8 days with occasional shaking and filtered. The filtrate was tested for the presence of alkaloids. The powdered seeds were treated again with rectified spirit and glacial acetic acid till the filtrate did not give a positive test for alkaloids. All the filtrates were combined and the solvent removed under reduced pressure. The ethanol extract was further concentrated to about 30 ml and kept in a vacuum desiccator overnight. The concentrate was basified with ammonia (0.88 density) by keeping the concentrate in a freezing mixture. The precipitate separated was extracted several times with ether till the ethereal extract did not give a positive test for alkaloids. The ethereal extracts were combined and dried over anhydrous sodium sulphate. On concentratin the extract, a tarry residue was obtained, which was extracted several times with chloroform till the chloroformic extract did not give a positive test for

alkaloids. The chloroformic extracts were combined, dried over anhydrous sodium sulphate, filtered and concentrated. The concentrate was kept overnight in a vacuum desiccator. It was treated with acetic anhydride (5 ml) and dry pyridine (0.5 ml) and kept overnight in a refrigerator in a tightly corked flask. The contents were filtered and the filtrate was basified with sodium bicarbonate at pH 8.5. The precipitate was extracted several times with ether till the ethereal extract no longer gave a positive test for alkaloids. The ethereal extracts were combined, dried over anhydrous sodium sulphate and filtered. On concentrating, the filtrate left a gummy mass which was kept overnight in a vacuum desiccator to remove excess of pyridine. The gummy mass was dissolved in minimum amount of chloroform and chromatographed over a column of acid washed chromatographic alumina, using petroleum ether, benzene, chloroform, methanol and their mixtures as eluants. The eluants were collected in fractions of 25-30 ml each and evaporated to dryness. The fractions obtained from chloroform-methanol mixtures (99:1 and 98:2 v/v) left colourless crystals on evaporation, which on repeated crystallisation from methanol gave colourless prisms, m.p. 158°C; yield (0.4 gms.).

Found: C, 71.06; H, 6.96; N, 4.02; $C_{21}H_{25}O_4N$ requires C, 70.98; H, 7.04; N, 3.94%.



MASS SPECTRUM OF THE ALKALOID.



SPECTRUM NO.	ORIGIN A-B Ray	LEGEND	REMARKS Polystyrene Conventional 6-3-64
SAMPLE LX		1.	
	PURITY	2.	
	PHASE Nujol	DATE 2-10-65	

SPECTRUM NO.
SAMPLE

The homogeneity of the base was confirmed by descending paper chromatography using Whatman filter paper No.1, upper layer of butanol-formic acid water (12:1:7 v/v) as the developer and Dragendorff's reagent as the spray reagent. The alkaloid gave a single spot.

The molecular weight of the base was determined by mass spectra (as shown in the diagram) and was found to be 355. The molecular formula of the alkaloid may, therefore, be $C_{21} H_{25} O_4 N$.

The infra red spectrum of the alkaloid shows the presence of a phenolic group which is also confirmed by an intense brown colouration given by the alkaloid with ferric chloride. The alkaloid was fairly soluble in methanol and ether and sparingly soluble in chloroform.

The alkaloid could not be identified as the amount of the material was very small.

CHAPTER V

INDUSTRIAL USE OF THE SEED OIL OF LITSEA CONSIMILIS

USE OF THE SEED OIL OF LITSEA CONSIMILIS IN NON-DRYING OIL MODIFIED ALKYD RESINS:

A number of reaction products of polyhydric alcohols and polybasic acids are known either as alkyds or glyptals. The fundamental basis for these resins is the product from the direct interaction of glycerol and phthalic anhydride or phthalic acid. This product is of little value in itself, and it is only when modifying ingredients are introduced that valuable synthetic resins are produced.

Kienle and Hovey (1); Carothers; Barry, Drummond and Morrell; Bradley; Savard and Diner; Honel; Bozza; Schlenkert (2); Wornum (3); Houwink and Klaassens (4); Siddle (5); Flory (6); and Kienle and Petke (7) have studied and explained the basic mechanism of alkyd resin formation.

Classification of alkyds:

From the varnish maker's point of view, alkyds may be divided into the following groups:

1. Unmodified glyceryl phthalate resins.
2. Natural resin modified resins.
3. Styrenated alkyds.
4. Synthetic resin modified resins.
5. Drying oil modified resins and
6. Non-drying oil modified resins.

Kolke (8) has suggested another classification; alkyds for air drying varnishes, compatible with stand oil, natural and synthetic resins and zinc oxide; alkyds for bakl
ng

varnishes yielding hard glossy films with similar compatibility to the air drying alkyds; and alkyds for use with nitrocellulose.

Alkyds modified with fatty acids are frequently described as short, medium or long oil types.

1. Unmodified alkyds:

Unmodified alkyds are the least popular of the range probably by the reason of their relatively poor solubility, necessitating the use of expensive solvents. A resin solution derived from glycerol and phthalic anhydride may be baked on to chromium plated steel to form a film which is capable of removal and application to any other surface without baking (9). Another use for unmodified alkyds is as adhesives.

2. Natural resin modified alkyds:

Incorporation of natural resin to an alkyd resin is named as Natural resin modified resins. Normally, the addition of natural resin is to improve gloss, hardness and speed of drying.

3. Styrenated alkyds:

These are new addition to the alkyd range. They usually involve reacting a preformed oil modified alkyd of suitable type with styrene. This reaction proceeds satisfactorily in the presence of a solvent such as xylol.

The styrenated alkyds are characterised by extremely

rapid drying properties. They lose their solvent in a few minutes becoming dry to touch in as little as a quarter of an hour. They harden subsequently by a slower oxidation and polymerization process.

The styrenated alkyds have better adhesion and outstanding colour retention properties.

4. Synthetic resin modified alkyds:

The addition of synthetic resin to alkyds are called as synthetic resin modified alkyds. These alkyds are particularly used for stoving finishes. They form harder films with little tendency to discoloration on stoving.

5. Drying oil modified alkyds:

Linseed oil, dehydrated castor oil and a number of semi-drying oils are commonly used in this type of alkyds. These alkyds have rapid drying properties, outstanding durability and as claimed by Wulf to be free from chalking tendency (10). Medium and long oil alkyds are mostly exployed in the preparation of varnishes and are also used as a vehicle in most of the durable air-drying coatings. The shorter types of alkyds are used in stoving lacquers and enamels or as resin plasticizers for cellulose lacquers.

6. Non-drying oil modified alkyds:

Alkyd resins modified with non-drying oils are used primarily as plasticizers in stoving finishes, particularly with urea or melamine formaldehyde resins or as plasticizers for cellulose lacquers. These alkyd resins have outstanding hardness, depending upon the percentage of urea resin

APPARATUS FOR THE PREPARATION OF ALKYD RESINS.

present, are very resistant to solvents, and retain a very pale colour even when the temperature of stoving is high (300-350°F). These alkyds are also useful as the basis of non-drying adhesives. The enamel finishes made from non-drying modified alkyd resins are found to possess better adhesion, flexibility, toughness, less tendency to chalk and better colour retention. A number of fatty acids i.e. adipic, lactic and succinic acids etc. have been used by different workers to prepare non-drying modified alkyd resins (11).

An attempt has been made to prepare non-drying oil modified alkyds using Litsea consimilis seed fat. These resins have been examined with a view to studying their properties and their suitability for varnish preparations.

EXPERIMENTAL

Preparation of the alkyd resins:

The proportions of the reactants used in the preparation of the resins are given in table No. 17. The seed oil of Litsea consimilis and linseed oil were mixed well in a three necked round bottomed flask, fitted with an electric stirrer, a thermometer, a tube for passing carbon-dioxide, a 50 ml separating funnel containing xylene and a water condenser as shown in the diagram.

The mixture of these two oils was heated for few minutes at 100°C with occasional shaking. Lead naphthenate

(equivalent to 0.1% of the total weight of the oils) was then added and the temperature raised to 180°C. Glycerol equivalent to one fourth of the weight of the oil was now added. The mixture was heated at about 220°C with constant stirring. The progress of the reaction was observed from time to time till an adequate partial esterification took place, which was assessed by determining the solubility in hot methanol (1:1 dilution). After complete partial esterification, the product was cooled to 180°C, and phthalic anhydride and rest of the glycerol were then added (as shown in the table no. 17). The temperature was again raised to 220°C. Sufficient amount of xylene was added from time to time through the separating funnel in order to maintain a constant temperature and to remove water of esterification azeotropically. At the same time, the mixture of the reactants was continuously stirred and a current of carbon dioxide passed to avoid oxidation. The progress of the reaction was noted by determining the acid value after every half an hour. The reaction was considered to be complete when only a very little fall in the acid value was observed. This fall in acid value has been recorded in table no. 18 and also represented graphically.

TABLE No. 17

COMPOSITION OF REACTANTS TAKEN FOR THE PREPARATION OF NON-DRYING OIL MODIFIED ALKYD RESINS:

Reactants	A	B	C	D	E
Raw linseed oil	140.25gms.	107.70gms.	96.93gms.	86.16gms.	64.62gms.
Litsea consimilis seed oil	-	-	10.77gms.	21.54gms.	43.08gms.
Glycerol	35.10gms.	27.00gms.	27.00gms.	27.00gms.	27.00gms.
Lead naphthenate	0.14gms.	0.107gms.	0.107gms.	0.107gms.	0.107gms.
Phthalic anhydride	71.25gms.	95.62gms.	95.62gms.	95.62gms.	95.62gms.
Glycerol	3.40gms.	19.65gms.	19.65gms.	19.65gms.	19.65gms.

TABLE No. 18

FALL IN ACID VALUE WITH TIME:

Time in minutes	Acid value				
	A	B	C	D	E
30	92.0	95.0	67.0	97.5	70.5
60	81.5	75.5	51.0	93.5	53.5
90	70.0	54.5	35.5	57.5	42.5
120	58.5	37.5	26.0	45.0	40.0
150	38.4	36.5	25.5	41.0	34.0
180	20.0	33.0	-	37.5	-
210	17.5	32.5	-	35.0	-

Properties of the alkyd resins:

The hydroxyl value, phthalic anhydride content and viscosity were determined by the following methods and the results are summarised in table no. 19.

Estimation of the Hydroxyl groups (12):

A known weight of the resin was heated for 60 minutes with 1 volume of acetic anhydride and 4 volumes of pure anhydrous pyridine:

$$R(OH)_n + n\ CH_3CO{\cdot}O{\cdot}COCH_3 = R(O{\cdot}COCH_3)_n + n\ CH_3COOH$$

The excess of unchanged acetic anhydride was then hydrolysed by addition of water, and the total free acetic acid estimated by titration with standard sodium hydroxide solution. Simultaneously a blank experiment was also performed. The difference in the volumes of sodium hydroxide solution required in two experiments is equivalent to the difference in the amount of acetic acid formed, i.e., to the acetic acid used in the actual acetylation. The number of hydroxyl groups can then be calculated by the following formula:

$$\% \text{ of OH} = \frac{(\text{ml of alkali used for blank} - \text{ml of alkali used for the sample}) \times \text{normality} \times 0.017 \times 100}{\text{weight of the sample}}$$

% of OH in resin A:

Weight of the resin taken	= 0.7285 gms.
Strength of NaOH solution	= N/10
Volume of NaOH used for resin	= 46.2 ml
Volume of NaOH used for blank	= 53.4 ml
Volume of NaOH consumed	= 53.4-46.2 ml

= 7.2 ml

% of OH = (ml of alkali used for blank-ml of alkali used for sample) x normality x 0.017 x 100 / Weight of the sample

= (53.4-46.2) x 1 x 0.017 x 100 / 10 x 0.7285

= 1.68

<u>% of OH in resin B:</u>

Weight of the resin taken = 0.5580 gms.

Strength of NaOH solution = N/10

Volume of NaOH used for resin = 44.8 ml

Volume of NaOH used for blank = 53.4 ml

Volume of NaOH consumed = 53.4 - 44.8 ml

= 8.6 ml

% of OH = (ml of alkali used for blank-ml of alkali used for sample) x normality x 0.017 x 100 / Weight of the sample

= (53.4-44.8) x 1 x 0.017 x 100 / 10 x 0.558

= 2.62

<u>% of OH in resin C:</u>

Weight of the resin = 0.5623 gms.

Strength of NaOH solution = N/10

Volume of NaOH used for resin = 44.8 ml

Volume of NaOH used for blank = 53.4 ml

Volume of NaOH consumed = 53.4-44.8 ml

= 8.6 ml

% of OH = (ml of alkali used for blank-ml of alkali used for sample) x normality x 0.017 x 100 / Weight of the sample

= (53.4-44.8) x 1 x 0.017 x 100 / 10 x 0.5623

= 2.60

% of OH in resin D:

Weight of the resin = 0.5797 gms.

Strength of NaOH solution = N/10

Volume of NaOH used for resin = 44.6 ml

Volume of NaOH used for blank = 53.4 ml

Volume of NaOH consumed = 53.4-44.6 ml

= 8.8 ml

$$\% \text{ of OH} = \frac{(\text{ml of alkali used for blank} - \text{ml of alkali used for sample}) \times \text{normality} \times 0.017 \times 100}{\text{Weight of the sample}}$$

$$= \frac{(53.4 - 44.6) \times 1 \times 0.017 \times 100}{10 \times 0.5797}$$

= 2.58

% of OH in resin E:

Weight of the resin = 0.5536 gms.

Strength of NaOH solution = N/10

Volume of NaOH used for resin = 44.9 ml

Volume of NaOH used for blank = 53.4 ml

Volume of NaOH consumed = 53.4 - 44.9 ml

= 8.5 ml

$$\% \text{ of OH} = \frac{(\text{ml of alkali used for blank} - \text{ml of alkali used for sample}) \times \text{normality} \times 0.017 \times 100}{\text{Weight of the sample}}$$

$$= \frac{(53.4 - 44.9) \times 1 \times 0.017 \times 100}{10 \times 0.5536}$$

= 2.61

Determination of Phthalic anhydride content (13):

Such an amount of the resin was dissolved in benzene (10 ml) which gave approximately 0.5 gms. of

potassium phthalate on reacting with 50 ml of an ethanolic solution of normal potassium hydroxide. The mixture was refluxed for four hours, with occasional shaking, and a soda lime tube was connected with the condenser to prevent the entrance of carbon dioxide.

The mixture was cooled and the precipitate thus formed was transferred to a sintered glass crucible (grade 4). The precipitate was washed several times with absolute ethanol-benzene mixture (1:1 v/v). It was finally sucked dry, heated for 1 hour at 110°C, for 4 hours at 140°C and then finally weighed as potassium phthalate. The phthalic anhydride content could then be calculated as follows:

<u>% of Phthalic anhydride in resin A:</u>

Weight of the resin taken = 0.7162 gms.

Weight of the crucible = 24.3988 gms.

Weight of the crucible + potassium phthalate = 24.9188 gms.

$\therefore$ Weight of potassium phthalate = 24.9188 - 24.3988

= 0.52 gms.

242 gms. of potassium phthalate contain 148 gms. of phthalic anhydride.

$\therefore$ 0.52 gms. " " " $= \frac{148 \times 0.52}{242}$

$= 0.318$ gms.

0.7162 gms. of the resin contain 0.318 gms. of phthalic anhydride.

$\therefore$ 100 gms. " " " $= \frac{0.318 \times 100}{0.7162}$

= 44.4%

<u>% of Phthalic anhydride in resin B:</u>

Weight of the resin = 0.9275 gms.

Weight of the crucible = 26.4218 gms.

Weight of the crucible + potassium phthalate = 26.9858 gms.

∴ Weight of potassium phthalate = 26.9858 - 26.4218

= 0.564 gms.

242 gms. of potassium phthalate contain 148 gms. of phthalic anhydride.

∴ 0.564 gms. " " " = $\frac{148 \times 0.564}{242}$

= 0.345 gms.

0.9275 gms. of the resin contains 0.345 gms. of phthalic anhydride.

∴ 100 gms. of " " = $\frac{0.345 \times 100}{0.9275}$

= 37.2%

<u>% of phthalic anhydride in resin C:</u>

Weight of the resin taken = 1.00 gms.

Weight of the crucible = 23.4628 gms.

Weight of the crucible + potassium phthalate = 24.0588 gms.

∴ Weight of potassium phthalate = 24.0588 - 23.4628

= 0.596 gms.

242 gms. of potassium phthalate contain 148 gms. of phthalic anhydride.

∴ 0.596 gms. " " " = $\frac{148 \times 0.596}{242}$

= 0.3645 gms.

1.00 gms. of the resin contains 0.3645 gms. of phthalic anhydride.

∴ 100 gms. " " = $\frac{0.3645 \times 100}{1.00}$

= 36.4%

% of phthalic anhydride in resin D:

Weight of the resin taken = 0.9916 gms.

Weight of the crucible = 32.5642 gms.

Weight of the crucible + potassium phthalate = 33.1642 gms.

Weight of potassium phthalate = 33.1642 - 32.5642

= 0.60 gms.

242 gms. of potassium phthalate contain 148 gms. of phthalic anhydride.

∴ 0.60 gms. " " " = $\frac{148 \times 0.60}{242}$

= 0.3669 gms.

0.9916 gms. of the resin contains 0.3669 gms. of phthalic anhydride.

∴ 100 gms. " " " = $\frac{0.3669 \times 100}{0.9916}$

= 37.0%

% of phthalic anhydride in resin E:

Weight of the resin taken = 1.00 gms.

Weight of the crucible = 28.6212 gms.

Weight of the crucible + potassium phthalate = 29.2332 gms.

Weight of potassium phthalate = 29.2332 - 28.6212

= 0.6212 gms.

242 gms. of potassium phthalate contain 148 gms. of phthalic anhydride.

∴ 0.612 gms. " " " = $\frac{148 \times 0.612}{242}$

= 0.3743 gms.

1.00 gms. of the resin contains 0.3743 gms. of phthalic anhydride.

∴ 100 gms. " " " = $\frac{0.3743 \times 100}{1.00}$

= 37.4%

The viscosities of 35% xylene solutions of different alkyd resins were determined at 40°C by Redwood Viscometer in the usual manner.

TABLE No. 19

PROPERTIES OF ALKYD RESINS:

Properties of the resin	A	B	C	D	E
Appearance of the resin	Clear	Clear	Slightly dark	Dark	Dark
Acid value	17.5	32.5	25.5	35.0	34.0
Viscosity of 35% solution in seconds	34	46	38	32	32
% of OH	1.68	2.62	2.60	2.58	2.61
% of Phthalic anhydride	44.4	37.2	36.4	37.0	37.4

Preparation of varnishes from these resins:

All the resin solutions were adjusted to the same viscosity (say at 70 seconds at 40°C by Red Wood Viscometer) either by adding xylene or the resin. Such requisite quantities of lead naphthenate and cobalt naphthenate were added to the resin solutions so as to contain 0.5% lead and 0.05% cobalt as the metal contents. The amount of lead naphthenate added during esterification was substracted from the final addition. These varnishes were examined for the following properties:

Determination of the Drying time:

The drying time was determined by Gardner Circular Drying Time Recorder. The different varnish compositions were applied on one square foot plane glass plate and then

the recorder was placed on the wet film. The instrument was started by plugging the cord into 110 volt, 50 or 60 cycle A.C. outlet. The electric timing motor rotated at a speed of one revolution per 24 hours, during which a weighed spherical pin, connected to the motor shaft by means of an arm, rode on the film and scribed an arc shaped pattern until the film was dried. A metal templet having hour marks from 0 to 24 hours engraved on the periphery, was then placed on the dried film with the zero mark on the templet coinciding with the starting point of the test. The drying time was then noted on the templet at the point where the pin no longer left any impressinn on the film. Thus several stages of these varnishes e.g., set to touch time, surface dry or dust free time and hard dry time were noted for the drying time. The varnish compositions D and E, which could not dry up in air were stove dried at 120°C.

Determination of gloss:

Gloss means the ability of a surface to reflect light regularly. It was determined by Hunter Portable Photoelectric Glossmeter. The glossmeter consists of a built in flash light lamp and a reflector, and a light meter suitably recalibrated to measure gloss. The device confirms to the conditions set up by A.S.T.M. method D 523 in respect to the angles of incidence and reflection,

namely, 60°, and the sizes of the light source and receptor A piece of polished black plate glass having a gloss of 95 is contained in the carrying case for calibration of the glossmeter. The current for the lamp is obtained from A.C. source at 110 voltage.

The varnishes were applied on 30 gauge steel panels measuring 6" x 6". Compositions A, B and C were air dried and compositions D and E were stove dried at 120°C and then the gloss was measured. The glossmeter was calibrated by placing the head on the standard polished black plate having a gloss of 95. The test panels were kept below the glossmeter head and the gloss was measured at different places, the mean of these values was taken as the actual gloss of the panel.

Determination of Scratch hardness:

Scratch hardness value was normally determined by Gardner Hardness Tester, which consists of a phonograph type needle at an angle of 90° and a pan on the needle to keep desired weights.

The varnish coated panels (5" x 1.5") were placed beneath the needle point, the weights were placed on the pan and the plate was drawn slowly under the point in the direction away from the fulcrum. The weights which switched on the tester light, showed the value of the scratch hardness of the panel.

Determination of Flexibility:

Bending test has been used to test the flexibility of a varnish coated panel. This test was carried out by bending a test panel over a rod or mandrel of $\frac{1}{8}$" in diameter. It gave an over all indication of elasticity, adhesion, cohesion, hardness and durability of the film (14-15). It was observed that all the varnish compositions gave quite hard and tough films.

Water Resistance:

The water resistance of varnish films was determined by the method given in A.S.T.M Designation D 154-47. The varnish coated panels were immersed in cold distilled water and the action of water on the film was noted after 24 hours. Immediately after performing the water immersion test, the panels were also tested for scratch hardness.

Action of Sodium hydroxide solution:

The panels were dipped in a 5% solution of sodium hydroxide at 25°C, and the time, during which the film disintregrated completely, was noted.

Effect of Sulphuric acid and Xylene solutions:

The panels were dipped separately in (i) a 2% aqueous solution of sulphuric acid and (ii) xylene for 5 minutes, in order to find out whether these reagents had any reaction on the varnish films.

The above mentioned properties have been summarised in table no. 20.

TABLE No.20.

PROPERTIES OF THE VARNISH:

Alkyd resin varnishes	Gloss % (60°)	Scratch hardness	Mandrel Bend ¼"	Drying time	Water immersion test after 24 hours.	Scratch just after water immersion	Effect of 2% H_2SO_4 solution in 5 minutes	Resistance against 5% aqueous NaOH solution at 25°C	Xylene action test in 5 minutes
A	100	1000 gms.	Pass	(i) 9.30 hrs. (ii) 12.00 hrs. (iii) 17.00 hrs.	Slight whitening	400 gms.	7 minutes	Very slight whitening	No effect
B	100	1000 gms.	Pass	(i) 1.50 hrs. (ii) 3.00 hrs. (iii) 4.00 hrs.	Very slight whitening	800 gms.	7 minutes	Slight whitening	No effect
C	100	1000 gms.	Pass	(i) 6.30 hrs. (ii) 10.00 hrs. (iii) 16.00 hrs.	Very slight whitening	400 gms.	5 minutes	Very slight whitening	No effect
D	90	1000 gms.	Pass	30 minutes at 120°C	No effect	600 gms.	10 minutes	Pronounced whitening	No effect
E	80	1000 gms.	Pass	30 minutes at 120°C	No effect	400 gms.	17 minutes	Pronounced whitening	No effect

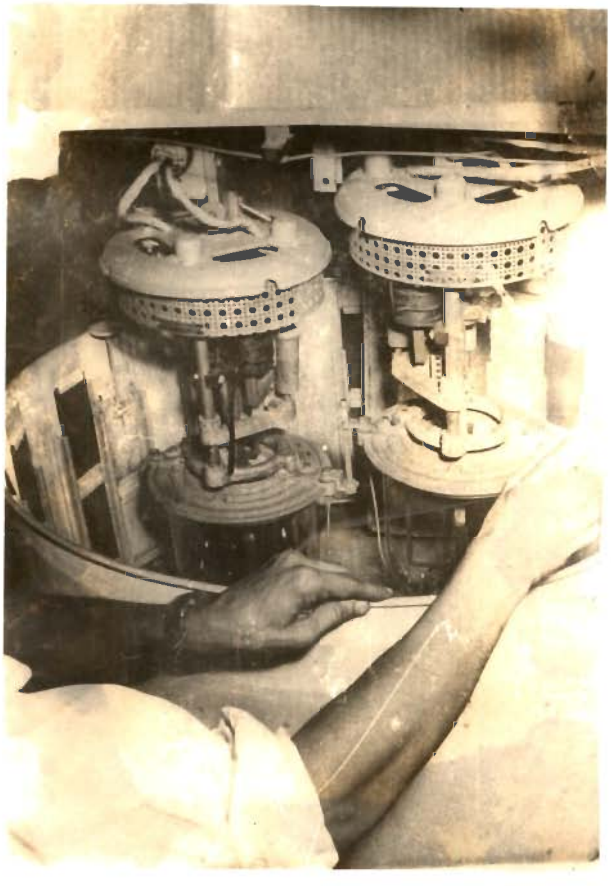
where: (i) set to touch (ii) surface dry and (iii) Hard dry.

Other test panels were exposed in a Twin Arc Weather-O-Meter for accelerated weathering and kept outside for natural weathering.

Details of Accelerated weathering:

The panels were mounted on test panel racks of the Twin Arc Weather-O-Meter and were exposed to 102 seconds ultraviolet light and then to a water spray for 18 seconds along with ultraviolet light exposure, and the sequence was repeated. The panel racks were rotated uniformly about the arc. The temperature of the cabinet was maintained at 58°C.

The test panels were taken out from the weatherometer after 50, 250 and 350 hours of weathering respectively, and they were then tested for gloss, scratch hardness and bending properties in the usual manner. The results have been recorded in table no. 21.



PHOTOGRAPHIC VIEW OF TWIN ARC WEATHER-O-METER FOR ACCELERATED WEATHERING.

TABLE No. 21

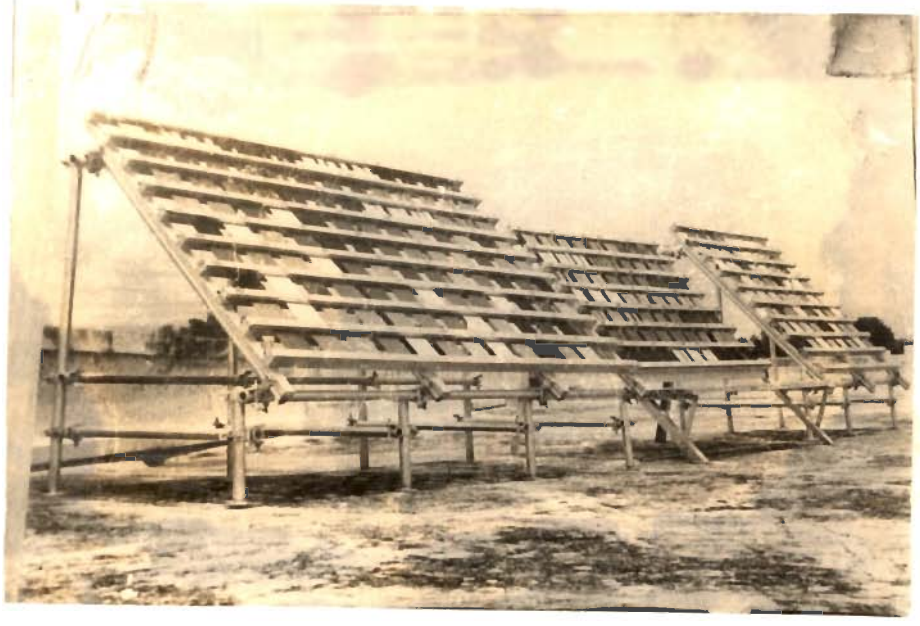
PROPERTIES OF TEST PANELS AFTER ACCELERATED WEATHERING:

Alkyd resin varnishes	After 50 hours			After 250 hours			After 350 hours		
	Gloss % (60°)	Scratch hardness in gms.	Mandrel Bend ⅛"	Gloss % (60°)	Scratch hardness in gms.	Mandrel Bend ⅛"	Gloss % (60°)	Scratch hardness in gms.	Mandrel Bend ⅛"
A	67	1500	Pass	31	2000	Pass	17	2000	Pass
B	69	1300	Pass	34	2200	Pass	24	2300	Pass
C	70	1500	Pass	56	2200	Pass	28	2300	Pass
D	52	1100	Pass	33	1900	Pass	33	2000	Pass
E	48	1100	Pass	31	1500	Pass	31	1800	Pass

Details of Natural weathering:

A number of panels were placed on an exposure rack at an angle of 45° (16) to the vertical facing south, so that the maximum amount of light fell on them.

The above panels were tested after 1, 2, 3 and 8 months' exposure for their scratch hardness and bending properties in the usual manner. The results are given in table no. 22.



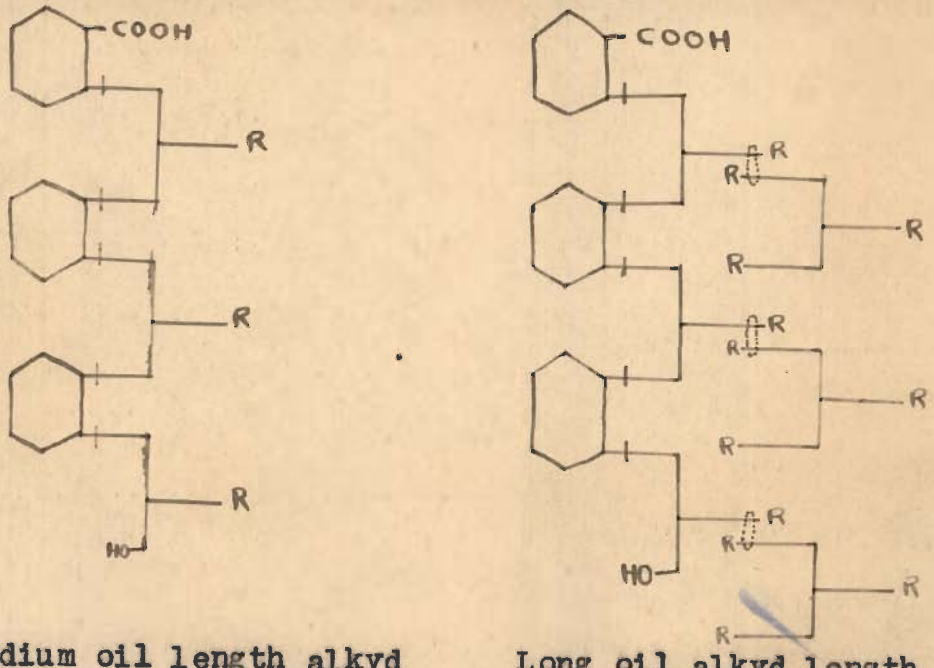
PHOTOGRAPHIC VIEW OF NATURAL WEATHERING.

PROPERTIES OF TEST PANELS AFTER NATURAL WEATHERING

Alkyd resin varnishes	After 1 month		After 2 months		After 3 months		After 8 months	
	Scratch hardness in gm.	Mandrel Bend ½"	Scratch hardness in gm.	Mandrel Bend ½"	Scratch hardness in gm.	Mandrel Bend ½"	Scratch hardness in gm.	Mandrel Bend ½"
A	2300	Pass	2400	Pass	2500	Pass	2500	Fail
B	2400	Pass	2500	Slightly Fail	2500	Fail	2500	Fail
C	2200	Pass	2200	Slightly Fail	2500	Fail	2500	Fail
D	2100	Pass	2200	Pass	2500	Pass	2500	Fail
E	2100	Pass	2200	Pass	2500	Pass	2500	Fail

DISCUSSION

Plasticizers generally reduce the brittleness of the paint film. They may be added either externally or internally (17). Internal plasticization involves modification of the macromolecules of the basic polymer by primary forces within the polymer molecules. Monobasic acids have been used for this purpose in the preparation of the alkyd resins (18). In fact, their additions space



Medium oil length alkyd Long oil alkyd length

(where ⬡ phthalic anhydride,]- Glycerol; – R fatty acid)

out phthalic acid and polyol residues in the poly-chain, and increases the mobility of macromolecules. This can very well be noticed from viscosity data of different compositions (greater the spacing out, lesser the viscosity; table no. 19). The addition of monobasic acids thus increases the solid resin content in the dried lacquer

coating. This may increase the durability of the film for the same number of coats.

Scratch resistance after 350 hours of accelerated weathering shows that the rate of hardening of the film modified with the seed fat of Litsea consimilis is much more slower than that of the straight linseed oil modified film for the same oil length (table no. 21). This indicates that the seed oil modified resin film would last longer than the straight linseed oil alkyd resin film. The same trend is observed in the case of natural weathering (table no. 22), compositions D and E can be very well compared with composition A as far as the hardness of the film is concerned. Therefore, in order to have a flexibility equivalent to long oil alkyds, one can replace much more linseed oil with lesser amounts of the seed fat. Upto an exposure period of two months, composition C gives lower values for the hardness when compared with composition B. From the bending test, it is quite evident that the replacement of linseed oil by the seed fat of Litsea consimilis (compositions C, D and E) increases the flexibility of the varnish films.

Scratch resistance of alkyd resins (compositions D and E) after 24 hours water immersion is quite good and is equivalent to composition A. Although, compositions B and C are air-drying and show similar properties, the

best advantage in the case of composition C is that it requires lesser cooking time. So the manufacturing process is more economical when the seed fat of Litsea consimilis is used. The compositions D and E (having 20% and 40% seed fat respectively) which are stove drying, can be made air-drying by blending them with pentaerythritol in place of glycerol. This, however, needs further investigation.

The foregoing results show that the linseed oil can successfully be replaced in parts by the seed fat of Litsea consimilis to produce plasticizing alkyds. These modified resins have superior adhesion, better scratch resistance and are more durable than the straight linseed oil alkyds.

USE OF LITSEA CONSIMILIS SEED FAT AS A LUBRICANT:

When one solid surface is moved relative to another solid surface with which it is in contact, there is a frictional resistance to motion. If the surfaces are perfectly smooth, the frictional resistance arises primarily from the operation of molecular forces of cohesion. With practical surfaces, which are not perfectly smooth, the interference of minute projections on the surfaces contributes to the resistance. This frictional resistance causes surface wear and the dissipation of energy as heat. To hold energy dissipation and surface wear to a minimum in all sorts of machines, a lubricant is used to keep the moving surfaces apart. Minerals such as graphite, talc, molybdenum disulphide; mineral oils; greases; and silicones and polyalkylene glycols have been used as lubricants. Due to high oiliness, a number of vegetable and animal oils such as castor oil, rape seed oil, olive oil, lard oil, tallow oil, neet's foot oil etc. are found to be good lubricants or lubricant additives (19) for different types of machines. The seed oils of some Litsea and Actinodaphne species are reported to possess lubricating properties for watches and other delicate machines (20). The seed fat of Litsea consimilis has not been so far tested for its possible use as a lubricant. This possibility has been explored by the author for the first time.

EXPERIMENTAL

The air dried powdered seeds were soxhletted with petroleum ether (b.p. 60-80°C). A brownish yellow coloured oil was obtained in 50.6% yield. The oil was purified by treating it with Fuller's earth and activated charcoal. The following physical constants of the oil were determined in order to test its usefulness as a lubricant:

Specific gravity at 35°C	= 0.8946
Acid value	= 4.257
Saponification value	= 253.4
Iodine value (Hanus)	= 28.62
Acetyl value	= 22.71
Reichert-Meissl value	= 1.43

The details for the determination of the above physical constants have been described previously (chapter 1, pages 18-24).

VISCOSITY:

The viscosity of the seed oil was determined at different temperatures by Redwood viscometer and the observations are summarised in table no. 23. The relationship between temperature and viscosity has also been expressed graphically.

VISCOSITY - TEMPERATURE CURVE

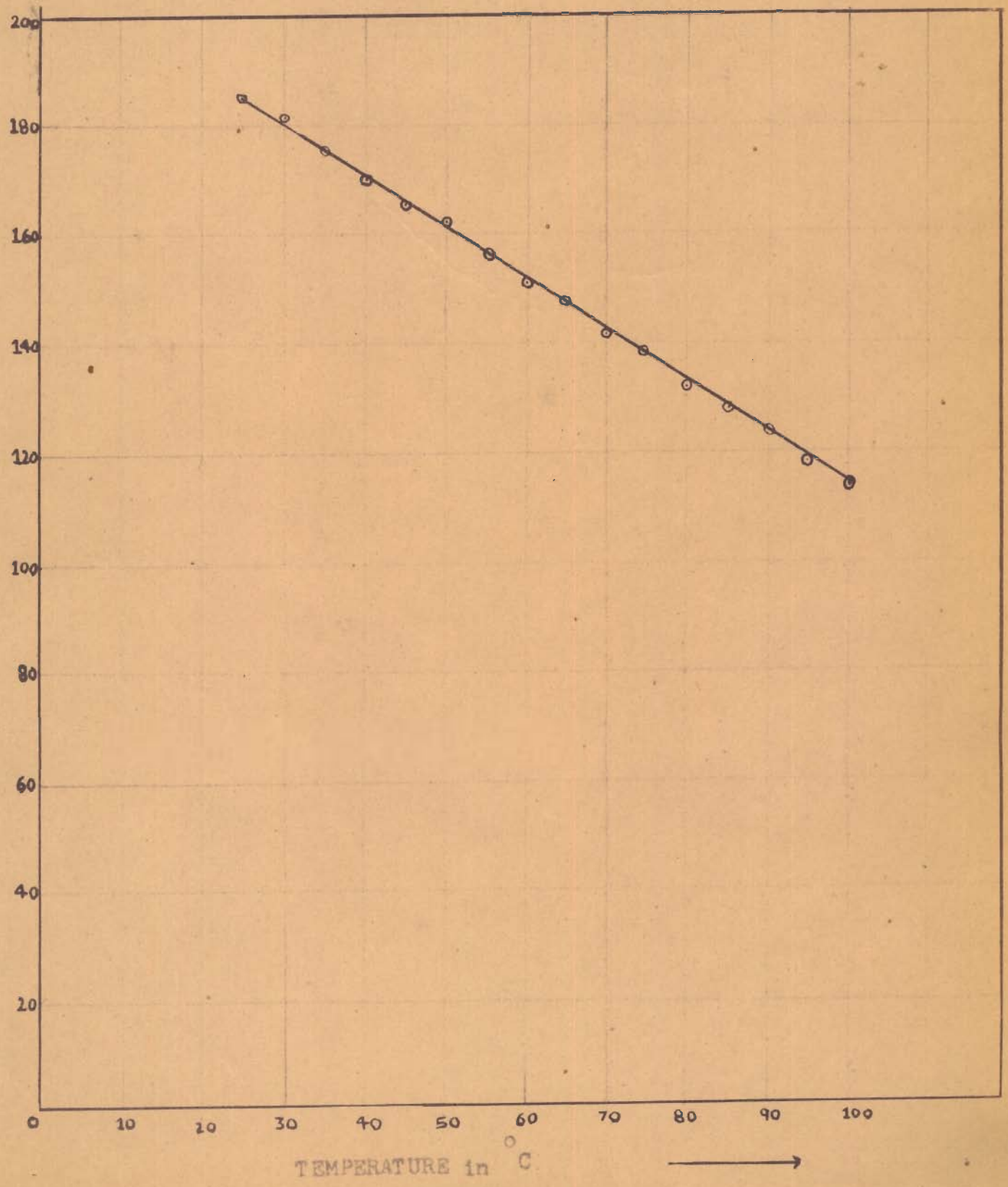


TABLE No.23

VISCOSITY OF THE SEED OIL AT DIFFERENT TEMPERATURES:

Temperature in ^{o}C	Viscosity in seconds
25	185
30	181
35	175
40	170
45	165
50	162
55	156
60	151
65	147.5
70	141
75	138
80	131.5
85	128
90	123
95	118
100	113.5

Determination of Moisture:

The moisture was determined by Dean and Stark standard apparatus. It consists of a 500 ml round bottomed glass flask, connected to a receiver through a reflux condenser. 100 gms. of the seed fat were poured into the flask through a measuring cylinder and the adhering

oil in the cylinder was poured in the flask by washing the cylinder with 50 ml of xylene. On distilling the oil on a water bath, the water which distilled azeotropically along with xylene was collected in a receiver, weighed and the percentage of moisture calculated as follows:

Weight of the oil taken = 100 gms.

Weight of water collected = 0.25 gms.

$\therefore$ % of moisture $= \frac{0.25 \times 100}{100}$

$= 0.25\%$

Determination of the percentage of ash:

5 gms. of the seed fat were ignited in a platinum dish till all the carbon was completely burnt off. The weight of the ash was determined and the percentage of ash calculated:

Weight of the oil taken = 5 gms.

Weight of the ash present = 0.055 gms.

% of ash $= \frac{0.055 \times 100}{5}$

$= 1.1\%$

Determination of the flash point:

The flash point of the oil was determined by Pensky Martens apparatus. It consists of a metal cup of a standard size in which the oil was heated at a definite rate. At intervals, the cover of the cup was removed for about a second and a small flamewas brought close to the

oil surface. The lowest temperature at which the vapours flashed, was called the closed flash point, and was found to be 135°C.

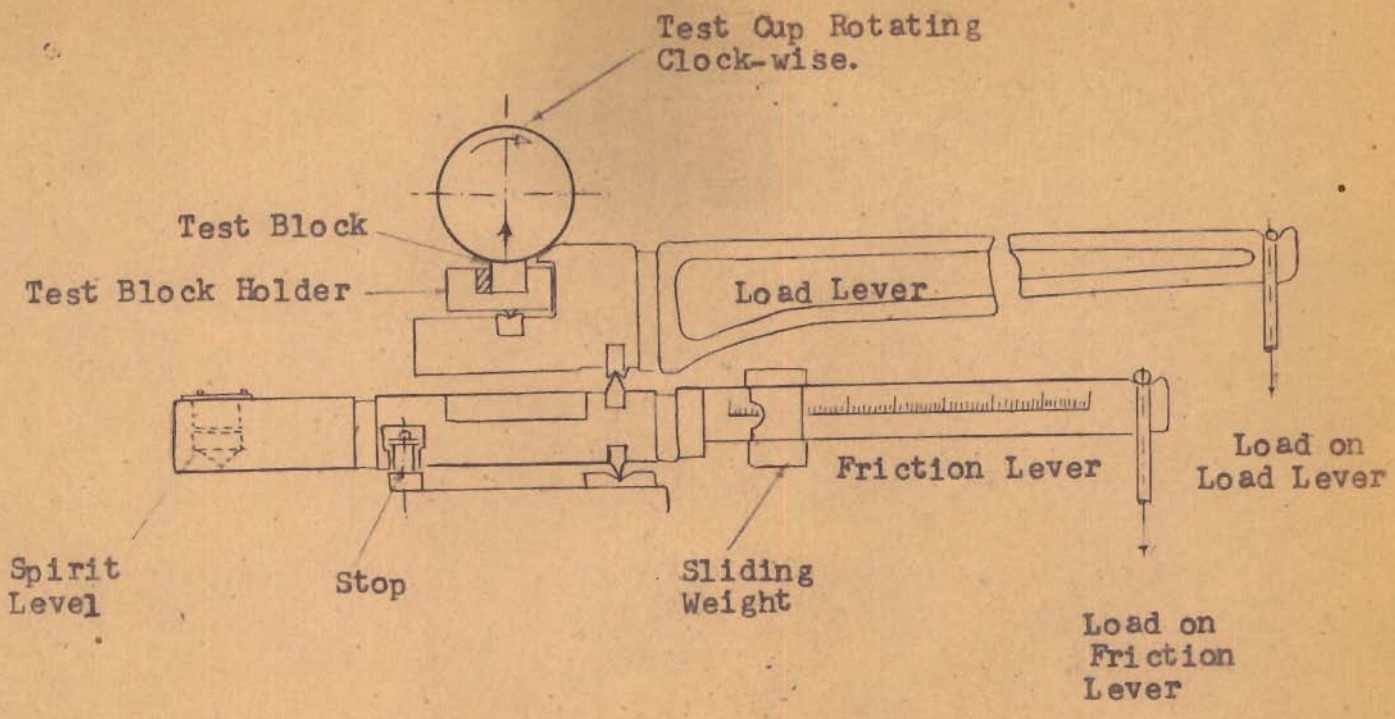
After determining the closed flash point, the temperature was raised further until a flash due to ignition of the oil vapour was obtained while keeping the cup open. This temperature, known as the open flash point, was found to be 150°C.

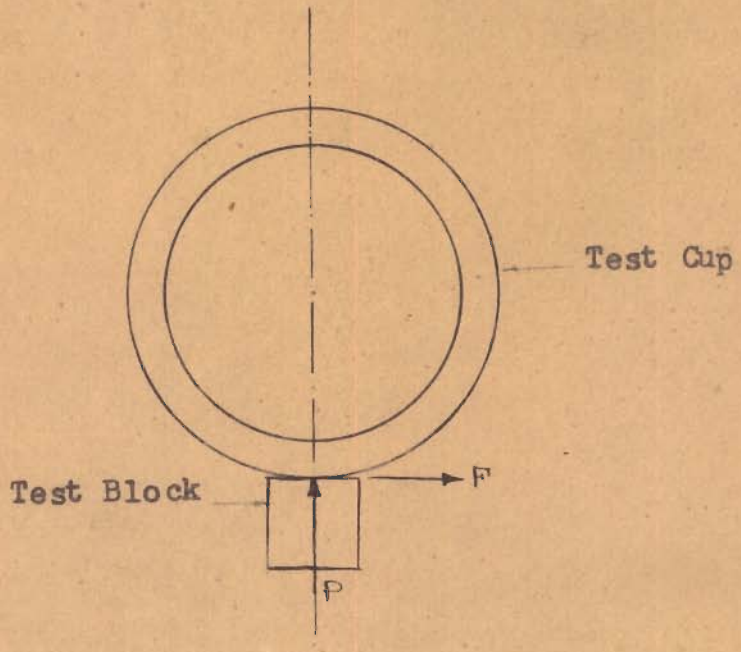
Determination of corrosion:

5 gms. of finely polished iron foils (1 mm. wide and 50 mm long) were suspended for 15 days in a corked bottle containing the seed fat of Litsea consimilis. After 15 days, the iron foils were taken out, dried and weighed. It was found that there was no change in the weight of the iron foils indicating that the seed fat did not produce any corrosion.

Determination of the coefficient of friction, test pressure and wear:

Timken wear and Lubricant Testing Machine was used for determining the coefficient of friction, test pressure and wear. The machine consists of two metal surfaces having a very small contact area. These metal surfaces are rubbed together in presence of a high pressure between them. A circular disc, approximately 2" in diameter, is rotated against a rectangular block, the area of contact being a very narrow band.



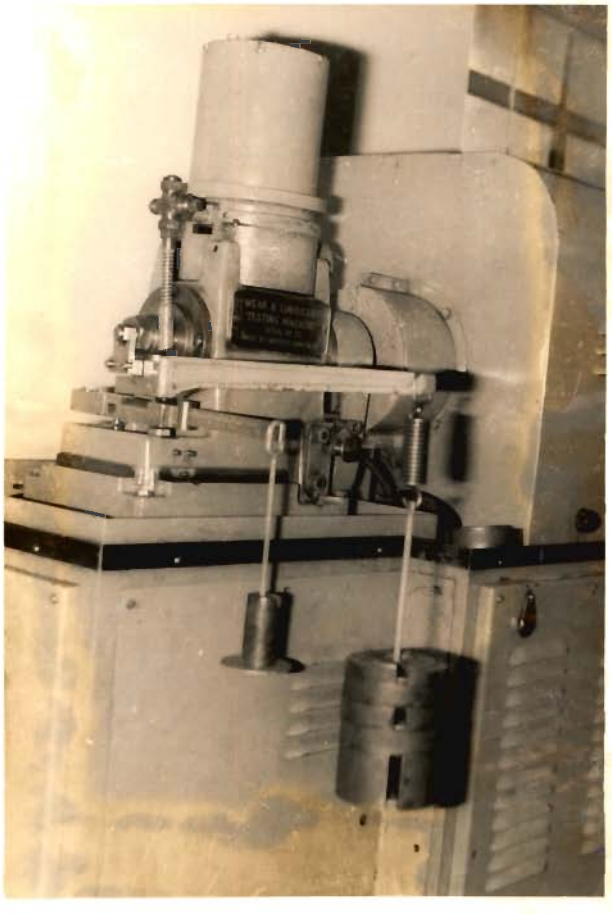


ENLARGED VIEW

Schematic view of the Apparatus used for Testing and the System of Forces Acting.

The general arrangement of the basic elements of the machine is given in the diagram. It consists of a system of two superimposed levers. The upper or the load lever carries a pivoted holder containing the test block. This test block bears against the test cup being held up by weights suspended from the end of the lever on which the block is carried. The lower or the frictional lever, to which weights are added to balance the frictional forces, is used to obtain a direct reading of friction in terms of weight. A built-in electric heater below the oil reservoir is used to heat the oil upto 210°F. The oil flows from the reservoir under gravity over the test block and is pumped back to the reservoir. The oil pump is engaged or disengaged by actuating a knob attached to a short lever. A flow valve regulates the flow of oil. A small hole in the cover of the reservoir is provided for reading the temperature of the oil on a thermometer.

The machine is driven by a variable speed motor. The speed can be varied upto 2000 revolutions per minute. There is a spirit level attached at the end of the friction lever which indicates the level of the lever system when it is assembled in position. Other spirit levels are also provided to check the level of the machine.



PHOTOGRAPHIC VIEW OF TIMKEN WEAR AND LUBRICANT TESTING MACHINE.

Test procedure:

The testing machine was cleaned and brought to correct level. Oil reservoir was filled with the seed fat of Litsea consimilis. The oil pump clutch was engaged. The test cup and block were cleaned and weighed. The test cup was fitted on machine spindle and test block was placed in its holder. Surfaces of cup and block were smeared fully with the oil. The lever system was assembled in position, and checked by built in spirit level at the end of the friction lever. The weight carrier was kept in readiness with the required weight fitted on it. The sliding weight on the friction lever was kept at zero initially.

To confirm that the test cup and the block were contacting properly, the machine was given a few revolutions by hand. The flow valve was opened by the same amount each time and the machine was started by switching on the motor. The speed of the machine and the temperature were maintained constant at 800 revolutions per minute and 100^{o}F respectively. The weight carrier was mounted on the load lever in position. Friction lever reading was taken when steady temperature was attained. The test was run for ten minutes.

After performing the test, the cup and block were again cleaned and weighed. The width of the scar on the test block was measured.

Results were calculated by the following equations:

(i) $P = 10(A+C)-2.5\,(B+R)$

(ii) $S = P/WXL$

(iii) $\mu = 9.45 \times (B+R)/10\,(A+C)-2.5(B+R)$

where A = Weight on the load lever

B = Weight on end of friction lever

C = load lever constant

P = test force (normal)

R = friction lever sliding weight reading

S = pressure on test block in lbs. per square inch.

W = width of scar on test block in inches.

L = length of scar on test block in inches = 0.5"

μ = coefficient of friction

B+R= friction lever weight

Observations:

Weights on the load lever	= 14.26 lbs.
Load lever constant	= 1.67 lbs.
Weights on end of friction lever	= 0.9 lbs.
Weight on sliding friction lever	= 0.1 lbs.
Width of scar on test block	= 0.0528"
Length of scar on test block	= 0.5"

Coefficient of friction (μ):

Coefficient of friction (μ) $= \dfrac{9.45 \times (B+R)}{10 \times (A+C)-2.5\,(B+R)}$

$= \dfrac{9.45 \times (0.9 + 0.1)}{10\times(14.26+1.67)-2.5(0.9+0.1)}$

$= 0.0602$

Test force (P):

Test force (P) = 10(A+C)-2.5(B+R)

= 10(14.26+1.67)-2.5(0.9+0.1)

= 156.8

Pressure on test block (S):

Pressure on test block (S) = $\frac{P}{WXL}$

= $\frac{156.8}{0.0538 \times 0.5}$

= 5829 p.s.i.

Observation for the wear:

Weight of the test cup (before test) = 57.6580 gms.

Weight of the test cup (after test) = 57.6560 gms.

Loss in weight due to wear in cup = 57.6580-57.6560

= 0.002 gms.

Weight of the test block (before test) = 24.2000 gms.

Weight of the test block (after test) = 24.1945 gms.

Loss in weight due to wear in block = 24.2000-24.1945

= 0.0055 gms.

Oiliness:

Oiliness is reciprocal of the coefficient of friction and is correlated with the following properties:

(i) Ability to prevent wear.

(ii) Resistance of the oil from being ruptured or displaced.

(iii) Load carrying abilities.

(iv) Ability to prevent failure by seizure, galling and scuffing.

From the value of the coefficient of friction, it may be indirectly concluded that the seed fat of Litsea consimilis possesses a satisfactory oiliness.

Oiliness, however, could not be determined due to non-availability of an oiliness testing machine.

Determination of cutting force:

The experiment was performed under the following two conditions:

(a) Dry condition

(b) Wet condition

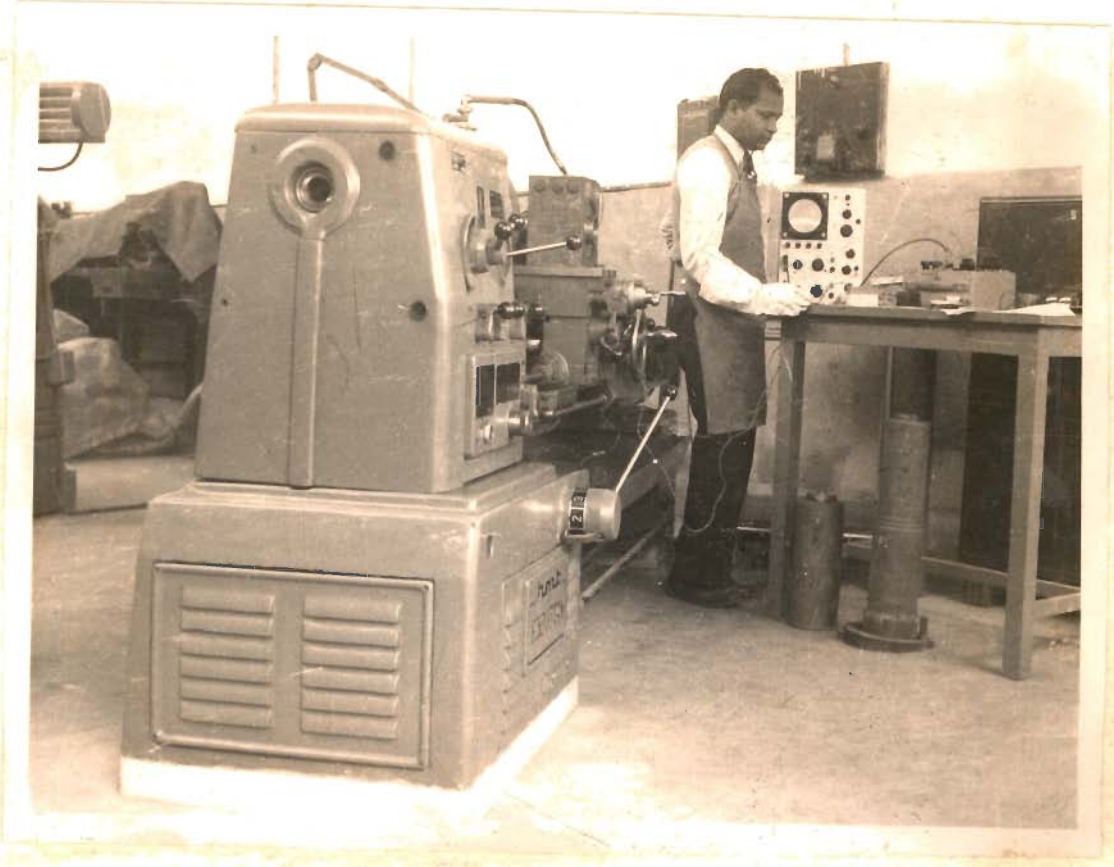
The cutting force was determined with the help of an oscilloscope, a strain bridge amplifier and a dynamometer as shown in the diagram.

Before starting the experiment, the oscilloscope was allowed to warm up for about 10 minutes and then the horizontal beam was fixed at some place. The horizontal beam was adjusted and balanced at the centre for all sensitivities. Throughout the whole experimentation, the sensitivity was kept at 10 m.volts/cm. as the calibration curve was obtained for this sensitivity.

Now the work piece of 3" diameter and 2' long was loaded on the H.M.T. lathe machine. The machine was allowed to run for about one minute, so that the machine reached the stabilized condition.

Procedure:

The cutting force under different conditions i.e.



PHOTOGRAPHIC VIEW OF LATHE MACHINE, OSCILLOSCOPE, STRAIN BRIDGE AMPLIFIER AND DYNAMOMETER.

dry, with water and with seed oil was measured at different speeds and the depths of cut. The flow of water and the seed oil was kept constant throughout the experiment. The observations are recorded in table no. 24 A.B.C.

TABLE No. 24A

CUTTING FORCE IN DRY CONDITION

R.P.M.	Depth of cut mm.	Feed in mm/rev.	Cutting forces cms.	lbs.	kg.
	1.0		0.3	65	29
64		0.05			
	1.5		0.5	110	50
	1.0		0.4	90	41
80		0.05			
	1.5		0.3	65	29
	1.0		0.2	42	19
125		0.05			
	1.5		0.3	65	29
	1.0		0.6	140	64
160		0.05			
	1.5		0.8	195	91

TABLE No. 24 B

CUTTING FORCE IN WET CONDITION WITH WATER

R.P.M.	Depth of cut mm.	Feed in mm./rev.	Cutting forces cms.	lbs.	kg.
64	1.0	0.05	0.2	42	19
	1.5		0.4	90	41
80	1.0	0.05	0.2	42	19
	1.5		0.2	42	19
125	1.0	0.05	0.2	42	19
	1.5		0.2	42	19
160	1.0	0.05	0.4	90	41
	1.5		0.6	140	64

TABLE No. 24 C

CUTTING FORCE IN WET CONDITION WITH SEED OIL

R.P.M.	Depth of cut mm	Feed in mm./rev.	Cutting forces cms.	lbs.	kg.
64	1.0	0.05	0.2	42	19
	1.5		0.3	65	29
80	1.0	0.05	negligible	-	-
	1.5		0.2	42	19
125	1.0	0.05	0.1	21	9.5
	1.5		0.2	42	19
160	1.0	0.05	0.3	65	29
	1.5		0.5	110	50

DISCUSSION:

The specific gravity and the viscosity of the seed oil of Litsea consimilis are nearly the same as those of vegetable lubricating oils (21,22). The viscosity-temperature curve shows that the viscosity of the seed oil varies only slightly with the rise in temperature. Therefore, the oil may be included in the family of vegetable lubricating oils.

It is quite interesting to observe that the closed flask point of the seed oil is 135°C which corresponds with those vegetable oils used as lubricants in spindles (23).

Considering the values of the coefficient of friction (22), test pressure and wear, it may be observed that the seed oil may be used as a good lubricating oil.

Cutting forces with the seed oil are reduced appreciably when compared with those obtained in dry condition and with water. This indicates that the cutting forces are reduced due to the lubricating properties of the seed oil. It may, however, be mentioned that the cutting forces with the seed oil are not reduced appreciably at high speeds as the seed oil produces fumes and unpleasant smell. Consequently, the seed oil can be used as a lubricant only in those machines where high temperatures are not developed.

From the above discussion, it is quite evident that

the seed oil of Litsea consimilis possesses all the characteristics of vegetable lubricating oils and can, therefore, be used profitably as a lubricant. It may, however, be added that the seed oil can only be used as a lubricant in those machines where high temperatures are not developed during their operation.

CHAPTER VI

CHEMICAL EXAMINATION OF THE SEEDS AND FRUITS OF SOLANUM FEROX LINN.

Solanum ferox Linn. is a moderately sized (2-4 ft.) evergreen shrub belonging to solanaceae family. It grows wildly in Garhwal (U.P.), Madras, Bihar, Orissa, Assam, Ceylon and China. The various parts i.e. roots, leaves, stems, flowers, fruits and seeds have been used medicinally in sore throat, cough, asthma, chest pains, dropsy and rheumatism (1).

The botanical description of the plant is as follows (2):

Stem herbaceous, stout, 2-4ft. high, densely clothed with long coarse, often stalked usually fulrous stellate hairs, and copiously armed with straight slender prickles. Leaves usually 2 at a node and unequal, 6-11 by 4-8 inch, broadly elliptic in outline, sinuately or pinnately cut into few short triangular lobes, softly stellately fulvous, hairy on both sides (very densely beneath), armed with long erect yellow slender prickles on the nerves on both sides, base cordate, truncate or more or less acute, often unequal-sided; main nerves 6-8 pairs; petiole 1.5 - 2.5 inch long, prickly and densely fulvous-hairy. Flowers solitary or 2-6 flowered leaf-opposed densely hairy cymes; peduncles very short; pedicles 1/4 - 1/2 inch long, densely stellately fulvous hairy and usually with a few prickles. Calyx campanulate 3/8 inch long, divided about 1/2 way down, densely stellately fulvous hairy, slightly enlarged in fruit; teeth deltoid, acute. Corolla 3/4 inch long,

5 partite; tube very short, 1/16 inch long; lobes ovate lanceolate acute, densely hairy outside. Anthers sessile or nearly so, linear-lanceolate, 1/3 inch long, opening by small pores. Ovary densely hairy with long hairs; style glabrous. Berry globose, 3/4-1$\frac{1}{4}$ inch in diameter, densely covered with long fulvous hairs. Seeds 1/10 inch in diameter, faintly brown with concentric rings.

Although the seed and fruit fats, and the alkaloidal contents of a number of solanum species have been investigated by various workers (3-14), no work appears to have been done on Solanum ferox Linn. It was, therefore considered worthwhile by the author to take up a systematic chemical examination of this plant.

CHEMICAL EXAMINATION OF THE SEED FAT:

From the seeds of Solanum ferox Linn., a yellow coloured oil was obtained in 2.7% yield. On keeping the oil for about fifteen days a yellowish sticky mass was obtained. It was crystallised from absolute alcohol and characterized as solanocarpone. The fatty acid composition of the oil was determined and found to be palmitic acid, 12.15%; stearic acid, 9.96%; oleic acid, 39.83% and linoleic acid, 38.06%.

EXPERIMENTAL

The seeds (1.5 kg.) were air dried, powdered and soxhletted with petroleum ether (b.p. 60-80°C). A yellow coloured oil was obtained in 2.7% yield. The oil was purified by treating it with Fuller's earth and activated charcoal. The purified oil, on allowing to stand for about fifteen days, deposited a yellowish sticky mass, which was filtered, washed successively with petroleum ether and benzene several times to remove the adhering fatty material. The crude product was repeatedly extracted with warm ethanol, and the ethanol extracts were combined. On concentrating and keeping the ethanol extract in a refrigerator overnight, a brown amorphous powder separated which on repeated crystallisation from hot absolute alcohol afforded cream coloured microscopic needles, yield (0.55 gms.), m.p. 78°C.

Found: C, 68.45; H, 8.76; $C_{28}H_{42}O_7$ requires C, 68.57; H, 8.57%.

The substance showed all the characteristic properties of $\Delta^{\alpha\beta}$ unsaturated lactones. It dissolved eaisly in benzene, acetone, ethyl acetate, chloroform and phenol; it was slightly soluble in methanol and ethanol; and was insoluble in ethyl ether, petroleum ether and water. It dissolved in alcoholic caustic soda producing an intense yellow colour, from which it was precipitated unchanged on acidification. It quickly decolourised a solution of bromine in chloroform and also alkaline and acidic solutions of potassium permanganate. It gave a positive Salkowski reaction, i.e. a solution of the lactone in chloroform gave with concentrated sulphuric acid a red and finally a green colouration. A solution of the lactone in chloroform on treatment with acetic anhydride followed by concentrated sulphuric acid gave a fine violet colouration. With concentrated sulphuric acid it produced an intense yellow colour which finally changed to deep red. With concentrated nitric acid it developed a yellow colour on heating. It gave a white precipitate with lead acetate, but no precipitate or colouration with silver nitrate or ferric chloride. With Tollen's reagent a light yellow colouration was produced which slowly changed to brown and then gradual reduction took place. It gave no colouration with an alkaline solution of sodium nitroprusside.

The above compound showed all the characteristic properties of solanocarpone (15). The further examination could not be undertaken due to availability of the material in a very small amount.

The oil after removal of the lactone possessed the following characteristics: specific gravity at 30°C, 0.9248; refractive index at 25°C, 1.4725; acid value, 3.6; saponification value, 200.4; saponification equivalent, 279.9; ester value, 196.8; iodine value (Hanus), 102.7; Hehner value, 95.95; Reichert-Meissl value, 1.54; Polenske value, 8.6; unsaponifiable matter, 1.1%.

The physical constants were determined as described previously (Vide chapter 1; pages 17-28).

(a) Determination of the percentage yeild:

Weight of the seed extracted	= 1500 gms.
Weight of the conical flask	= 46.4212 gms.
Weight of the conical flask+oil	= 86.9212 gms.
∴ Weight of the oil	= 40.5 gms.
∴ % yield	$= \frac{40.5 \times 100}{1500}$
	= 2.7%

(b) Specific gravity:

Weight of the specific gravity bottle	= 6.8548 gms.
Weight of the specific gravity bottle + water	= 17.5769 gms.
Weight of the specific gravity bottle + oil	= 16.7707

∴ Weight of the water taken = 10.7221 gms.

Weight of the oil taken = 9.9159 gms.

∴ Specific gravity of the oil at 35°C $= \frac{\text{Weight of the oil taken}}{\text{Weight of the water taken}}$

$= \frac{9.9159}{10.7221}$

= 0.9248

(c) Refractive index:

By Abbe's refractometer

Refractive index at 25°C = 1.4725

(d) Determination of the acid value:

Weight of the oil taken = 3.1166 gms.

Strength of NaOH solution = N/10

Volume of NaOH solution used = 2.0 ml

∴ Acid value $= \frac{2.0 \times 56.1}{10 \times 3.1166}$

= 3.6

(e) Determination of the saponification value:

Weight of the oil taken in conical flask 'A' = 1.3717 gms.

Volume of approximately N/2 alcoholic potassium hydroxide solution taken in each flask = 25 ml

Volume of N/5 hydrochloric acid used:

(i) for blank 'B' = 63.5 ml

(ii) for oil 'A' = 39.0 ml

∴ Volume of N/5 hydrochloric acid required for the saponification of the oil = 63.5-39.0 ml

= 24.5 ml

∴ Saponification value = $\frac{24.5 \times 56.1 \times 1000}{5 \times 1000 \times 1.3717}$

= 200.4

(f) Determination of the saponification equivalent:

Saponification equivalent = $\frac{56100}{\text{Saponification value}}$

= $\frac{56100}{200.4}$

= 279.9

(g) Determination of the ester value:

Ester value = Saponification value-Acid value = 200.4-3.6

= 196.8

(h) Determination of the iodine value (Hanus):

Weight of the oil taken in iodine flask 'A' = 0.2410 gms.

Volume of the Hanus solution taken in each flask = 25 ml

Strength of sodium thiosulphate solution = N/10

Volume of sodium thiosulphate solution used:

(i) for blank 'B' = 53.7 ml

(ii) for oil 'A' = 34.2 ml

∴ Volume of thiosulphate used (Volume of N/10 iodine reacted) = 53.7-34.2 ml

= 19.5 ml

∴ Iodine value = $\frac{127 \times 19.5 \times 100}{10 \times 1000 \times 0.2410}$

= 102.7

(i) Determination of Hehner value:

Weight of the oil taken for saponification = 0.6280 gms.

Weight of the insoluble acid present = 0.6026 gms.

Hehner value = $\frac{\text{Weight of the insoluble acids} \times 100}{\text{Weight of the taken for saponification}}$

$= \frac{0.6026 \times 100}{0.6280}$

$= 95.95\%$

(j) Determination of Reichert-Meissl value:

Weight of the oil taken = 5 gms.

Strength of sodium hydroxide solution = N/10

Volume of sodium hydroxide solution used for the oil = 2.4 ml

Volume of sodium hydroxide solution used for the blank = 1.0 ml

Reichert-Meissl value = (ml of alkali solution used by the oil-ml of alkali solution used by blank) x normality x 1.1 x 10

$= (2.4-1.0) \times \frac{1}{10} \times \frac{1.1}{1} \times \frac{10}{1}$

$= 1.54$

(k) Determination of Polenske value:

Volume of alkali used = 8.6 ml

Polenske value = ml of alkali used x normality x 10

$= 8.6 \times \frac{1}{10} \times 10$

$= 8.6$

(l) Estimation of the unsaponifiable matter:

Weight of the oil taken for saponification = 2.4680 gms.

Weight of the unsaponifiable matter = 0.0272 gms.

$\therefore$ % of unsaponifiable matter $= \frac{0.0272 \times 100}{2.4680}$

$= 1.1\%$

Saponification of the seed fat:

A requisite amount of the oil was saponified with ethanolic potassium hydroxide. The ethanol-free aqueous solution of the soap was thoroughly extracted with ethyl ether to remove the unsaponifiable matter. The soap was treated with dilute sulphuric acid and the liberated fatty acids were extracted and examined in the usual manner. They possessed the following characteristics: iodine value (Hanus), 103.4; mean molecular weight, 276.3.

The mixed fatty acids were separated into solid (22.7%; iodine value, 3.4; mean molecular weight, 270.2) and liquid acids (77.3%; iodine value, 136.1; mean molecular weight, 280.6) using Hilditch's modification of Twitchell's lead salt method (16). The solid and liquid acids were separately converted into methyl esters (17) and then fractionally distilled under reduced pressure. The weight, iodine value, saponification equivalent and mean molecular weight of different fractions (S_1-S_4, L_1-L_4 and residues) were determined, and fatty acid compositions of each of the fractions were calculated in the usual manner (Vide chapter 1; pages 36-44). The results are recorded in table nos. 25 and 26.

TABLE No. 25.

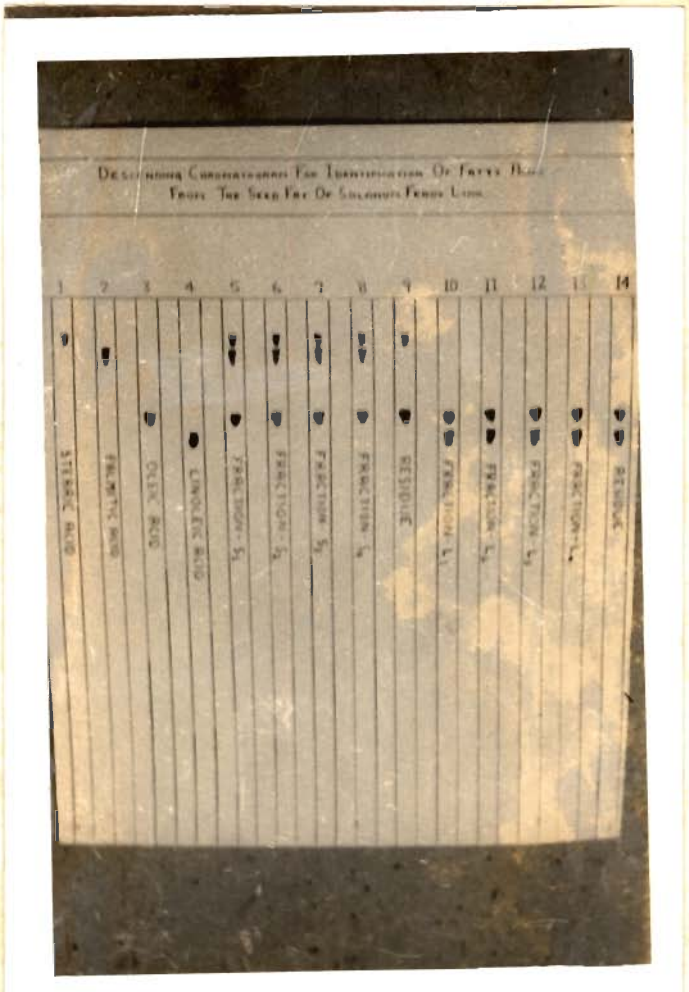
DISTILLATION OF METHYL ESTERS OF SOLID ACIDS AT 11 mm :

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.)		
						Palmitate	Stearate	Oleate
S_1	170-175	1.46	1.2	270.0	269.8	1.20	0.24	0.02
S_2	175-180	1.24	1.4	274.3	275.2	1.00	0.22	0.02
S_3	180-185	1.32	2.9	283.4	284.1	0.65	0.63	0.04
S_4	185-190	1.54	4.6	290.7	290.1	0.37	1.09	0.08
Residue	-	0.56	5.1	296.6	296.8	-	0.53	0.03
Loss	-	0.08	-	-	-	-	-	-
Total	-	6.20	-	-	-	3.22	2.71	0.19
% as ester	-	-	-	-	-	52.60	44.30	3.10
% as acid	-	-	-	-	-	52.50	44.40	3.10

TABLE No. 26.

DISTILLATION OF METHYL ESTERS OF LIQUID ACIDS AT 12 mm.

Fraction number	Temp. °C	Weight (gms.)	I.V.	S.E.	Mean M.W.	Methyl esters (Wt. gms.) Oleate	Linoleate
L_1	180-185	5.56	111.2	293.1	292.4	3.94	1.62
L_2	185-190	4.44	121.4	294.0	293.1	2.62	1.82
L_3	190-195	4.50	135.1	295.2	295.1	1.95	2.55
L_4	195-200	5.24	140.4	295.4	295.1	1.95	3.29
Residue	-	1.26	160.3	295.6	295.4	0.18	1.08
Loss	-	0.24	-	-	-	-	-
Total	-	21.24	-	-	-	10.64	10.36
% as ester	-	-	-	-	-	50.67	49.33
% as acid	-	-	-	-	-	50.70	49.30



CHARACTERIZATION OF FATTY ACIDS BY DESCENDING PAPER CHROMATOGRAPHY.

Characterization of acids:

The liquid ester fractions L_1 - L_4 and the residue were saponified and the liberated acids on oxidation with a cold dilute solution of alkaline potassium permanganate (18), gave a white solid, which was resolved into dihydroxystearic acid (m.p. and mixed m.p. 131°C), and tetrahydroxystearic acid (m.p. and mixed m.p. 170°C) by ethyl acetate, thus confirming the presence of oleic and linoleic acids in these fractions.

Similarly, the solid ester fractions S_1 - S_4 and the residue were saponified and the liberated fatty acids on oxidation gave only dihydroxystearic acid (m.p. and mixed m.p. 131°C), thereby indicating the presence of oleic acid in the fractions S_1 - S_4 and the residue. The unoxidised portion of the fatty acids was extracted with ethyl ether and the residue obtained on removal of the solvent, gave palmitic acid (m.p. and mixed m.p. 62°C) and stearic acid (m.p. and mixed m.p. 68°C) on fractional crystallisation from dilute acetone thereby confirming the presence of palmitic acid in the fractions S_1 - S_4 and the stearic acid in the fractions S_1 - S_4 and the residue.

The fatty acids liberated from different ester fractions were also confirmed by reversed phase descending paper chromatography (19) (Vide chapter 1; pages, 45-47).

CHEMICAL EXAMINATION OF THE UNSAPONIFIABLE MATTER:

Carpesterol has been isolated from the unsaponifiable matter of the seed oil of Solanum ferox Linn., and has been identified by its physical and chemical properties and by the preparation of its acetate and benzoate derivatives.

EXPERIMENTAL

Isolation and purification of the sterol:

Dry seeds of Solanum ferox Linn. were powdered and soxhletted with petroleum ether (b.p. 60-80°C). The oil obtained after removal of petroleum ether was purified by treating it with Fuller's earth and activated charcoal, and freed from volatile essential oil as far as possible by heating in Vacuo. It was saponified by refluxing on water bath for 6 hours with ethanolic potassium hydroxide solution. After saponification, the product was diluted with water and ethanol distilled off on water bath with the addition of corresponding amounts of water from time to time. The ethanol-free aqueous solution of the soap was extracted vigorously several times with ether. The ethereal extract was washed with distilled water, dried over anhydrous sodium sulphate and the solvent distilled off. On keeping the concentrate overnight in a refrigerator, a white solid separated, which on repeated crystallisation from methanol afforded shining white plates, yield (1.1 gms.);

m.p. 248°C.

Found: C, 78.69; H, 9.42; $C_{36} H_{54} O_4$ requires C, 78.54; H, 9.81%.

The substance was fairly soluble in boiling ethanol and methanol, sparingly soluble in hot benzene, chloroform, acetone, ethyl ether and petroleum ether. The compound developed a reddish purple colour in Liebermann-Burchard test. The compound was identified as carpesterol (15) by preparing its acetyl and benzoyl derivatives.

Preparation of the acetyl derivative:

The sterol (0.2 gms.) was refluxed over a water bath for about two hours with acetic anhydride (2 ml) and pyridine (1 ml). The reaction mixture was poured to crushed ice and kept in a refrigerator. The separated solid was filtered off, washed with water and crystallised from ethanol in colourless silky needles, m.p. 193°C.

Found: C, 77.24; H, 9.62; $C_{38} H_{56} O_5$ requires C, 77.02; H, 9.46%.

Preparation of the benzoyl derivative:

The sterol (0.2 gms.) was refluxed over a water bath for about two hours with benzoyl chloride (2 ml) and pyridine (1 ml). The reaction mixture was poured into ice cold water and kept in a refrigerator overnight. The separated solid was filtered off, washed with 2% aqueous potassium hydroxide solution (till free from

benzoic acid) and then with water and finally crystallised from ethanol in colourless silky needles, m.p. 216°C. Found: C, 78.96; H, 8.69; $C_{43}H_{58}O_5$ requires C, 78.89; H, 8.86%.

CHEMICAL EXAMINATION OF THE FRUITS:

From the fruits of Solanum ferox Linn., solanine was isolated in 0.3% yield. Solanine on acid hydrolysis gave solanidine and a mixture of glucose, galactose and rhamnose. Solanine and solanidine were characterized by their physical and chemical properties and by preparing their derivatives. The sugars were characterized by descending paper chromatographic technique.

EXPERIMENTAL

The alkaloid was isolated following the method of Pfankuch (20). The defatted fruits of Solanum ferox Linn. (4 kg.) were soxhletted with a mixture of 98% ethanol (4 litres) and glacial acetic acid (80 ml) for 36 hours. The ethanolic extract was concentrated to approximately 250 ml under reduced pressure and sodium sulphate (140 gms.) was added to it. The mixture was warmed on a water bath for 40 minutes, when a flocculent precipitate of proteins appeared. After cooling and adding 20% sulphuric acid (50 ml), the mixture was filtered and the residue washed with distilled water (250 ml). The filtrate was basified with strong ammonia and kept overnight in a refrigerator, when a precipitate which settled down, was filtered off and washed with 2% ammonia (60 ml). The crude precipitate was redissolved in dilute acetic acid, filtered and reprecipitated with ammonia.

The process was repeated four times. The product was dried at 100°C, finely powdered and extracted with hot amyl alcohol until the residue no longer gave a purple colour with concentrated sulphuric acid and formaldehyde. The amyl alcohol extract was concentrated under reduced pressure until the solution formed a gel. After removing the last traces of amyl alcohol, the gel was dissolved in 0.2% acetic acid, and then made alkaline with ammonia. On warming, a flocculent precipitate so obtained, was separated by centrifuging, and recrystallised from ethanol to give needle shaped crystals, yield (12.1 gms.), m.p. 282°C (after drying at 100°C with a shrinkage at 235°C), $[\alpha]_D^{20}$ - 59°40 (pyridine) (21).

Found: C, 62.12; H, 8.51; N, 1.58; $C_{45}H_{73}O_{15}N$ requires C, 62.28; H, 8.42; N, 1.61%.

The compound gave a positive test for alkaloids and was sparingly soluble in water, readily soluble in hot ethanol and amyl alcohol, but almost insoluble in ethyl ether, petroleum ether, chloroform and benzene. It did not reduce Fehling's solution but reduced ammonical silver nitrate. It formed crystalline hydrochloride and a acetyl derivatives.

Preparation of the hydrochloride (22):

The compound (0.4 gms.) was dissolved in hot ethanol and dilute hydrochloric acid (10 ml) was then added. On

adding ether to the above mixture and standing, the hydrochloride separated in a crystalline form. The crystals were separated and dried, m.p. 212°C

Preparation of the acetyl derivative (23):

The compound (0.4 gms.) was treated with acetic anyhydride (4 ml) and pyridine (2 ml). The mixture was refluxed on a sand bath for two hours. The reaction mixture was poured to crushed ice and kept in the refrigerator overnight. The separated solid was washed with water and crystallised from ethanol in needles, m.p. 204-205°C; $[\alpha]_D^{20}$ - 34°96.

Hydrolysis of the compound with hydrochloric acid (24):

The compound (2.0 gms.) was dissolved in 2.5% hydrochloric acid (2 litres) and heated on a boiling water bath for two hours. The solution was cooled, made slightly alkaline with ammonia and a flocculated precipitate was obtained by gently warming. The mixture was kept overnight in a refrigerator, the precipitate obtained was filtered and washed with 2% ammonia. The product and the hydrolysate were examined separately.

Examination of the crystalline product:

The crude product was recrystallised from ethanol in needle shaped crystals m.p. 219°C; $[\alpha]_D^{21}$ - 28°5 (ethanol).

Found: C, 81.46; H, 10.92; N, 3.54; $C_{27}H_{43}O_3N$ requires C, 81.61; H, 10.83; N, 3.52%.

It was slightly soluble in water, but soluble in ethanol and ethyl ether and did not reduce Fehling's solution and ammonical silver nitrate. The crystalline compound was characterized as solanidine by preparing its hydrochloride, methiodide and mono acetyl derivatives; and by ascending paper chromatographic technique.

Preparation of the hydrochloride:

The compound (0.2 gms.) was dissolved in hot ethanol and dilute hydrochloric acid (5 ml) was added. On adding ether to the above mixture and standing, the hydrochloride separated, which was crystallised from ethanol in rhombic crystals, m.p. 345°C (decomp). When this hydrochloride was heated, a molecule of water was eliminated and solanidene (25) was formed, m.p. 167°C.

Found: C, 85.51; H, 10.78; N, 3.63; $C_{27}H_{41}N$ requires C, 85.48; H, 10.81; N, 3.69%.

Preparation of the methiodide:

The compound (0.2 gms.) was refluxed on a water bath with methyl alcohol (6 ml) and methyl iodide (0.4 gms.). On removal of the solvent, the methiodide separated, which was crystallised from ethanol in brown needles, m.p. 280°C (decomp.).

Preparation of the monoacetyl derivative:

The compound (0.2 gms.) was treated with acetic anhydride (2 ml) and pyridine (0.5 ml). The mixture was

refluxed for about two hours on a water bath. The reaction mixture was poured to crushed ice and kept in a refrigerator overnight. The separated solid was washed with water and crystallised from ethanol, m.p. 206-208°C.

Ascending paper chromatography of the compound (26):

Whatman filter paper no. 1 (sheets 12 x 6") was impregnated with a mixture of formamide and acetone (3:7 v/v). The paper was dried in air for few minutes. 0.005-0.01 ml of a 2% chloroform solution of the compound was chromatographed on the paper. The chromatogram was developed for about ten hours at room temperature with a solution of formamide which was saturated with benzene-chloroform mixture (1:2 v/v). After development, the chromatogram was dried for 20 minutes in air and then for 2 hours at 110°C. It was sprayed with a 25% chloroform solution of antimony chloride. It was dried for 30 minutes at room temperature and then for 3 minutes at 75°C. The compound was located as a red spot with R_f value 0.56 and was characterized as solanidine.

Examination of the hydrolysate by descending paper chromatography (27):

0.004-0.01 ml of a 2% aqueous solution of different reference sugars and the hydrolysate were together chromatographed on Whatman filter paper no. 1 (sheets 24 x 12"). The chromatogram was developed with the upper layer of n-butanol-acetic acid-water (4:1:5 v/v) for

24 hours at room temperature. The chromatogram was air dried, sprayed with benzidine reagent (0.5 gms. benzidine, 10 ml acetic acid, 10 ml 45% trichloro acetic acid and 100 ml 95% ethanol), again air dried and the spots of sugars were located by heating the chromatogram at 90°C for about 5 minutes. The R_f values of a number of reference sugars were also determined and are recorded in table no. 27. Glucose, galactose and rhamnose were thus characterized from the hydrolysate.

TABLE No. 27

R_f VALUES OF SUGARS AT 25°C:

Serial number	Sugars	R_f value
1	Lactose	0.09
2	Maltose	0.11
3	Sucrose	0.14
4	Galactose	0.16
5	Glucose	0.18
6	Fructose	0.23
7	Xylose	0.28
8	Rhamnose	0.37

The gluco-alkaloid was thus confirmed as solanine. Solanine and its hydrolysis product, solanidine also responded to colour and precipitation tests (28), as shown in table no. 28.

TABLE No. 28

COLOUR AND PRECIPITATION TESTS FOR SOLANINE AND SOLANIDINE:

Serial No.	Reagent	Solanine (solid)	Solanidine (solid)
1.	Conc. sulphuric acid warmed	Yellow → red	Yellow → brown
2.	Resorcinol in glacial acetic acid + sulphuric acid warmed	Wine red → blood red → brownish red	Yellow → orange red → brown with green fluorescence
3.	Vanillin in glacial acetic acid + sulphuric acid warmed	Yellow → brownish yellow → reddish brown → blood red brown	Yellow → orange red → red → brownish red → dark brown → black
4.	Vanillin in glacial acetic acid + sulphuric acid cold	Brown ring at the junction of liquid, purple in acetic acid layer	As for solanine
5.	Iodine in potassium iodide solution	No precipitate	No precipitate
6.	Picric acid	No precipitate	No precipitate
7.	Tannic acid	Turbidity	No precipitate
8.	Phosphomolybdic acid	brown precipitate	No precipitate

CHAPTER VII

CHEMICAL EXAMINATION OF EUPHORBIA HIRTA LINN. AND EUPHORBIA THYMIFOLIA LINN.

CHEMICAL EXAMINATION OF EUPHORBIA HIRTA LINN:

Euphorbia hirta Linn. (Family: Euphorbiaceae; Hindi, Dudhi) is a small herb which grows throughout the hotter parts of India. This plant has been medicinally used in the diseases of the bowel complaints, cough, dysentery, colic pains, bronchial affections and asthma (1).

The botanical description of this plant is as follows(2):

An erect or decumbent roughly hairy herb, 8" to 2 ft. high, with opposite unequal sided serrulate elliptic-oblong obovate or oblong. Lanceolate leaves, 0.75-1.5" long, with acute or cuneate tip. Involucres minute, 0.04-0.05" long, crowded in capitate finally peduncled axillary cymes, hairy, campanulate, with four shortly stipitate red tipped glands without a limb or with a minute flushy rounded green or white limb. Pubescence often curly. Leaves sometimes sub-rhomboid, the upper extremity nearly always acute; one variety prostrate with leaves under 1" long and with a black patch in the centre of each, lower surface pale and hispidly hairy on the 3-4 rather strong subflabellate nerves, hairs sometimes red or brown, those on the stem usually coarse and finer silky. Petiole 0.12-0.20" long, stipules of glands minute. Cymes always congested, at first sub-sessile but peduncle elongate and finally sometimes forked and sometimes 1" long, from nearly all the alternate axils. Involucre minute, only 0.04" long.

Styles 2 fid to base, capsule hairy, 0.05" long, seed oblong, reddish, 3 keeled and faintly transversely rugose. Stem contains a white milky juice called latex.

Although some varieties of Euphorbia hirta viz., procumbens, pedilantus calcaratus and pedilantus tehuacanus have been chemically examined by earlier workers (3), no systematic chemical examination of Euphorbia hirta Linn., seems to have been undertaken so far. It was, therefore, considered worthwhile to take up a systematic chemical examination of this plant.

Petroleum ether and ethanol extracts of the stems of Euphorbia hirta Linn. were submitted to chromatography over Brockmann alumina, and using petroleum ether, benzene, chloroform and their mixtures as eluants, myricyl alcohol, taraxerol, friedelin, β-sitosterol, hentriacontane and β-amyrin were isolated, while the ethanol extract of the flowers of Euphorbia hirta Linn. gave only ellagic acid. The above compounds were characterized by their physical and chemical properties and by the preparation of their derivatives.

EXPERIMENTAL

The air dried, powdered stems (2 kg.) were successively soxhletted with petroleum ether (b.p. 60-80°C) and ethanol for 30 hours.

Examination of the petroleum ether extract of the stems:

The petroleum ether extract was concentrated and left in a refrigerator overnight, when a white solid separated, which was filtered and dissolved in petroleum ether and then chromatographed over Brockmann alumina using petroleum ether, benzene, chloroform and their mixtures as eluants as shown in the table no. 29. Eluants were collected in fractions of 30-40 ml each and evaporated to dryness.

TABLE No. 29

Fractions	Eluants	Residue left on evaporation of solvents
1-14	Petroleum ether(b.p. 60-80°C)	Waxy material
15-24	Petroleum ether+Benzene(5:1 v/v)	Colourless solid
25-32	Petroleum ether+Benzene(3:2 v/v)	Colourless solid
33-40	Petroleum ether+Benzene(1:1 v/v)	Nil
41-49	Benzene	Short needles
50-58	Benzene+Chloroform (3:2 v/v)	Shining flakes
59-66	Benzene+Chloroform (1:1 v/v)	Shining flakes
67-72	Chloroform	Nil

Fractions 1-14 gave a waxy material which on repeated crystallisation from ethanol afforded silky needles (1.2 gms.), m.p. 84°C.

Found: C, 82.14; H, 14.12; $C_{30}H_{62}O$ requires C, 82.19; H, 14.15%.

The compound was soluble in benzene, chloroform, ethyl ether and petroleum ether and was insoluble in water. It was identified as myricyl alcohol by mixed melting point with an authentic sample and by preparation of its acetyl and chloro derivatives.

Preparation of the acetyl derivative (4):

The compound (0.2 gms.) was refluxed with acetic anhydride (4 ml) and pyridine (1 ml) on a water bath for about half an hour. On keeping the contents in a refrigerator overnight, a solid product was obtained, which was washed several times with distilled water, and on crystallisation from ethanol afforded a colourless crystalline derivative, m.p. 71.5°C.

Found: C, 79.84; H, 13.46; $C_{32}H_{64}O_2$ requires C, 80.00; H, 13.33%.

Preparation of the chloro derivative (4):

The compound (0.2 gms.) was refluxed with phosphorous pentachloride and phosphorous oxychloride on a water bath for about two hours. The solid, which separated, was crystallised from methanol, and gave a crystalline derivative, m.p. 64°C.

Found: C, 78.92; H, 13.46; Cl, 7.50; $C_{31}H_{63}Cl$ requires C, 79.06; H, 13.39; Cl, 7.54%.

The fractions 15-32 gave a colourless solid which when crystallised from methanol-chloroform mixture afforded

needle shaped crystals (1.1 gms.), m.p. 281°C.
Found: C, 84.46; H, 11.71; C_{30} H_{50} O requires C, 84.51; H, 11.74%.

This compound gave colour reactions characteristic of triterpenes including Noller's test. A sequence of colour (Yellow ——→ orange ——→ red ——→ purple) was developed when the compound was treated with thionyl chloride in presence of tin. The compound was identified as taraxerol by mixed melting point with an authentic sample and by preparation of its acetate and benzoate derivatives.

Preparation of the acetate (5):

The compound (0.2 gms.) was refluxed over water bath for 45 minutes with acetic anhydride (10 ml). The reaction mixture was poured over crushed ice and filtered, the solid separated was crystallised from methanol in colourless crystals, m.p. 304°C.
Found: C, 81.94; H, 11.18; C_{32} H_{52} O_2 requires C, 82.06; H, 11.11%.

Preparation of the benzoate(5):

The compound (0.2 gms.) was mixed with chloroform (1 ml), pyridine (0.2 ml) and benzoyl chloride (0.4 ml). On leaving the mixture overnight at room temperature, a crude solid product was obtained. It was dissolved in a minimum amount of benzene and submitted to chromatography over Brockmann alumina using benzene as the eluant. On

evaporation of the solvent and recrystallisation from chloroform-methanol mixture, glistering prisms were obtained. m.p. 292°C.

Found: C, 83.51; H, 10.26; $C_{37}H_{54}O_2$ requires C, 83.77; H, 10.18%.

The fractions 41-49 gave short needles, which on crystallisation from benzene afforded colourless needles (2.4 gms.), m.p. 260-261°C.

Found: C, 84.44; H, 11.82; $C_{30}H_{50}O$ requires C, 84.51; H, 11.74%.

The compound gave no colour reaction in Salkowski or Liebermann-Burchard reaction or with the tetranitromethane reagent. The compound was identified as friedelin by mixed melting point and by preparing its derivatives i.e. friedelinol, oxime, oxime acetate and friedelin-2,4-dinitrophenyl hydrazone.

Preparation of friedelinol (6):

A solution of friedelin (0.5 gms.) in isoamyl alcohol (50 ml) was heated with sodium metal (7 gms.) for two hours on an oil bath. The solvent was steam distilled and the residue on recrystallisation from a mixture of benzene-ethyl acetate gave colourless plates, m.p. 299°C.

Found: C, 84.00; H, 12.32; $C_{30}H_{52}O$ requires C, 84.11; H, 12.15%.

Preparation of the oxime (7):

1.0 gms. of friedelin was dissolved in 20 ml of benzene containing 5 ml of ethanol, and 0.35 gms. of hydroxylamine hydrochloride dissolved in 2.5 ml of ethanol was then added. 0.3 gms. of potassium hydroxide dissolved in 2.5 ml of ethanol was added to the reaction mixture through the reflux condenser, and the contents were refluxed for one hour, cooled and poured into 40 ml of water. The resulting solution was acidified with sulphuric acid and the product was filtered, washed and on recrystallisation from benzene-ethyl acetate mixture (2:1 v/v) gave thin hexagonal plates (0.75 gms.), m.p. 292°C.

Found: C, 81.42; H, 11.48; N, 3.21; $C_{30}H_{51}NO$ requires C, 81.63; H, 11.56; N, 3.17%.

Preparation of friedelin oxime acetate (7):

0.6 gms. of friedelin oxime was dissolved in 20 ml of acetic anhydride and refluxed for two hours. The acetate which separated from the cold solution was recrystallised from ethyl acetate, from which it separated in the form of hexagonal plates, m.p. 239°C.

Found: C, 79.43; H, 11.14; N, 3.16; $C_{32}H_{53}NO_2$ requires C, 79.50; H, 10.97; N, 2.89%.

Preparation of friedelin-2,4-dinitrophenyl hydrazone (7):

0.5 gms. of friedelin was dissolved in 60 ml of

hot methyl alcohol and 6.3 gms. of 2, 4-dinitrophenyl hydrazine were added. The mixture was warmed till it gave a homogeneous solution. Two drops of concentrated hydrochloric acid were added and the mixture boiled for 5-10 minutes. On cooling, the solution deposited an orange coloured mass, which on crystallisation from benzene afforded orange coloured crystals, m.p. 297-298°C (decomp.).

Found: C, 71.41; H, 9.08; N, 9.28; $C_{36}H_{54}N_4O_4$ requires C, 71.28; H, 8.91; N, 9.24%.

The fractions 50-66 gave shining flakes which on repeated crystallisation from methanol-ethyl acetate mixture yielded, colourless flakes (1.4 gms.), m.p. 136-137°C.

Found: C, 84.15; H, 12.16; $C_{29}H_{50}O$ requires C, 84.05; H, 12.08%.

The compound was fairly soluble in benzene, methanol and ethanol; and readily soluble in chloroform, ethyl ether and ethyl acetate. The compound gave a positive Salkowski reaction and Liebermann-Burchard reaction. It formed an acetate (8), m.p. 126°C, $[\alpha]_D^{25}$ - 42.0 (chloroform), (Found: C, 81.51; H, 11.36; $C_{31}H_{52}O_2$ requires C, 81.57; H, 11.40%), a benzoate (8), m.p. 144°C, $[\alpha]_D^{25}$ - 14.5 (chloroform), (Found: C, 83.31; H, 10.36; $C_{36}H_{54}O_2$ requires C, 83.39; H, 10.42%) and a digitonide (9), m.p. 230°C (decomp.), (Found: C, 61.73; H, 8.53; $C_{29}H_{50}O \cdot C_{55}H_{90}O_{29}$ requires C, 61.91; H, 8.60%). The compound was

identified as β-sitosterol by mixed melting point and by the formation of a mixed chromatogram of the compound with an authentic sample of β-sitosterol on a column of Brockmann alumina .

The details of the preparation of the acetate, benzoate, digitonide derivatives and the formation of a mixed chromatogram of the compound with an authentic sample of β-sitosterol have already been described (Vide chapter 1; page, 51-53).

Examination of the ethanol extract of the stems:

The ethanol extract was concentrated under reduced pressure and keeping it overnight in a refrigerator left a greenish waxy material which was dissolved in petroleum ether and then chromatographed over Brockmann alumina using petroleum ether, benzene, chloroform and their mixtures as eluants as shown in table no. 30. Eluants were collected in fractions of 30-40 ml each and evaporated to dryness.

TABLE No. 30

Fractions	Eluants	Residue left on evaporation of solvents
1-10	Petroleum ether (b.p. 60-80°C)	Waxy material
11-17	Petroleum ether+Benzene(3:2 v/v)	White solid
18-25	Petroleum ether+Benzene(1:1 v/v)	White solid
26-30	Benzene	Nil
31-35	Benzene + Chloroform (3:2 v/v)	Nil
36-40	Benzene + Chloroform (1:1 v/v)	Nil
41-44	Chloroform	Nil

Fractions 1-10 gave a waxy material which on repeated crystallisation from a mixture of methanol-acetone-benzene (1:1:1 v/v) gave a colourless solid (2:2 gms.), m.p. 68°C. Found: C, 85.44; H, 14.71; $C_{31}H_{64}$ requires C, 85.32; H, 14.67%.

Its infra red absorption spectrum did not indicate the presence of any functional groups. It did not absorb bromine and on oxidation with air in presence of potassium permanganate and boric acid at 200°C gave an alcohol (10), m.p. 58°C. The compound was identified as hentriacontane (11).

The fractions 11-25 gave a white solid which on crystallisation from chloroform-methanol mixture afforded colourless needles (1.3 gms.), m.p. 199°C; $[\alpha]_D^{25}$ + 87.0° (chloroform).

Found: C, 84.46; H, 11.78; $C_{30}H_{50}O$ requires C, 84.51; H, 11.74%.

The substance gave a positive Liebermann-Burchard reaction for a triterpene. It formed an acetate (12), m.p. 237°C; $[\alpha]_D^{25}$ + 80.0° (chloroform), which produced a yellow colouration with tetranitromethane.

(Found: C, 81.92; H, 11.04; $C_{32}H_{52}O_2$ requires C, 82.06; H, 11.11%); a benzoate (12), m.p. 232°C; $[\alpha]_D^{25}$ + 101.1° (chloroform); (Found; C, 83.61; H, 11.24; $C_{37}H_{54}O_2$ requires C, 83.77; H, 10.18%) and a p-nitrobenzoate (12), m.p. 258°C; $[\alpha]_D^{25}$ + 95.6° (chloroform), (Found: C, 77.06;

H, 9.41; N, 2.48; $C_{37}H_{53}NO_4$ requires C, 77.22; H, 9.22; N, 2.43%). The compound was identified as β-amyrin by the formation of a mixed chromatogram of the compound with an authentic sample of β-amyrin.

The details of the preparation of the acetate, benzoate, p-nitrobenzoate derivatives and the formation of the mixed chromatogram with β-amyrin have been described previously (chapterIII; pages, 96-97).

Examination of the flowers:

Fresh flowers of Euphorbia hirta Linn. (2 Kg.) were soxhletted with ethanol for 30 hours. Ethanol was distilled off under reduced pressure and the sticky mass left taken up in water (100 ml). The aqueous solution was extracted with petroleum ether to remove the fatty material and chlorophyll, and then extracted with ethyl ether in a liquid-liquid extractor. Ether was removed and the residue left taken up in methanol, filtered and distilling off the methanol afforded a yellow solid, which on crystallisation from pyridine gave yellow crystals (4.2 gms.), m.p. $> 360^{\circ}C$.

Found: C, 55.64; H, 2.02; $C_{14}H_6O_8$ requires C, 55.62; H, 1.98%.

The compound gave a green colour with ferric chloride solution, dissolved in aqueous sodium hydroxide with a bright yellow colour and gave a positive Griessmeyer's reaction. The compound developed a red colour when heated

with nitric acid containing a little nitrous acid. It was identified as ellagic acid by mixed melting point determination with an authentic sample and by preparation of its tetra-acetate and a ellagorubin.

Preparation of the tetra-acetate (13):

The compound (0.2 gms.) was acetylated with acetic anhydride (4 ml) and pyridine (0.5 ml) at 30°C for 30 hours. The reaction mixture was poured over crushed ice and filtered, the separated solid was crystallised from dioxane-petroleum ether mixture in colourless small prisms, m.p. 342-343°C.

Preparation of ellagorubin (14):

Ellagic acid pyridine free (3.0 gms.), 5% aqueous solution of sodium hydroxide (20 ml) and benzyl chloride (8 ml) were mixed and immersed in a water bath maintained at 70°C. 20% aqueous sodium hydroxide (10 ml) was then added drop by drop with mechanical stirring for 1 hour. Stirring was continued for an additional half an hour. 50% aqueous potassium hydroxide (5 ml) was then added and stirring was continued for 1 hour. After cooling at 0°C for 2 hours in a refrigerator, the aqueous layer was decanted from the oily black product which had separated from the reaction mixture. The black product was washed successively with water (40 ml), methanol (60 ml) and ethyl ether (60 ml). The residual gum was then washed

with acetone (100 ml) and the brown acetone solution free from sodium and potassium salts (as they are not soluble in acetone) was filtered from the deep blue undissolved solid. The blue solid was suspended in water (300 ml) containing few drops of concentrated ammonia, heated to boiling and filtered. The treatment of unoxidised solid once more with boiling water (200 ml) gave a colourless insoluble crystalline solid (0.16 gms.), recrystallisation of the solid with dioxane-methanol mixture afforded colourless needles, m.p. 235-236°C. The deep blue aqueous filtrates were combined and on acidification with hydrochloric acid gave orange red precipitate, which was filtered, dried and recrystallised from acetone in orange red prisms (1.4 gms.), m.p. 218-219°C.
Found: C, 76.26; H, 4.43; $C_{42}H_{30}O_8$ requires C, 76.13; H, 4.53%.

It gave an intense blue-violet colouration with alcoholic ferric chloride solution. The compound was identified as ellagorubin by its acetylation and methylation products.

Acetylation of the compound:

The compound was acetylated for 5 minutes by boiling with acetic anhydride. The acetyl derivative was crystallised successively with dilute acetic acid and from methanol-acetone mixture in golden yellow rectangular plates, m.p. 195°C (Ellagorubin diacetate).

Found: C, 73.84; H, 4.63; $C_{46}H_{34}O_{10}$ requires C, 74.0; H, 4.59%.

Methylation of the compound:

Methylation of the compound with excess of ethereal diazomethane gave an orange methyl ether which separated in rhombs from chloroform-hexane mixture, m.p. 241°C.

Paper chromatography of the compound with ellagic acid (15):

The compound was further confirmed as ellagic acid by descending paper chromatography and also by co-chromatography of the compound with an authentic sample of ellagic acid.

A 2% methanol solution of the compound along with an authentic sample of ellagic acid was chromatographed (0.005-0.01 ml) on Whatman filter paper no. 1. The chromatogram was developed for 3 hours in the descending technique with an aqueous solution of 60% formamide buffered at pH 3.5 with formic acid. The chromatogram was then taken out, air dried and exposed to ammonia vapours for half an hour in a chamber. The compound and ellagic acid were located as yellow spots with an identical R_f value (0.58). Similarly the co-chromatography of the compound with an authentic sample of ellagic acid was done, and only a single yellow spot with R_f value 0.58 was obtained on the chromatogram.

CHEMICAL EXAMINATION OF EUPHORBIA THYMIFOLIA LINN:

Euphorbia thymifolia Linn. (Family: Euphorbiaceae; Hindi; Chotidudhi) is a small herb, which grows throughout India in the plains and lower hills. This plant has been medicinally used in diseases of the bowel complaints, ringworm, snake bite and skin diseases (16).

The botanical description of the plant is as follows:

A small annual herb, more or less hispidly pubescent; stems prostrate, divaricately branched, slender, cylindric, more or less hairy. Leaves opposite, very small, numerous, 1/8 - 1/4 by 1/10 - 1/6 inch, obliquely oblong or elliptic oblong, rounded at the apex, glabrous above, slightly pubescent beneath, base rounded, very unequal-sided; petioles very short; stipules fimbriate. Involucres axillary, solitary or 2-3 in an axil, campanulate, 1/30 inch long; stalk very short; glands minute; capsules 1/16 inch long, obtusely keeled, pubescent; styles short, 2-fid. Seeds 1/20 inch long, quadrangular, bluntly pointed.

No chemical examination seems to have been undertaken except the isolation of 5, 7, 4-trihydroxy flavone-7-glucoside from the leaves and stems (17); and the chemical examination of essential oil constituents from this plant (18). It was, therefore, considered of interest to undertake a systematic chemical examination of Euphorbia thymifolia Linn.

Myricyl alcohol, taraxerol and tirucallol have been isolated from the petroleum ether extract and hentriacontane from the ethanol extract of Euphorbia thymifolia Linn.

EXPERIMENTAL

The air dried, powdered plant (2 kg.) was soxhletted with petroleum ether (b.p. 60-80°C) and ethanol successively for 30 hours.

Examination of the petroleum ether extract:

The petroleum ether extract was concentrated and left in a refrigerator overnight, when a white solid separated, which was filtered and dissolved in petroleum ether and then chromatographed over Brockmann alumina using petroleum ether, benzene, chloroform and their mixtures as eluants as shown in the table no. 31. Eluants were collected in fractions of 30-40 ml each and evaporated to dryness.

TABLE No. 31

Fractions	Eluants	Residue left on evaporation of solvents
1-12	Petroleum ether (b.p. 60-80°C)	Waxy material
13-20	Petroleum ether+Benzene(5:1 v/v)	Colourless solid
21-29	Petroleum ether+Benzene(3:2 v/v)	Colourless solid
30-34	Petroleum ether+Benzene(1:1 v/v)	Nil
35-48	Benzene	White solid
49-54	Benzene+Chloroform(5:1 v/v)	White solid
55-58	Benzene+Chloroform(3:2 v/v)	Nil
59-63	Benzene+Chloroform(1:1 v/v)	Nil
64-68	Chloroform	Nil

Fractions 1-12 gave a waxy material which on repeated crystallisation from ethanol afforded silky needles (1.0 gms.), m.p. 85°C.

Found: C, 82.24; H, 14.05; $C_{30}H_{62}O$ requires C, 82.19; H, 14.15%.

The compound was soluble in benzene, chloroform, ethyl ether and petroleum ether and was insoluble in water. It was identified as myricyl alcohol by mixed melting point determination with an authentic sample and by preparation of its acetyl derivative (4), m.p. 72°C; (Found: C, 79.92; H, 13.39; $C_{32}H_{64}O_2$ requires C, 80.0; H, 13.33%), and a chloro derivative (4), m.p. 64°C; (Found: C, 78.90; H, 13.42; Cl, 7.51; $C_{31}H_{63}Cl$ requires C, 79.06; H, 13.39; Cl, 7.54%).

The fractions 13-29 gave a colourless solid which when crystallised from methanol-chloroform mixture afforded needle shaped crystals (1.2 gms.), m.p. 282°C.

Found: C, 84.42; H, 11.65; $C_{30}H_{50}O$ requires C, 84.51; H, 11.74%.

This product gave colour reactions characteristic of triterpenes including Noller's test. A sequence of colour (Yellow ⟶ orange ⟶ red ⟶ purple) was developed when the compound was treated with thionyl chloride in presence of tin. The compound was identified as taraxerol by mixed melting point determination and by

preparation of its acetate (5), m.p. 304°C; (Found: C, 81.91; H, 11.13; $C_{32}H_{52}O_2$ requires C, 82.05; H, 11.11%) and a benzoate (5), m.p. 291°C; (Found: C, 83.62; H, 10.21; $C_{37}H_{54}O_2$ requires C, 83.77; H, 10.18%).

The fractions 35-54 gave a white solid which was rechromatographed over Brockmann alumina using petroleum ether (b.p. 60-80°C) as the eluant. The product on repeated crystallisation from methanol afforded needle shaped crystals (0.8 gms.), m.p. 133-134°C; $[\alpha]_D^{20}$ + 4°.5 (benzene).

Found: C, 84.41; H, 12.00; $C_{30}H_{50}O$ requires C, 84.51; H, 11.74%.

It gave a positive Liebermann-Burchard reaction and was characterized as tirucallol by preparing its acetate and benzoate derivatives.

Preparation of the acetate (19):

The compound (0.1 gms.) was heated at 100°C for 90 minutes with acetic anhydride (5 ml) and pyridine (5 ml). The reactants were poured in water and the solid product was separated, which on crystallisation with acetone gave long needles, m.p. 164°C; $[\alpha]_D^{20}$ - 16°.7 (benzene).

Found: C, 81.76; H, 11.15; $C_{32}H_{52}O_2$ requires C, 82.05; H, 11.11%.

Preparation of the benzoate (19):

The compound (0.15 gms.), pyridine (1 ml) and benzoyl chloride (1 ml) were heated at 100°C, giving a colourless

solid which was purified by running an ethereal solution of it through a column of Brockmann alumina and then crystallised from acetone-methanol mixture to give tirucallyl benzoate in flat plates, m.p. 149-150°C; $[\alpha]_D^{20}$ + 10°.8 (benzene).

Found: C, 84.00; H, 10.64; $C_{37}H_{54}O_2$ requires C, 83.77; H, 10.18%.

Examination of the ethanol extract:

The ethanol extract was concentrated under reduced pressure and keeping it overnight in a refrigerator left a greenish waxy material which on repeated crystallisation from a mixture of benzene-acetone-methanol (1:1:1 v/v) gave a colourless solid (1.8 gms.), m.p. 69°C.

Found: C, 85.48; H, 14.76; $C_{31}H_{64}$ requires C, 85.32; H, 14.67%.

Its infra red absorption spectrum did not indicate the presence of any functional groups. It did not absorb bromine and on oxidation with air in presence of potassium permanganate and boric acid at 200°C gave an alcohol (10), m.p. 58°C. The compound was identified as hentriacontane (11).

FREE ORGANIC ACIDS OF EUPHORBIA HIRTA LINN AND EUPHORBIA THYMIFOLIA LINN:

Euphorbia hirta Linn. and Euphorbia thymifolia Linn. were chemically examined with a view to characterizing free non-volalite organic acids present in them. Succinic and fumaric acids were detected in the stems and leaves of Euphorbia hirta Linn. and only fumaric acid in Euphorbia thymifolia Linn.

Considerable work has been done on organic acids in plants (20-22). Hajarnavis (23) has studied the pattern of free organic acids in Euphorbia tirucalli and Euphorbia antiquorum. No work seems to have been done so far on the organic acids present in Euphorbia hirta Linn. and Euphorbia thymifolia Linn. These plants were, therefore, chemically examined for the characterization of free organic acids occuring in the stems and leaves.

EXPERIMENTAL

The free organic acids from the stems and leaves were extracted with 80% neutral ethanol following the method of Dhabolkar, Joshi and Bharucha (21). 0.5 gms. of the dry stems and leaves of both the plants were separately grounded with 80% neutral ethanol (100 ml) in a mortar and the extracts were kept in a refrigerator overnight. The contents were filtered and concentrated to 5 ml and then examined by descending paper chromatographic technique (24).

Characterization of free organic acids:

The ethanolic extracts of stems and leaves were chromatographed on Whatman filter paper No.1 (sheets 24 x 12") along with a number of reference organic acids (0.002 ml of 1% aqueous solutions). The upper layer of n-butanol-formic acid-water (10:2:5 v/v) was used as the solvent phase. The chromatogram, after development by descending technique for 24 hours, was first air dried overnight at room temperature and then again dried at 75-80°C for about one hour. The acids were located as lemon yellow spots on the chromatogram by spraying it with bromo phenol blue (0.08 gms. in 100 ml of 95% ethanol). The R_f values of a number of reference organic acids were also determined and are recorded in table no. 32.

TABLE No. 32

R_f VALUES OF ORGANIC ACIDS:

Serial number	Organic acids	R_f value
1.	Oxalic acid	0.12
2.	Tartaric acid	0.21
3.	Citric acid	0.32
4.	Malic acid	0.36
5.	Malonic acid	0.41
6.	Succinic acid	0.66
7.	Fumaric acid	0.75

The free organic acids present in the stems and leaves of these plants have been given in table no. 33.

TABLE No. 33

FREE ORGANIC ACIDS IN EUPHORBIA HIRTA LINN AND EUPHORBIA THYMIFOLIA LINN.

Name of the plant	Part of the plant	O	T	C	M	Mal	S	F
Euphorbia hirta Linn.	Stems	-	-	-	-	-	+	+
	Leaves	-	-	-	-	-	+	+
Euphorbia thymifolia Linn	Stems	-	-	-	-	-	-	+
	Leaves	-	-	-	-	-	-	+

where +, present; -, absent; O, oxalic; T, tartaric; C, Citric; M, malic; Mal, maloniс; S, succinic; F, fumaric acids.

Although succinic acid was detected in both these plants during flowering condition, Euphorbia thymifolia Linn. did not indicate its presence even in the matured condition. The absence of citric, tartaric, and malic acids etc. shows that the acids are not present in the free state but they may be associated with inorganic cations to form salts or they might have been converted into some other acids in the Kreb's cycle which is working in the plants. It is, however, interesting to note that the pattern of free organic acids in Euphorbia hirta Linn. and Euphorbia thymifolia Linn. is different from other Euphorbia species studied by previous workers (23).

ABSTRACT

Investigations have been carried on four Indian medicinal plants viz.,Litsea consimilis (Family: Lauraceae); Solanum ferox Linn. (Family: Solanaceae); Euphorbia hirta Linn. and Euphorbia thymifolia Linn. (Family: Euphorbiaceae), which have not been chemically examined so far. From these plants, various organic compounds have been isolated and chemically examined.

Litsea consimilis:

The seed fat was chemically examined and found to consist of the glycerides of capric 1.7%; lauric 59.1%; myristic 11.5% and oleic acids 27.7%. This seed fat was found to be different from the seed fats of other Litsea species (Indian genera) which were reported by earlier workers (1) to contain the glycerides of lauric and oleic acids only. The unsaponifiable matter was characterized as β-sitosterol by determining its physical and chemical properties, and preparing its derivatives. The essential oil constituents were separated from the seeds and identified as α-d-pinene, cineole, ocimene, dipentene, linalool, citronellal and citral. Although, the majority of the constituents of this essential oil were found to be quite different from the essential oil constituents of the seeds of other Litsea species already studied (2), some of them were found to be identical with those occurring in the essential oils of the leaves of Litsea cubeba (3) and Litsea zeylanica (4).

The fatty acid composition of the fruit coat fat was found to be capric 4.51% ; lauric 52.54% and oleic acids 42.95%. The unsaponifiable matter contained β-sitosterol. The amino acid make-up of the fruit coat was determined by descending paper chromatographic technique and found to be histidine, glutamic acid, alanine, tyrosine, valine, phenyl alanine and leucine. The amino acid make-up of the fruit coat was found to be slightly different from those of the seeds (5) which contained proline and cystine in addition to the amino acids found in the fruit coats.

The bark fat on chemical analysis has been found to contain the glycerides of capric 2.15%; lauric 61.04% and oleic acids 36.81%. The petroleum ether extract of the bark on concentrating and submitting to chromatography over Brockmann alumina gave β-amyrin and β-sitosterol.

The defatted bark on treatment with rectified spirit and acetic acid gave an aporphine alkaloid which was characterized as laurotetanine. From the seeds, another alkaloid was isolated, the structure of which could not be elucidated due to its poor yield.

The lubricating properties of the seed fat of Litsea consimilis were examined. It can fruitfully be employed as a lubricant in machines where the temperatures developed during the operation are not very high. The seed fat was also used in the preparation of alkyd resins.

It was observed that the linseed oil can successfully be replaced in parts by this seed fat to produce plasticizing alkyds. These modified resins have superior adhesion, better scratch resistance and are more durable than the straight linseed oil alkyds.

Solanum ferox Linn.:

The seed fat of Solanum ferox Linn. on extraction with petroleum ether, gave a yellow coloured oil, which on keeping left a sticky mass. It was crystallised from absolute alcohol and characterized as solanocarpone. The fatty acid composition of the oil was determined and found to be palmitic 12.15%; stearic 9.96%; oleic 39.83% and linoleic acids 38.06%. The unsaponifiable matter contained carpesterol. From the fruits of this plant, the alkaloidal content was isolated and characterized as solanine.

Euphorbia hirta Linn. and Euphorbia thymifolia Linn.:

On chromatographing the petroleum ether and ethanol extracts of the stems of Euphorbia hirta Linn. over Brockmann alumina, myricyl alcohol, taraxerol, friedelin, β-sitosterol, hentriacontance and β-amyrin were isolated, while the ethanol extract of the flowers gave only ellagic acid. The above compounds were characterized by their physical and chemical properties and by the preparation of their derivatives.

Myricyl alcohol, taraxerol and tirucallol have

been isolated from the petroleum ether extract and hentriacontane from the ethanol extract of Euphorbia thymifolia Linn.

The stems and leaves of these plants were chemically examined for the characterization of free organic acids occuring in them. Succinic and fumaric acids were found to be present in the stems and leaves of Euphorbia hirta Linn., while only fumaric acid was detected in the stems and leaves of Euphorbia thymifolia Linn. The free organic acid pattern of Euphorbia hirta Linn. and Euphorbia thymifolia Linn. was found to be different from other Euphorbia species investigated by other workers (6).

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