

CHEMICAL INVESTIGATION OF *STERCULIA LYCHNOPHORA* HANCE FRUITS

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สำนักหอสมุดกลาง มหาวิทยาลัยศรีนครินทรวิโรฒ

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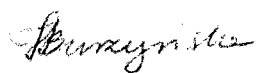
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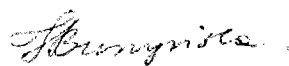
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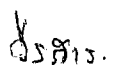
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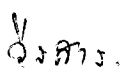
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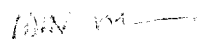
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Surachai Ungkanasayun

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Chapter I

INTRODUCTION

Sterculia lychnophora Hance (order Malvales, family Sterculiaceae) synonymous *Scaphium marcropodum* Beaumée (Phungtha-lai : พงทะลาย) (เดิม สมิติกานนท์ 2523 : 316) a small to medium tree of tropical rain forest, is found in Indo-China, Thailand, the Malay Peninsula, Sumatra and Borneo. According to Perry (Perry. 1980 : 399), "its seeds are used as medicine. The drug is the mucilaginous substance obtained from soaking the seeds in water for a few hours or overnight. It is remedial for all fevers, phthisis, hemorrhage from the nose, stomach, bowels or bladder, to counteract poison, sunstroke, opthalmia, toothache, intestinal worms, hemorrhoids, dry cough, fever in the marrow, to bring on the eruption of smallpox, and it is difficult to enumerate all of its medicinal virtues ..."

There is no report in literature on the chemical constituents of *Sterculia lychnophora*, however there are many reports on the same and different genera within the family. Many compounds belonging to the classes of lipids, steroids, alkaloids, flavonoids and coumarins were isolated and their structures have been established.

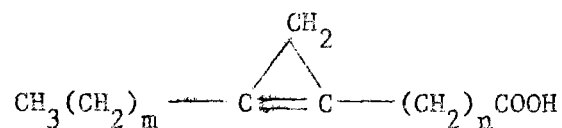
We have undertaken chemical investigation of *Sterculia lychnophora* fruits as a part of a research project under Thailand Institute of Scientific and Technological Research Programme titled "Development in Quality Improving and Controlling for Medicinal Plants, *Sterculia lychnophora* (Phungtha-lai), for Export".

RELATED LITERATURE

Investigation of Toxic Cyclopropenoid Fatty Acids in Plants of the Order Malvales.1. Occurrence and Isolation

Chemical and pharmacological properties of cyclopropenoid fatty acids (CPFA) present in many plants of the Sterculiaceae and other plant families of the same order Malvales¹ were studied very intensively.

The general formular of the CPFA is shown below.

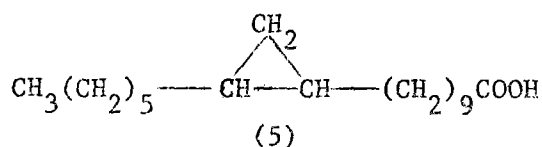
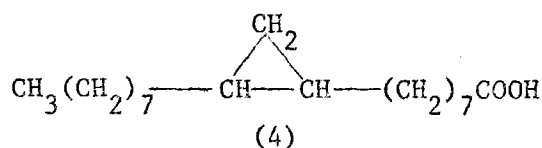
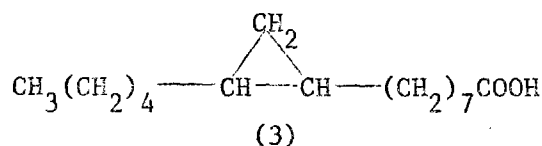


Two most common representatives of this class of compounds, sterculic acid (1)(m = n = 7) and malvalic acid (2)(m = 7, n = 6), are found in many seed oils of the order Malvales which comprises several important sources of food for man and animals. They frequently occur together.

The content of CPFA can vary substantially from one species to another. For example, *Ceiba pentandra* (Bombacaceae), *Sterculia monosperma* (Berry. 1980 : 224 - 227), *Firmiana platanifolia* (Shimadate, Iino and Hosoyama. 1976 : 33 - 93), *Sterculia foetida* (Schnieder, Loke and Hopkins. 1968 : 585 - 590) (Sterculiaceae) and *Gossypium herbaceum* (Mattson. 1973 : 189) (Malvaceae) were found to contain 10.1%, 51.6%, 15%, 55.5% and 1% of CPFA respectively in their seed oils. CPFA are also found in roots of some plants of this order, e.g. *Sterculia foetida* and *Ceiba pentandra* or kapok (Kaimal and Lakshminarayana. 1970 : 2225 - 2229).

Presence of CPFA is considered as a chemotaxonomic feature of the order Malvales (Shortland. 1963 : 288).

Although CPFA are usually found in plants, some closely related unusual fatty acids with cyclopropane ring, e.g. cis-9, 10-methylenehexadecanoic acid (3), dihydrosterculic acid (4) and lactobacillic acid (5) were also isolated from some bacteria (Dauchy and Asselineau. 1960 : 2635 - 2640 ; Asselineau. 1961 : 109 - 119).



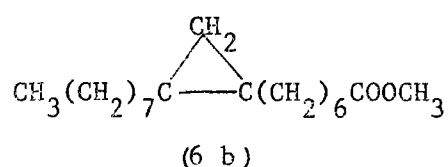
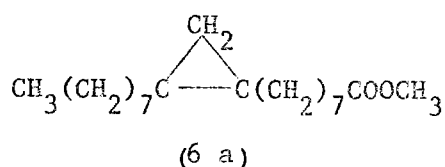
In general, the isolation procedures used by the various workers in their studies of CPFA follow the urea fractionation (Nunn. 1952 : 313 - 318) or the low temperature fractional crystallization of crude lipids (Kircher. 1964 : 4 - 8 ; Nordby and others. 1962 : 183 - 185 ; Varma, Nath and Aggarwal. 1954 : 10).

Sterculic acid (1) is a first CPFA found in Nature. Nunn (Nunn. 1952 : 313 - 318) established its structure in 1952. It was later synthesized from acetylenic precursor, stearolic acid, by the general method of Simmons and Smith (Simmons and Smith. 1969 : 4256 -

4264). Stearolic acid and methylene iodide reacted in the presence of a zinc-copper couple (Shank and Shechter. 1959 : 1825 - 1826) in refluxing ether. Products were isolated by fractional crystallization of the urea adducts. Synthetic sterculic acid was obtained in very low yield (4%). Its properties were identical with those of an authentic sample.

Homologue of sterculic acid, malvalic acid (2), was isolated by two groups of workers (Craven and Jeffrey. 1959 : 676 - 677 ; Macfarlane, Shenstone and Vickery. 1957 : 830 - 831).

Because of the unusual biological properties of CPFA, several syntheses of methyl sterculate (6 a) and methyl malvalate (6 b) from acetylenic precursor have been later developed.

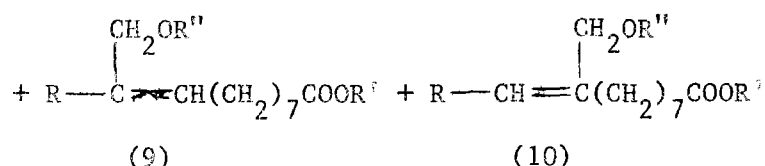


Gensler and his co-workers (Gensler and others. 1970 : 2472 - 2474) published a method for 6 steps synthesis of methyl sterculate with an overall yield of 30% starting from methyl stearolate.

Williams and Sgoutas (Williams and Sgoutas. 1971 : 3064 - 3065) improved the key step of above synthesis, decarbonylation, obtaining methyl sterculate with 60 - 65% overall yield.

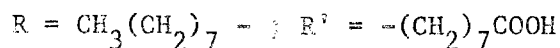
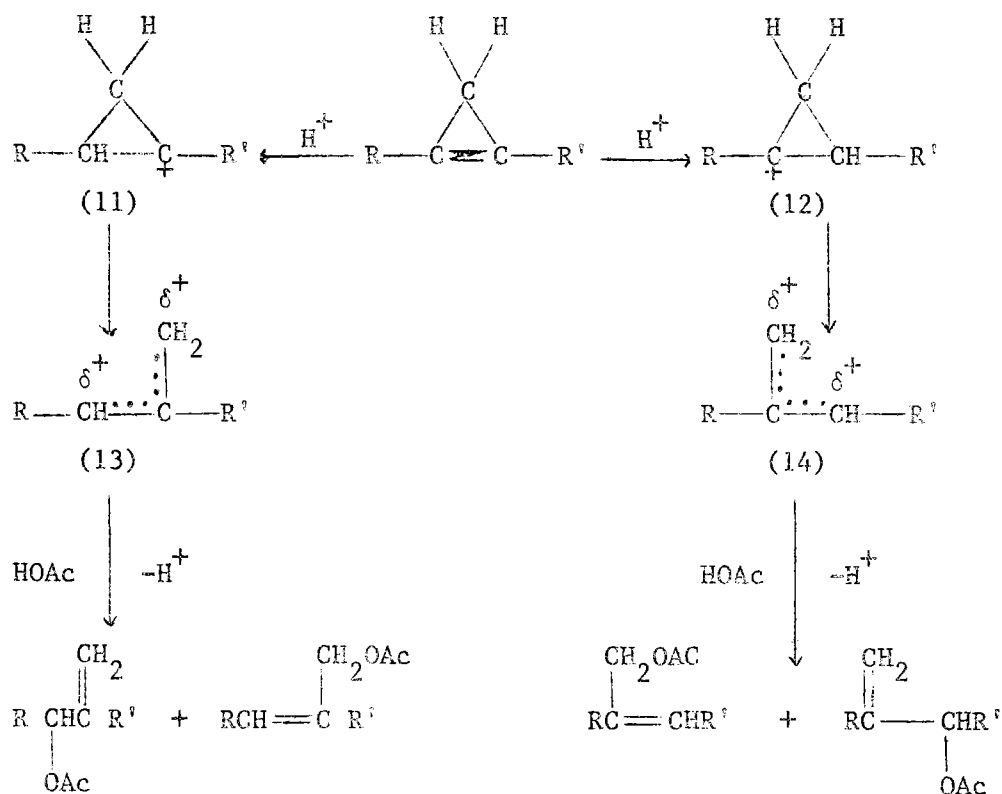
Synthesis of methyl malvalate was performed from acetylenic precursor, 1-decyne, to reach the desired product in 9 steps (Gensler and others. 1970 : 2301 - 2305).

2.1 Polymerization

$$\begin{array}{ccc} \text{R}-\text{C} \begin{array}{c} \diagup \text{CH}_2 \\ \diagdown \end{array} \text{C}-(\text{CH}_2)_7\text{COOH} \rightarrow \text{R}-\text{C} \begin{array}{c} \text{CH}_2 \\ || \end{array} \text{CH}(\text{XH}_2)_7\text{COOR}^{\text{f}} + \text{R}-\text{CH} \begin{array}{c} \text{CH}_2 \\ || \end{array} \text{C}(\text{CH}_2)_7\text{COOR}^{\text{f}} \\ \text{OR}^{\text{g}} & & \text{OR}^{\text{g}} \\ (7) & & (8) \end{array}$$


Cyclopropene rearrangement in polymerization of sterculic acid in presence of acetic acid was also studied (Rinchart, Culbertson and

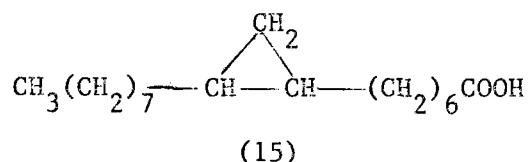
Tarimu. 1961 : 223 - 225). The mechanism presumably proceeds by initial protonation of the double bond of the cyclopropene ring to give cyclopropyl carbonium ion. In the most straightforward path two isomeric cyclopropyl carbonium ions (11 and 12) are formed, which then collapse to their respective allylic carbonium ions (13 and 14), which in turn react with acetate ion to give four products.



2.2 Hydrogenation

Hydrogenation of sterculic acid in ethanol in presence of palladium on calcium carbonate yielded dihydrosterculic acid (4) (Nunn. 1952 : 313 - 318).

Dihydromalvalic acid (15) was obtained similarly by hydrogenation of malvalic acid (2) with use of a palladium catalyst (Macfarlane, Shenstone and Vickery. 1957 : 830 - 831).



Hydrogenation studies of oils containing both sterculic and malvalic acids showed that prolonged hydrogenation in ethanol produced dihydro derivatives and branched-chain products only.

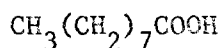
The use of Adams' catalyst in glacial acetic acid gave both branched-chain and straight-chain hydrogenolysis products (Carter and Frampton. 1964 : 517).

2.3 Oxidation

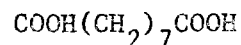
Pelargonic (16) and azelaic (17) acids were the only fission products obtained from oxidation of sterculic acid with hydrogen peroxide in acetic acid.

These two acids can be also obtained from oxidation with potassium permanganate in acetone (Carter and Frampton. 1964 : 517).

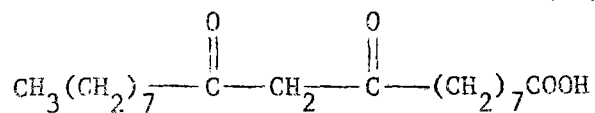
Ozonolysis of sterculic acid in ethyl acetate at low temperature followed by reduction with hydrogen in the presence of palladium on charcoal yielded 9, 10-diketnonadecanoic acid (18) (Nunn. 1952 : 313 - 318).



(16)



(17)



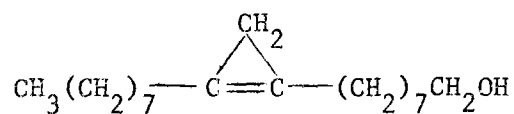
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2.4 Reduction

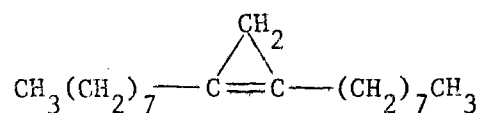
The cyclopropene ring was kept intact when sterculic acid was allowed to react with lithium aluminium hydride. Sterculyl alcohol (19) was the only product obtained from this reaction.

The hydrocarbon, sterculene (20) was obtained by reduction of methyl sterculyl ether (21) (Nunn. 1952 : 313 - 318).

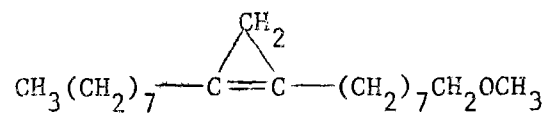
Sterculic acid can be easily reduced by hydrazine hydrate yielding dihydrosterculic acid. The reaction of CPFA was faster than other unsaturated fatty acids (Bhople, Rao and Rao. 1975 : 72).



(19)



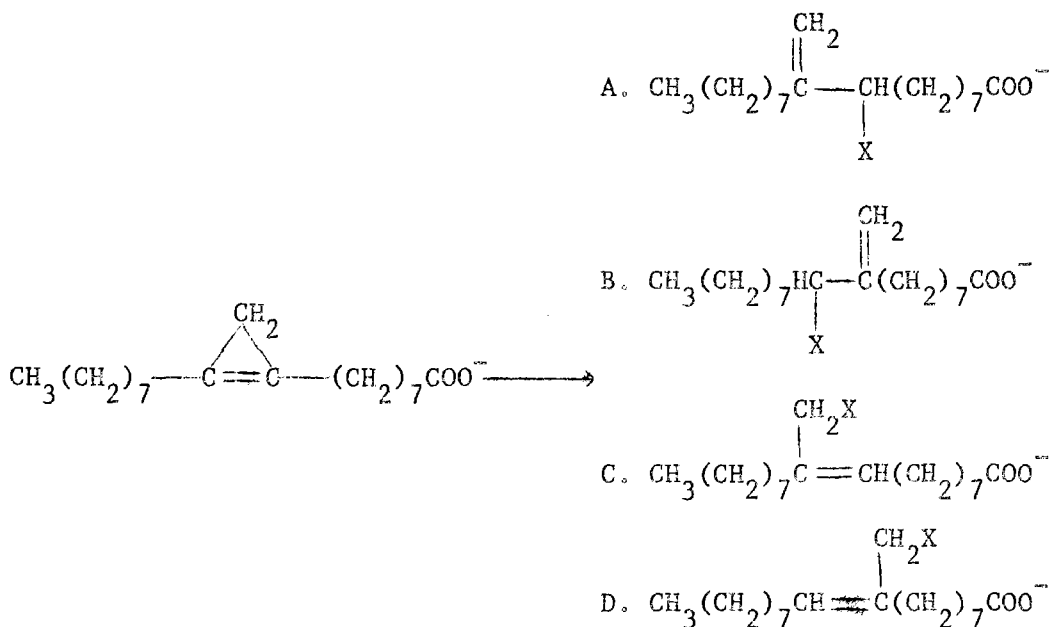
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(21)

2.5 Halogenation

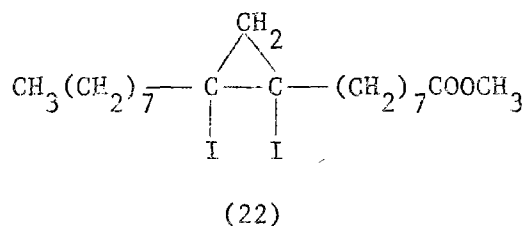
Hydrogen halides react easily with CPFA resulting in general, four isomeric monounsaturated derivatives represented by structures shown below.



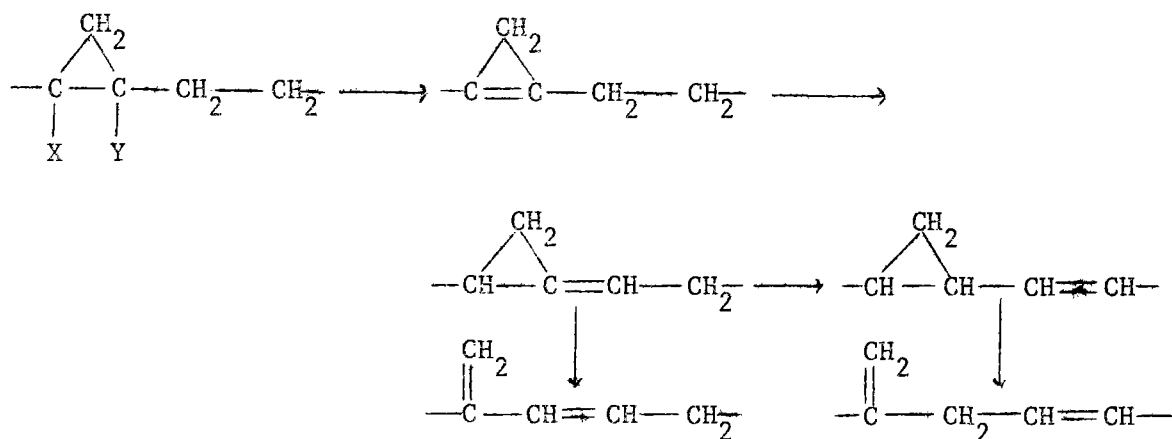
X = Br or Cl

The mechanism is analogue to that postulated for the polymerization of sterculic acid (Bailey and others. 1963 : 69).

Diiodide derivative was formed by heating methyl sterculate (6 a) under reflux in acetone with sodium iodide. A series of products was obtained upon heating the diiodo ester (22) at various conditions (Fawcett and Smith. 1960 : 871 - 872).

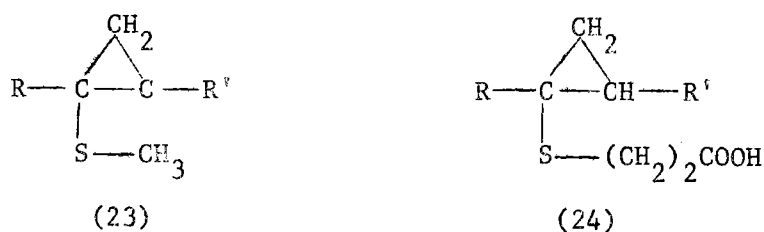


Dihalogen derivatives can give rise to the following products represented below, depending upon the severity of the method used for their decomposition (Carter and Frampton. 1964 : 517).



2.6 Reaction with Mercaptans

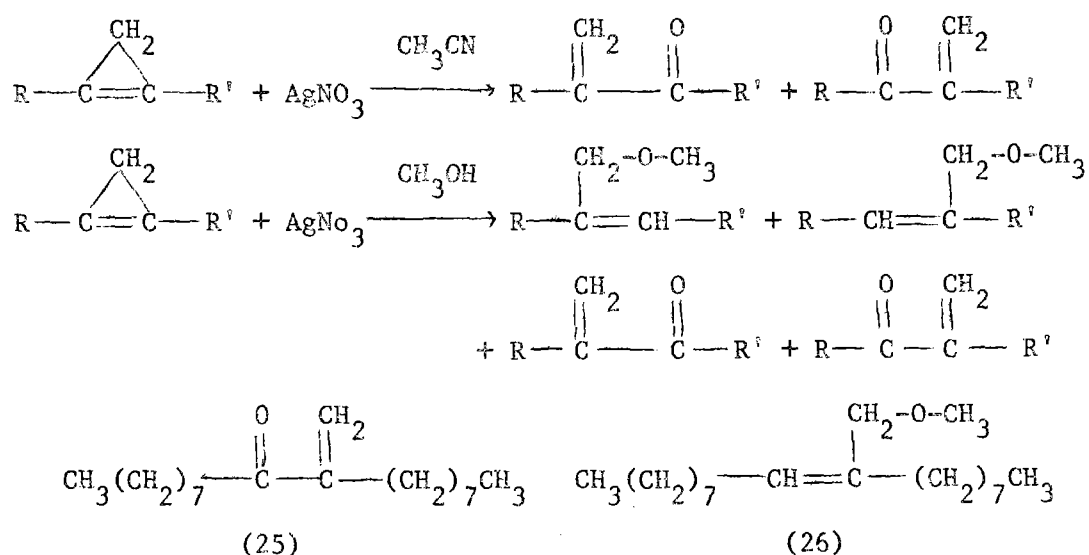
The sulphydryl group of methyl mercaptan or β -mercaptopropionic acid can add to the double bond of the cyclopropene ring of CPFA ester or sterculene to form the cyclopropylmethyl (23) and 2-carboxyethyl (24) derivatives (Kircher. 1964 : 4 - 8).



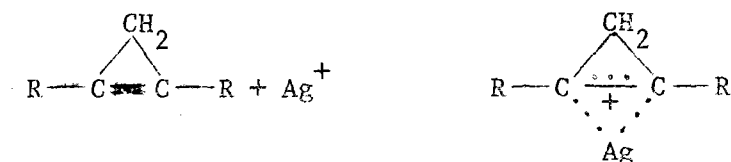
2.7 Reaction with Silver Nitrate

Sterculene reacts slowly with dilute solution of silver nitrate in acetonitrile with formation of a silver mirror and production of 9-methylene-10-octadecanone (25). The reaction is rapid in alcohols,

especially in methanol. Sterculene is transformed into 9-alkoxymethyl-9-octadecene (26) together with smaller amounts of the α , β -unsaturated ketone (25). The CPFA esters such as methylsterculate can also react with silver nitrate under the same conditions (Kircher. 1965 : 899 - 903).



The initial reaction is the formation of a silver ion-complex which has been described by Dewar (Dewar. 1951 : 71 - 79).



The complex arises from an overlap of the π electrons of the olefin with the 5p orbitals of the silver ion and the interaction of the 4d electrons of the latter with the anti-bonding orbitals of an olefin.

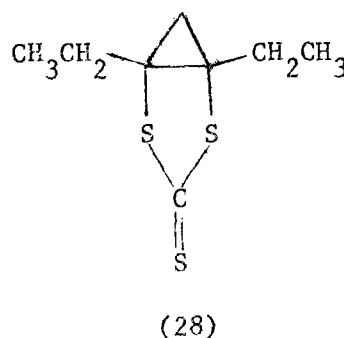
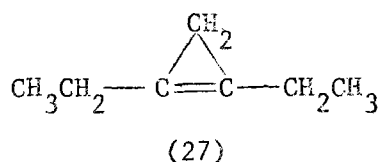
Silver nitrate derivatives of CPFA are frequently used in quantitative analysis of CPFA by gas-liquid chromatography (Berry.

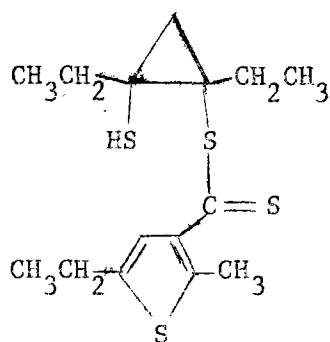
1980 : 224 - 227 : Kaimal and Lakshminarayana. 1970 : 2225 - 2229 : Schnieder, Loke and Hopkins. 1968 : 585 - 590 : Shimadate, Iino and Hosoyama. 1976 : 33 - 39).

2.8 Halphen Reaction

The original method of this reaction (Carter and Frampton. 1964 : 513 citing Halphen. 1898 : 358) was the treatment of the oil under examination with carbon disulfide containing 1% free sulphur using amyl alcohol as a solvent. The method has been developed later to give reproducible color development by using other solvents, e.g. pyridine (Deutschmann and Klaus. 1960 : 1809 - 1810), DMSO, butanol and DMSO-butanol (Coleman and Firestone. 1972 : 1288 - 1293).

Halphen reaction is specific for CPFA and sensitive up to 0.1 per cent (Magne. 1965 : 332 - 336). The presence of CPFA in oil produces pink-to-red color under this reaction. The structures of two colored products formed in the reaction of 1, 2-diethylcyclopropene (27) were established as 28 and 29 (Carter and Frampton. 1964 : 514 citing Zahorsky and Rinehart. 1964).





(29)

3. Determination of CPFA

The presence of CPFA in an oil is usually detected by Halphen test (Colemann and Firestone. 1972 : 1288 - 1293 ; Hammonds, Cornelius and Tan. 1971 : 599 - 602), hydrobromic acid titration and gas-liquid chromatography analysis. IR and NMR spectra are also informative, especially if CPFA content in an oil is high.

The IR spectrum shows two absorption bands at 1010 cm^{-1} and 1869 cm^{-1} characteristic of the cyclopropene group (Kaimal and Lakshminarayana. 1970 : 2225 - 2229 ; Rinehart, Tarimu and Culbertson. 1959 : 5007 - 5008). In ^1H NMR spectrum, a singlet at δ 0.8 ppm due to two cyclopropene protons occurs (Kaimal and Lakshminarayana. 1970 : 2225 - 2229).

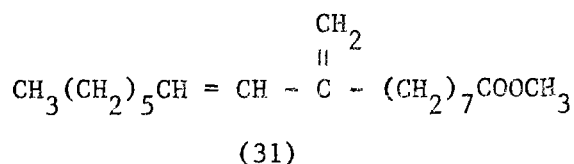
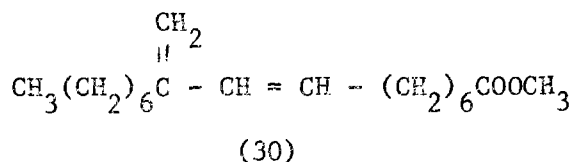
The quantitative analysis is usually based on hydrobromic acid titration and gas-liquid chromatography. The latter yields data on the chain length of the individual fatty acids.

Harris, Magne and Skau (Harris, Magne and Skau. 1963 : 718 - 720) showed that in the absence of interfering substances, CPFA derivatives can be precisely determined by a stepwise titration with hydrogen

bromide in acetic acid at 3°C and 55°C. The preliminary titration at 3°C overcomes the interference of non-cyclopropenoid substances, such as epoxides which can be titrated selectively at low temperature. Experiments on many non-cyclopropenoid vegetable oils, however, indicated that all glyceride oils, including cottonseed oil, contain traces of interfering substances resulting in small titrations not only at 3°C but also at 55°C. The accuracy of this method is therefore inadequate for the analyses of cottonseed oil and other oils having low cyclopropenoid moiety content since the 55°C titration error involved. In the latter report (Harris, Magne and Skau. 1964 : 309 - 311), a method for elimination of interfering substances was reported. The method is based upon their selective adsorption on activated alumina, so that the 55°C titration values for refined and crude cottonseed oils will be an accurate measure of the cyclopropenoid moiety content.

Gas-liquid chromatography was used as qualitative and quantitative methods of analysis for CPFA. Since CPFA in oil are present as the glycerides, therefore they have to be converted to methyl esters before gas-liquid chromatographic examination. Methyl sterculate was noted to give two peaks which relative sizes were apparently dependent upon experimental condition (Kircher. 1964 : 4 - 8). The spectral properties and Halphen response of methylsterculate were studied before and after chromatography and were found to be altered considerably. After chromatography the Halphen test became negative and IR absorption of cyclopropene group disappeared. The investigator believed that isomerization of the sterculate during chromatography resulted in the formation of a mixture

of methyl 10-methyleneoctadec-8-enoate (30) and methyl 9-methyleneoctadec-10-enoate (31) (Carter and Frampton. 1964 : 519 citing Masson. 1959).

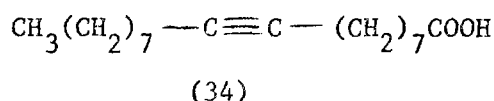
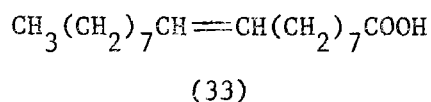
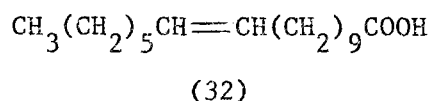


As a result of Kircher's studies (Kircher. 1965 : 899 - 903) on $\text{AgNO}_3\text{-CH}_3\text{OH}$ derivatives of methyl sterculate, gas-liquid chromatography became more suitable method for quantitative analysis of CPFA. Many investigators (Berry. 1980 : 224 - 227 ; Kaimal and Lakshminarayana. 1970 : 2225 - 2229 ; Schnieder, Loke and Hopkins. 1968 : 585 - 590 ; Shimadate, Iino and Hosoyama. 1976 : 33 - 39) have adopted his procedure which is nowadays commonly used for the determination of CPFA content in oils by gas-liquid chromatography.

4. Biogenesis of CPFA

Smith and Bu'lock (Smith and Bu'lock. 1964 : 433) had shown that the CPFA of Malvales were formed by addition of a one-carbon unit of the fatty acid chain; thus a C_{18} chain affords the C_{19} cyclopropene, sterculic acid. For the formation of the C_{18} cyclopropene, malvalic acid, involves additional step of chain-shortening (α -oxidation).

Similar biosynthetic pattern was proposed as an explanation of the presence of CPFA such as lactobacillic acid in *Lactobacillus arabinos* to involve the addition of a one-carbon fragment such as formate or the methyl carbon of a methionine group across the double bond of cis-vaccenic acid (32) (Liu and Hofmann. 1963 : 189 - 191). By comparing the biosynthesis of lactobacillic acid, Smith and Bu'lock mentioned that oleic acid (33) would be the precursor of dihydrosterculic acid and that an acetylene octadec-9-ynoic acid (stearolic) (34) should correspond to sterculic acid (Smith and Bu'lock. 1964 : 433)



5. Biological Effects

The cyclopropene configuration present in malvalic and sterculic acids have been proved to be toxic to nonruminants. Masson and others (Masson and others. 1957 : 751) have demonstrated that 25 mg/day of sterculic acid in the rations of laying hens caused pink egg discoloration. When the cyclopropene ring was destroyed by mild hydrogenation or with gaseous sulphur dioxide or hydrogen chloride, both cotton seed oil and *Sterculia foetida* oil no longer gave the Halphen test and pink egg

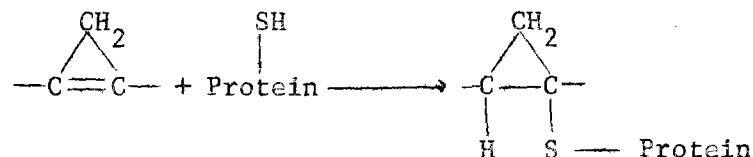
discoloration. Nordby and others (Nordby and others. 1962 : 183 - 185) showed that the acid or ester group was not required for biological activity.

Some other biological effects have been noted. Daily dietary intake of 100 mg of *Sterculia foetida* oil by the hens caused 100% embryo mortality in fertile eggs by the 19th day of incubation; an intake of 200 mg of the oil caused the hens to stop laying. The workers also observed that feeding the oil to sexually maturing pullets retarded comb, ovary and oviduct development, and caused a lack of egg production, enlargement of the gall bladder and liver, and a decrease in the iodine number of the chicken's body fat (Schnieder. 1962 : 1005 ; Schnieder and others. 1962 : 403 - 407).

The biological effects on other experimental animals, to be fed with *Sterculia foetida* oil, have been observed. Those effects are delaying sexual maturity in the female rat and sterility in the house fly; interference in lipid metabolism; co-carcinogenicity and carcinogenicity in rainbow trout and mortality in adult rats (Berry. 1980 : 224 - 227).

In hypothesis, proposed by Kircher (Kircher. 1965 : 899 - 903), the biological effects are due to the reaction of the cyclopropene ring with the sulphydryl groups present in physiologically active proteins that are intimately associated with lipids, such proteins are present in membranes, hormones and enzymes, or co-factors involved in fatty acid metabolism. The enhanced reactivity of the double bond in the cyclopropene ring over that of double bonds present in the ordinary unsaturated fatty acids may cause the former to add sulphydryl group readily under

physiological conditions; conditions under which the ordinary unsaturated fatty acids are unreactive.



This irreversible addition of protein sulphydryl groups to the cyclopropene ring would greatly alter the physical and biochemical properties of the protein molecule, and by this alteration, biological effects are produced.

Alkaloids from Plants of the Sterculiaceae Family

The word "alkaloid" is derived from the term "vegetable alkali", used originally to describe a group of bases of botanical origin. Most alkaloids are basic nitrogen-containing heterocyclic compounds derived from higher plants, often having marked physiological activity (Hughes and Genest. 1973 : 118 - 170). Biosynthetically, they are related to the amino acids. Basicity of an alkaloid depends on the availability of the lone electron pair on nitrogen atom. The basic nitrogen may be primary, secondary, tertiary or quaternary or it may be present as an amino oxide.

Alkaloids are usually classified on structural grounds because of heterocyclic ring systems common to each alkaloid² group (e.g. pyridine, 35, or quinoline, 36), but sometimes also because of their occurrence in certain plant families (e.g. lycopodium alkaloids).

Alkaloids are widely distributed in plants, where they occur mostly as organic salts. However, alkaloids were also found in animals (e.g. from Canadian beaver and Columbia arrow poison frog) and in micro-organisms (e.g. in some bacterias). Up to middle of 1973, 4959 alkaloids were isolated, of which 3293 had been known structures (Kirk and Othmer. 1973 : 883 - 943).

The existing literature on alkaloids isolated from plants of the Sterculiaceae family is rather limited. Only few plants were investigated since now.

Among the alkaloids characterized, more than half belong to the group of cyclopeptide alkaloids of frangulanine (37) and integerrine (38) types (The alkaloids of the frangulanine type are shown in Figure 1, and of the integerrine are in Figure 2). A 14-membered ring system and aryl ether resulting from p-hydroxystyrylamine and β -hydroxyamino acid are characteristic of these two groups of bases. The carboxyl of β -hydroxyamion acid is coupled in a peptide linkage with the amino of another amino acid (ring bond amino acid) whose carboxyl in turn is linked with amino of the styrylamine in an amide linkage. The "side chain" arises in another peptide linkage of the amino of β -hydroxyamino acid with another amino acid (Basic end amino acid).

The difference between frangulanine and integerrine alkaloids consists on nature of β -hydroxyamino acid involved in etheral linkage namely, those with β -hydroxyleucine are frangulanine type, while those with β -hydroxyphenylalanine are integerrine type.

The amino acids commonly present in these alkaloids are such as leucine, isoleucine, and phenylalanine. Tryptophan, proline and valine also occur.

Although cyclopeptide alkaloids are found in plants of Sterculiaceae, Pandaceae, Rubiaceae, Urticaceae, Hymenocardiaceae, and Celastraceae, they are particularly common in plants of the Rhamnaceae family. They occur in the leaves, bark, roots, and presumably in other parts but often in very small amounts difficult to capture (Tschesche and Kaubmann. 1975 : 165 - 204).

Among plants of the Sterculiaceae family, *Melochia tomentosa* L. was studied most intensively because of its controversial biological properties. Natives of Curacao use *Melochia tomentosa* L. to relieve throat inflammation (Kapadia and others. 1975 : 6814). Morton (Kapadia and others. 195 : 6814 citing Morton. 1968 : 2268) implicated that esophageal cancer, relatively common in Curacao, can be caused by decoctions of this, and other plants recognized in folk medicine. An extract of *Melochia tomentosa* roots was indeed reported to be tumorigenic (Kapadia and others. 1975 : 6814 citing O'Gara and others. 1974 : 445).

As the result of chemical investigations, three cyclopeptide alkaloids, i.e. melonovine A and B (39 and 40 respectively) and scutianine B (41) were isolated from roots, and their structures established (Kapadia and others. 1977 : 1431 - 1433). Melonovine A and B are new compounds while scutianine B has been isolated previously from *Scutia*

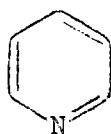
baxifolia (Rhamnaceae) (Tschesche, Ammermann and Fehlhaler. 1971 : 4405 - 4408).

Adouétines X, Y, Y', Z (42, 43, 44 and 45 respectively) were found in *Walteria americana* L. (Pais and others. 1968 : 1145). Their structure elucidation was based on results of acid hydrolysis and mass spectrometry. Chromatographic separation of adouétine Y and Y', proved to be difficult. Only adouétine Y was isolated as a pure compound. Chemical structure of adouétine Y' was established indirectly by mass spectrometry and acid hydrolysis of an equimolar mixture of adouétines Y and Y'.

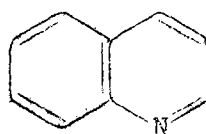
Tschesche and Reutel (Tschesche and Reutel. 1968 : 3817 - 3818) have isolated two cyclopeptide alkaloids, namely franganine (46) and frangufoline (17), from *Melochia corchorifolia*.

As it was already mentioned, cyclopeptide alkaloids occur in plants in very small quantities. The yeild of total base mixture from dried plant material varies from 0.01 to 1% and depends not only upon the plant source but also upon the method of isolation.

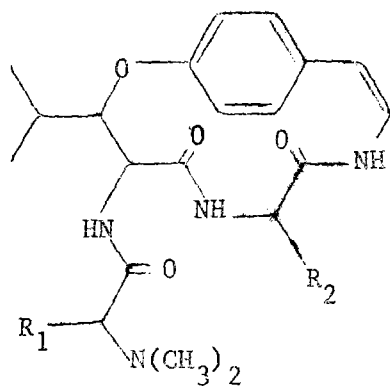
The further purification and the separation of individual bases is conveniently achieved by chromatographic methods. The alkaloids isolated from an individual plant often differ only from the lipophyllic side chain of the amino acids, or from the degree of methylation of the basic nitrogen (Tschesche and Kaubmann. 1975 : 165 - 204).



(35)

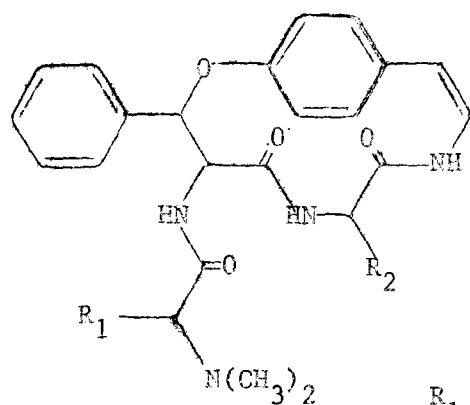


(36)

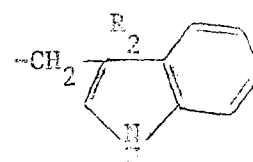
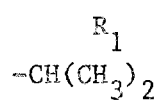


	R_1	R_2
(37); Frangulanine	$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$
(39); Melonovine A	$-\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$
(40); Melonovine B	$-\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{C}_6\text{H}_4\text{OH}$
(41); Scutianine B	$-\text{CH}_2\text{C}_6\text{H}_5$	$-\text{CH}_2\text{C}_6\text{H}_5$
(42); Adouétine X	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$	$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$
(44); Adouétine Y'	$-\text{CH}_2\text{C}_6\text{H}_5$	$-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$
(46); Franganine	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$
(47); Frangufoline	$-\text{CH}_2\text{C}_6\text{H}_5$	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$

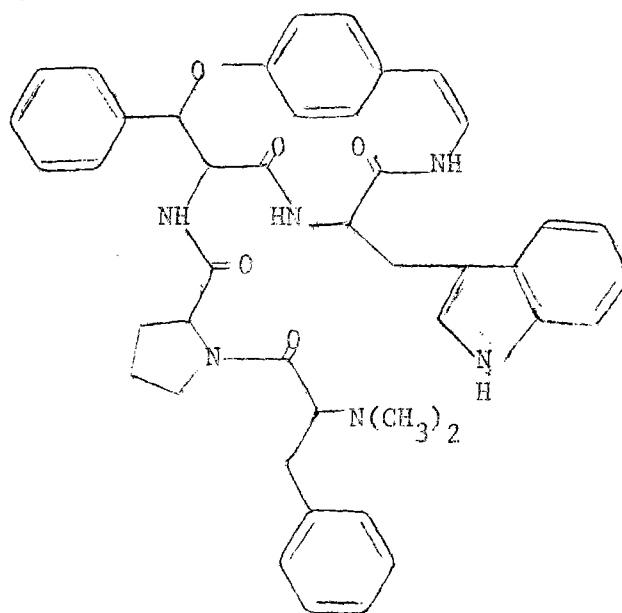
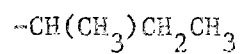
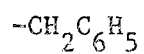
Figure 1 Cyclopeptide Alkaloids of Frangulanine Type



(38) : Integerrine



(43) : Adouetine Y



(45) : Adouetine Z

Figure 2 Cyclopeptide Alkaloids of Integerrine Type

As a result of their similar chromatographic properties, isolation of pure bases by repeated chromatography is often tedious and sometimes not possible, e.g. adouétine Y and Y' (Pais and others. 1968 : 1145).

The identification and characterization of peptide alkaloids is primarily based on spectral studies; UV, IR, NMR and mass spectrum which the latter is particularly informative. The structures of the bases of frangulanine and integerrine types can largely be determined by their mass spectra (Fehlhaber. 1968 : 19487k). Fragmentation is independent of the nature of amino acid. Fehlhaber established a general fragmentation scheme which can be used for structure elucidation of such an alkaloid. However, the position of the substituents on the aromatic ring can not be determined nor can leucine be distinguished from isoleucine.

In examples in which mass spectra do not differentiate between leucine and isoleucine the NMR spectra sometimes can do so (Tschesche and Kaubmann. 1975 : 165 - 204).

Furthermore, important data can be obtained from analysis of degradation products of an alkaloid. Degradation is usually done by hydrogenation of the double bond of the bases followed by complete hydrolysis with 6 N hydrogen chloride in a sealed tube at 120°C. The amino acids in the hydrolysate are identified by standard methods—amino acid analyzer or paper chromatography.

Although most of bases isolated from plants of the Sterculiaceae family are cyclopeptide alkaloids, some others, belonging to various alkaloids groups, were also found.

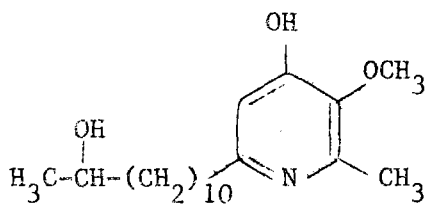
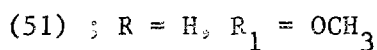
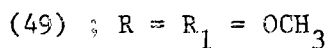
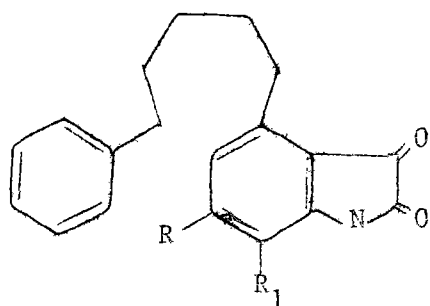
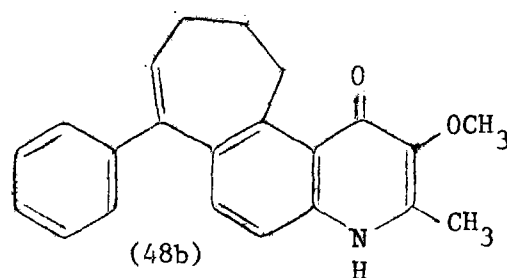
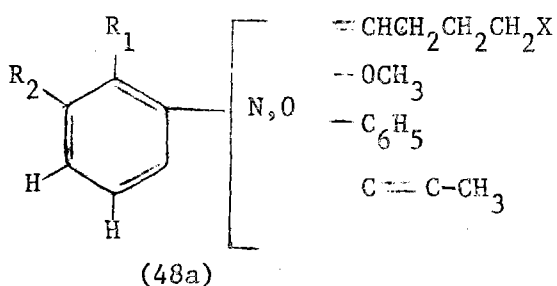
A new quinolinone alkaloid was isolated from benzene extract of *Melochia tomentosa* roots (Kapadia and others. 1975 : 6814 ~ 6819). As little as 82 mg (0.01% yield based on weight of dried roots) of this compound was available for structure elucidation. The partial formula, represented by 48a, was proposed as a result of detailed analysis of UV, IR, ^1H and ^{13}C NMR spectra. The available data failed to suggest a suitable environment for nitrogen-containing moiety.

Melochinone was found to be neutral compound, inert to action of acids, alkali and reducing reagents, i.e., lithium aluminium hydride and hydrogen in presence of Palladium on charcoal at 350°C. Since degradation experiments were unsuccessful, X-ray crystallographic analysis was undertaken resulting the complete structure 48 b.

Three novel isatin alkaloids, which can be considered as uncyclized analogues of melochinone, were isolated by column chromatography on silica gel from the same plant material. Melosatin A, B (Kapadia and others. 1977 : 535 ~ 536) and C (Kapadia, Shukla and Basak. 1980 : 2441 ~ 2447) (49, 50 and 51 respectively) are present in trace quantities: 0.001%, 0.00025% and 0.0003% respectively. These crystalline phenylpentylisatins constitute a novel class of alkaloids from *Melochia tomentosa*. Their structure elucidation was based on mass and spectral analysis. Furthermore, structure of melosatine A has been corroborated by synthesis (Kapadia and others. 1977 : 535 ~ 536).

Medina and Spiteller (Medina and Spiteller. 1979 : 376) have reported structure elucidation of a pyridone alkaloid, melochinin (52), isolated from *Melochia pyramidata* L.

Total alkaloids and total non-alkaloid nitrogenous bases **were** extracted from the leaves of *Sterculia platanifolia*, but further separation and structure elucidation were not completed (Trakman and others. 1966 : 2417a).



There is no reports in literature on pharmacological properties of alkaloids isolated from plants of the Sterculiaceae family. The difficulty of obtaining considerable quantities of these alkaloids in a pure condition for pharmacological testing has delayed such studies.

Flavonoids from Plants of the Sterculiaceae Family

The flavonoids are a group of some 750 naturally occurring plant constituents whose structures are derived in one way or another from the aromatic nucleus of flavan or 2-phenylbenzopyran (53). The simplest member is flavone (54), but this is untypical of the series, since the vast majority have phenolic hydroxyls attached at the 5-, 7-, 3'- and 4'-position (for numbering, see Formula 53). Flavonoids are usually divided into classes depending on the oxidation level of the central pyran ring³, the two most important classes being the flavonols or 3-hydroxyflavones (e.g. quercetin, 55) and the anthocyanidins (e.g. cyanidin, 56).

Flavonoids occur in plants in combined or protected form; usually one or more hydroxyl groups are involved in glycosidic linkages. Unprotected phenolic aglycones are presumably toxic to the living cell. Many flavonoid glycosides have been described. Attachment of sugar renders these compounds water-soluble, and flavonoids are mostly located in the cell vacuole. Less frequently, flavonoids occur with the majority of hydroxyl groups protected by methylation (e.g., in citrus fruits) and such compounds are lipid-soluble and are presumably contained in the cytoplasm.

Biological function of natural flavonoids together with the essential oils of flower scents consists in attracting birds and insects to the plant.

The anthocyanidins are intensely colored substances and their glycosides, the anthocyanins are responsible for most scarlet, red, mauve, purple, and blue colors in flowers, fruits and leaves of higher plants.

Other flavonoid classes, particularly chalcones, aurones, and flavonols, make a significant contribution to yellow coloration in many species (Harborne. 1973 a : 344 - 380).

Chemical investigation of several plants of the Sterculiaceae family has shown the presence of various flavonoids of anthocyanidins (i.e., cyanidin and pelargonidin), flavonols (i.e., kaempferol, quercetin, quercetagenin, gossypetin and herbacetin), and flavone (i.e. luteolin) classes. All pigments isolated from plants of this family and summarized in the Table 1.

Most of characterized flavonoids are O-glycosides, however some C-glycosides (carbon-carbon linked) are found in *Theobroma cacao* (Forsyth and Quesnel. 1957 : 177 - 179) and *Sterculia parviflora* (Lowry. 1971 : 689 - 690).

Because of their universal distribution and ease of detection by simple chromatographic methods, flavonoids are often considered as taxonomic markers in plants systematics (Harborne. 1973 a : 344 - 380).

Studies of the Sterculiaceae family showed that flavonoid pattern often lends support to the assignment of a separate species status, i.e. *Sterculia pallens* which contains quercetin, its 3-glucoside and 3-diglucoside (Ranganathan and Nagarajan. 1980 : 309 - 310) is distinctly

different from *Sterculia colorata* containing unusual 6-oxygenated flavone derivatives (Nair, Ramesh and Subramanian. 1976 : 839).

Isolation of two rare tetramethyl ethers of quercetin from *Sterculia foetida* has been reported (Anjaneyulu and Murty. 1981 : 87 - 88). First one, quercetin 3ⁱ, 4ⁱ, 5, 7-tetramethyl, though earlier known as a degradation product from several of its natural glycosides, from a natural source was isolated for the first time. Second one, rutusine, has not been previously found in Sterculiaceae family.

A novel compound, glucuronide 6-hydroxy luteolin (5, 7, 3ⁱ, 4ⁱ tetrahydroxy-6-O-β-D-glucuronyl flavone) was obtained from *Sterculia colorata* (Nair, Ramesh and Subramanian. 1976 : 839).

A new glycoside, melocorin (3, 4ⁱ, 5, 7-tetrahydroxy-8-O-β-D-glucuronosyl flavone) has been isolated from leaves of *Melochia corchorifolia* together with herbacetin and gossypetin (Nair, Ramesh and Subramanian. 1977 a : 1045). The authors came to the conclusion that according to flavonoid composition, *Melochia corchorifolia* differs from *Sterculia colorata* (Nair, Ramesh and Subramanian. 1976 : 839) and *Sterculia foetida* (Nair, Ramesh and Subramanian. 1977 b : 14) containing 6-oxygenated flavone.

Kaempferol-3-O-galactoside, a rare biflavonoid was only kaempferol glycoside found in *Melochia umbellata* and *Eriolaena hookeriana* (Gunasegaran and others. 1980 : 821). Although, in the absence of uncommon flavonoids, two species can not be distinguish by their flavonoid pattern, they differ from *Melochia corchorifolia*.

In general, flavonoids are isolated by extraction with hot aqueous alcohol or with hot alcohol. Alcoholic extracts, after washing to remove chlorophylls, lipids, and so far, are concentrated, and flavonoids are then obtained by crystallization (Gunasegaran and Subramanian. 1979 : 72 - 73 ; Ranganathan and Nagarajan. 1980 : 309 - 310), but mostly are purified by chromatography. Flavonoids are readily detected on chromatograms, without use of chromogenic sprays; many of them are colored, the remainder can be seen by means of UV light. Paper chromatography remains the most widely used technique for the separation and purification of flavonoids.

Anthocyanins, cations probably present in association with organic acid ions, are unstable at neutral or alkaline pH and are isolated as chlorides, by using methanolic HCl as the extracting medium (Lowry. 1971 : 689 - 690 ; Yoshitama and others. 1972 : 303 - 306).

The structural elucidation of flavonoids is based on spectral studies (i.e., UV, IR, NMR and MS); of these, UV and NMR spectroscopy are probably the most informative. In characterizing flavonoid glycosides, Rf measurements are very important, particularly for the anthocyanins, which cannot be characterized by melting point or elementary analysis.

Identification of carbohydrates present in some pigments involves acidic and enzymatic hydrolysis, H_2O_2 oxidation and UV spectral studies. Configuration of glycosidic linkage (α and β) can be determined by enzymatic hydrolysis and from ^1H and ^{13}C NMR spectra.

Table 1 Flavonoids Isolated from Plants of the Sterculiaceae Family

Plant	Part	Solvent used for extraction	Isolated compounds
<i>Eriolaena hookeriana</i>	leaves	hot 90% EtOH	Kaempferol-3-O- β -D-galactopyranoside (Gunasegaran and others. 1980 : 821)
<i>Firmiana platanifolia</i> Schott et Endl.	leaves	1% HCl-MeOH	Chrysanthemins (cyanidin-3-monoglucoside) (Yoshitama and others. 1972 : 303 - 306)
<i>Melochia corchorifolia</i> Linn.	leaves	hot 90% EtOH	3, 4', 5, 7-tetrahydroxy-8-O- β -D-glucuronosylflavone (herbacetin-8-O-glucuronide, melocorin) Gossypetin-8-O- β -D-glucuronide (hibifolin) Kaempferol-3-O- β -D-galactoside (trifolin) (Nair, Ramesh and Subramanian. 1977 a : 1045)
<i>Melochia umbellata</i>	leaves	hot 90% EtOH	Kaempferol-3-O- β -D-galactopyranoside (Gunasegaran and others. 1980 : 821)
<i>Pterospermum acerifolium</i> Willd.	flowers	hot 90% EtOH	Kaempferol-3-O- β -D-galactoside Luteolin-7-O-glucoside : luteolin (Rizvi and Sultana. 1972 : 856 - 858)
	leaves	hot 90% EtOH	Kaempferol-3-O- β -D-galactoside Luteolin-7-O- β -D-glucoside Luteolin-7-O- β -D-glucuronide

Table 1 (Continue)

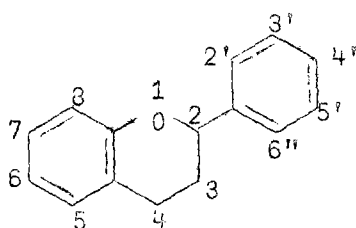
Plant	Part	Solvent used for extraction	Isolated compounds
<i>Pterospermum suberifolium</i> Lam. non. Roxb. (Syn : <i>P. canescens</i>)	flowers	hot 90%	Kaempferol-3-O- β -D-galactoside
		EtOH	Kaempferol-3-rutinoside
	leaves	hot 90%	Kaempferol-3-O- β -D-galactoside
		EtOH	Kaempferol
			Quercetin
			Quercetin-3-O-arabinoside
<i>Pterospermum xylocarpum</i> (Syn : <i>P. Heyneanum</i>) Wall)	flowers	hot 90%	Kaempferol-3-O- β -D-galactoside
		EtOH	Quercetin-3-O- β -D-galactoside
			Luteolin
	leaves	hot 90%	Kaempferol-3-O- β -D-galactoside
		EtOH	Quercetin-3-O- β -D-galactoside
			(Gunasegaran and Subramanian. 1979 : 72 - 73)
<i>Sterculia colorata</i>	leaves	hot EtOH	5, 7, 3', 4'-tetrahydroxy-6-O- β -D-glucuronylflavone
			Glucuronides of apigenin, luteolin, scutellarein, 6-hydroxyluteolin and vitexin (Nair and others. 1976 : 839)

Table 1 (Continue)

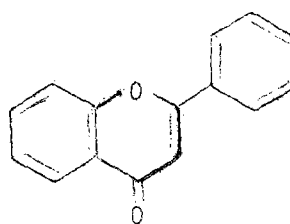
Plant	Part	Solvent used for extraction	Isolated compounds
<i>Sterculia foetida</i> Linn.	leaves		Scutellarcin-6-0-glucuronide (isoscutellarin) Procyanidin-3-0- β -glucoside 6-glucuronosyloxyluteolin (Nair, Ramesh and Subramanian. 1977b : 14)
	leaves	1% HCl-MeOH	Cyanidin-3-glucoside (Lowry. 1971 : 689 - 690)
	bark	MeOH	Quercetin-3, 7, 3', 4'-tetramethyl ether (retusine) Quercetin-5, 7, 3', 4'-tetramethyl ether (Anjeneyulu and Murty. 1981 : 87)
<i>Sterculia kunstleri</i> king	leaves	1% HCl-MeOH	Pelargonidin-3-glucoside (Lowry. 1971 : 689 - 690)
<i>Sterculia pallens</i>	leaves	hot 80% EtOH	Quercetin-3-0-glucoside Quercetin-3-0-diglucoside (Ranganathan and Nagarajan. 1980 : 309 - 310)
<i>Sterculia parviflora</i>	leaves	1% HCl-MeOH	Cyanidin-3-galactoside Cyanidin-3-arabinoside Pelargonidin-3-galactoside

Table 1 (Continue)

Plant	Part	Solvent used for extraction	Isolated compounds
<i>Theobroma cacao</i>			Pelargonidin-3-arabinoside (Lowry. 1971 : 689 - 690) Cyanidin-3- β -D-galactoside Cyanidin-3- α -L-arabinoside (Forsyth and Quesnel. 1957 : 177 - 179)

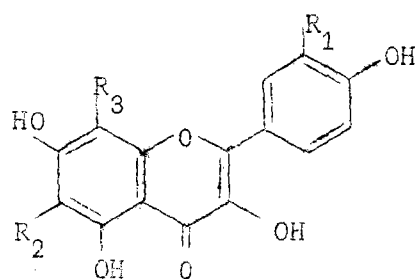


Flavan
(53)



Flavone
(54)

Flavonols



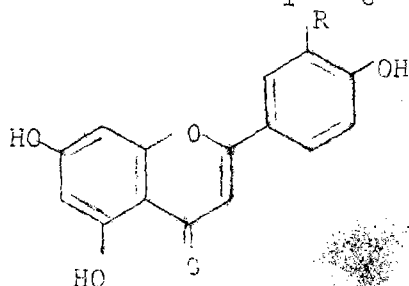
Kaempferol, $R_{1-3} = H$

Herbacetin, $R_1 = R_3 = OH$, $R_2 = H$

Quercetin (55), $R_1 = OH$, $R_2 = R_3 = H$

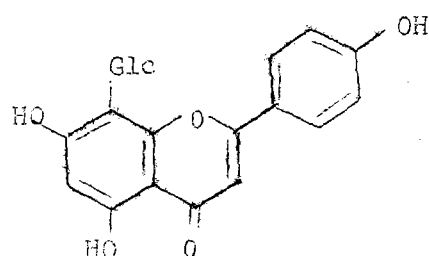
Quercetagetin, $R_1 = R_2 = OH$, $R_3 = H$

Gossypetin, $R_1 = R_3 = OH$, $R_2 = H$

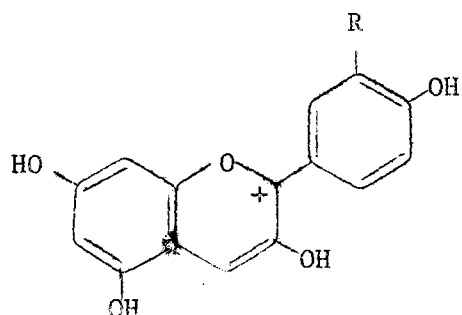


Apegenin, $R = H$

Luteolin, $R = OH$

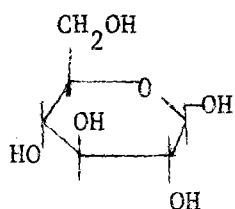


Vitexin

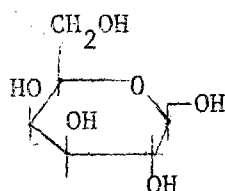
Anthocyanidins

Pelargonidin, R = H

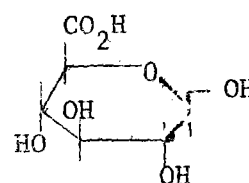
Cyanidin (56), R = OH

CARBOHYDRATES OCCURRING IN PIGMENTS

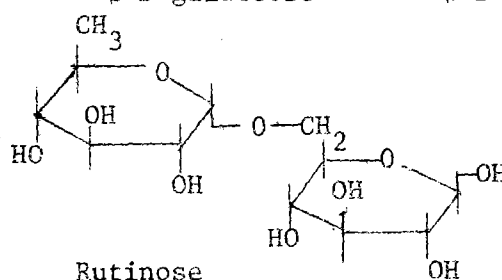
β -D-glucose



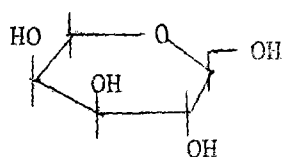
β -D-galactose



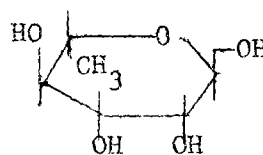
β -D-glucuronic acid



Rutinose



α -L-arabinose



β -L-rhamnose

Miscellaneous Studies

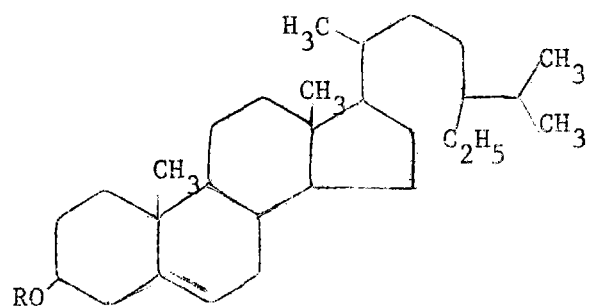
Phytochemical investigation of the flowers of *Pterospermum acerifolium* (Rizvi and Sultana. 1972 : 856 - 858), a large tree belonging to the Sterculiaceae has revealed the presence of 24 β -ethylcholes-5-en-3 β -O- α -cellobioside (57) ; 3, 7-diethyl-7-methyl-1 : 5-pentacosanolide ; dilignocerate of n-hexacosane-1, 26-diol ; friedelan-3 α -ol and its β -isomer ; β -amyrin (58) ; β -sitosterol ; n-tricontanol ; and n-hexacosane-1, 26-diol in light petroleum ether extracts of the dried flowers. Fatty acid composition has also been investigated. The identified fatty acids are listed below:

Myristic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$
Palmitic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
Stearic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
Arachidic acid	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$
Behenic acid	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$
Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
Linoleic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

The detailed chemical investigation of *Melochia tomentosa*, a tumorigenic medicinal plant has resulted the isolation and characterization of 6-methoxy-7, 8-methylenedioxy coumarin (59) (Shukla and others. 1976 : 1788), which has not been reported previously in the Sterculiaceae family.

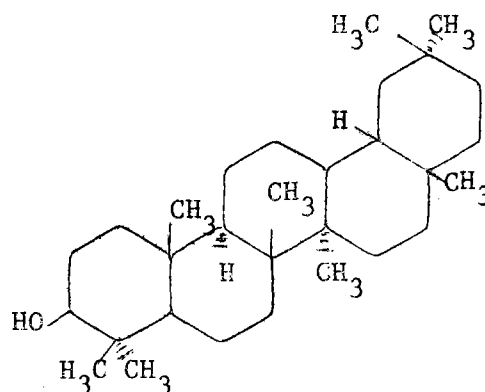
Lupeol (60), lupenone (61), betulin, β -sitosterol (62), β -sitosterol-3-O- β -D-glucoside (63) and sterculoside are isolated from

bark of *Sterculia foetida* Linn. (Anjaneyulu and Murty. 1981 : 87 - 88).

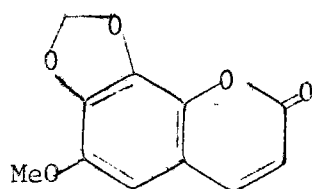


(57)

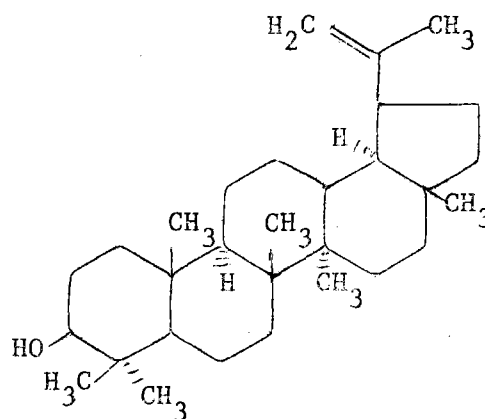
R = Cellobiose



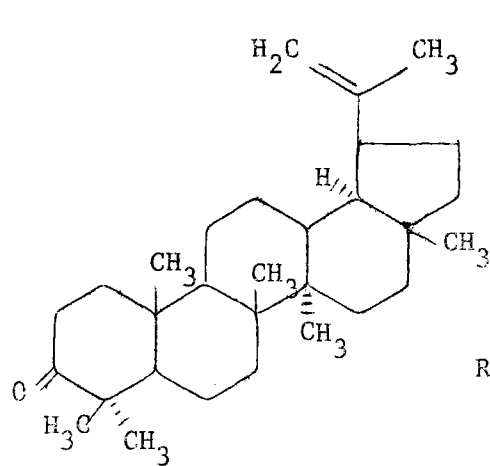
(58)



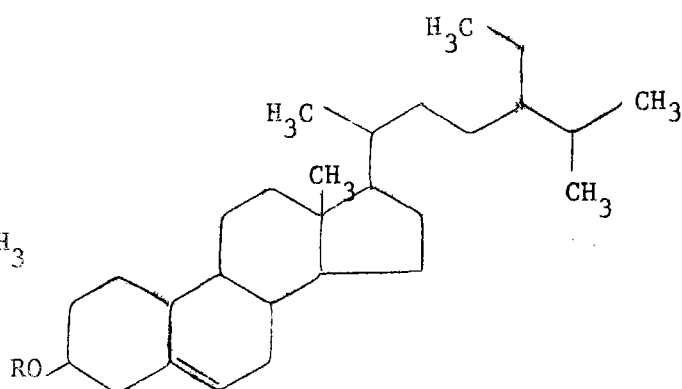
(59)



(60)



(61)



(62) ; R = H

(63) ; R = glucose

Objective of the Research

To the best of our knowledge there is no reports on chemical constituents of *Sterculia lychnophora*. Literature survey covering the research on other closely related plants revealed some general trends in their chemical composition. Unusual toxic lipids, namely cyclopropenoid fatty acids, commonly occur in plants of Sterculiaceae family. Some alkaloids are also found.

These indications were very useful in establishing our own research strategy. As the result of the preliminary studies including phytochemical screening and qualitative analysis of lipid fraction (see Experimental Part page 55 and 59 respectively) we found that total ethanolic extract of *Sterculia lychnophora* fruits is positive to alkaloid tests. Furthermore lipid fraction contains cyclopropenoid fatty acids.

The best approach in medicinal plant research requires pharmacological screening of extracts. Further chemical investigation can be concentrated on biologically active fractions thus the chances for isolation of some pharmacologically active principles are increased.

As pharmacological studies were not included in the scope of this project, we decided to concentrate our efforts on chemical investigation of classes of compounds usually showing pharmacological activity instead of trying to isolate any natural product.

Thus we selected lipid and basic fractions as worth to investigate, trying to determine the level of toxic cyclopropenoid fatty acids and isolate alkaloids from ethanolic extract.

We employed gas-liquid chromatography as a method for qualitative and quantitative analyses of lipids. Spectroscopic studies including IR, ^1H and ^{13}C NMR were also used.

Isolation of an alkaloid required combining several techniques, namely column, thin-layer, preparative thin-layer chromatography and crystallization as a final step. Structure elucidation of an isolated compound usually comprises spectroscopic studies including UV, IR, ^1H and ^{13}C NMR, and MS.

Scope of the Research

Investigation of alkaloids and cyclopropenoid fatty acids in *Sterculia lychnophora* fruits were of main interest.

Structure elucidation of an isolated compound requires advance spectroscopic studies and preparation of some chemical derivatives. The availability of some techniques depends on quality of equipment and an amount of isolated compound.

As the yield of basic fraction presumably containing alkaloids was very low, furthermore it was composed by several Dragendorff's positive compounds, thus only preliminary studies of alkaloids could be done in the scope of this work.

Importance of the Research

Presented research is one among other tasks of a project under Thailand Institute of Scientific and Technological Research Programme

titled "Development in Quality Improving and Controlling for Medicinal Plants for Export".

Sterculia lychnophora seeds and fruits are commonly used as a drug in Thailand and also exported, thus chemical studies and establishing the norms for quality control are necessary.

The presence of cyclopropenoid fatty acids in fruits implicates their toxicity. Although it has been clearly mentioned that drug is mucilaginous substance prepared by soaking seeds or fruit in water, while we were studying lipid and ethanolic fractions. The medicine probably does not contain or has very low content of cyclopropenoid fatty acids as most lipids are present in kernel while only outer parts of seed and fruit are swollen in water.

Nevertheless the evidence of the presence of cyclopropenoid fatty acids in *Sterculia lychnophora* fruits is of taxonomic importance. Alkaloid studies contribute to the basic research on natural products from medicinal plants and might result in isolation of a pure pharmacologically active compound (or compounds) of known or new structure.

Notes

¹The order Malvales comprises four families:

1. Bombacaceae
2. Malvaceae
3. Sterculiaceae
4. Tiliaceae

(Carter and Frampton. 1964 : 513).

²Some alkaloids, viz. ephedrine, muscarine and mescaline are not nitrogen-containing heterocyclic compounds, and some other, viz. colchicine is not basic (Hughes and Genest. 1973 : 118).

³Some ten classes of flavonoids are recognized, i.e. anthocyanidins, leucoanthocyanidins, flavonols, flavones, glycoflavones, biflavonyls, chalcones, aurones, flavanones, and isoflavones (Harborne. 1973b : 52 - 53).

Chapter II

RESULTS AND DISCUSSION

Phytochemical screening of the fruits revealed the presence of alkaloids (see Experimental Part page 55). Dried ground fruits (7.58 kg) were extracted thoroughly with petroleum ether (bp. 40 - 60°C) and then 95% ethanol. The petroleum ether extract gave, after evaporation under vacuum at 40°C, brown oil (357 g, 4.71% yield). The ethanolic extract resulted dark brown syrupy residue (746.4 g, 9.85% yield).

Chemical Investigation of Lipid Fraction

Sterculia lychnophora oil gave a positive Halphen test, indicating the presence of cyclopropenoid fatty acids (CPFA). Quantitative analysis of CPFA in the *S. lychnophora* oil was done by gas-liquid chromatography of $\text{AgNO}_3\text{-CH}_3\text{OH}$ derivatives of methyl esters. Lipid composition was established through the retention times by comparison of the examined chromatograms with the corresponding chromatograms obtained for the same derivatives of the *Sterculia foetida* seed oil which is well known to contain both malvalic and sterculic acid and widely employed as a reference standard (Berry, 1980 : 224 - 227 ; Racourt, Jurriens and Schmitz, 1967 : 35 - 42 ; Schnieder, Loke and Hopkins, 1968 : 535 - 590). The weight per cent of any given component was determined by dividing the area of its peak by the sum of the areas of peaks of all detected components and multiplying by 100. The results are shown in Table 2 and 3 respectively

and gas chromatogram are presented in Appendix A.

The *S. lychnophora* oil is found to contain 0.5 and 5.4% of malvalic and sterculic acids respectively, while *S. foetida* seed oil contains 9 and 52.75% of malvalic and sterculic acids respectively.

S. lychnophora oil shows the high content (65%) of other unsaturated fatty acids, among them linoleic acid is the main component (40%) while oleic and linolenic acids are present in 24 and 0.9% respectively.

Table 2 Composition of *Sterculia lychnophora* Oil as Determined by Gas-Liquid Chromatography

Retention time (minute)	Fatty acid	Area per cent
2.5	-	0.11
3.5	Myristic	0.32
4.5	-	0.19
6.5	Palmetic	23.29
12.5	Stearic	5.29
15.0	Oleic	23.96
19.5	Linoleic	39.94
26.0	Linolenic	0.99
37.0	a	0.5
49.0	b	5.39
70.0	c	0.01
102.0	d	0.01
Total	Malvalic acid	0.51
Total	Sterculic acid	5.4
Total	Cyclopropenoid fatty acids	5.91

- a Ether derivative of methyl malvalate
- b Ether derivative of methyl stercolate
- c Ketone derivative of methyl malvalate
- d Ketone derivative of methyl stercolate

Table 3 Composition of *Sterculia foetida* Seed Oil as Determined by Gas-Liquid Chromatography.

Retention Time (minute)	Fatty Acid	Area per cent
2.5	-	0.14
3.5	Myristic	0.24
7	Palmetic	17.75
12.5	Stearic	2.90
15	Oleic	6.60
19.5	Linoleic	7.85
27	Linolenic	2.76
38	a	9.00
53.5	b	48.30
78	c	0.01
105	d	4.45
Total	Malvalic acid	9.01
Total	Sterculic acid	52.75
Total	Cyclopropenoid fatty acids	61.76

a Ether derivative of methyl malvalate

b Ether derivative of methyl stercolate

c Ketone derivative of methyl malvalate

d Ketone derivative of methyl stercolate

Occurrence of CPFA was also supported by ^{13}C , ^1H NMR and IR spectra.

In ^{13}C NMR proton noise decoupled spectrum of a compound, each non-equivalent carbon gives a singlet which can be assigned on the basis of its chemical shift. Furthermore, the number of hydrogen atoms directly attached to a carbon can be often determined from so-called off-resonance decoupled spectrum. In such a spectrum signal of carbon bearing three hydrogens is splitted into quartet, signal of methylene carbon appears as a triplet and methine carbon as a doublet. The signal of quaternary carbon and the other carbons having no directly attached hydrogen shows as a singlet in both types of spectra.

These data are very useful in structure elucidation of natural products including lipids (Wenkert and others. 1972 : 82 - 87). ^{13}C NMR spectra of fatty acids show signal of carboxyl carbon at 180 ppm. and olefinic carbons at 130.9 ± 0.5 and 127.4 ± 0.5 ppm. The glycerol esters of fatty acids show signal at 173 ppm corresponding to the central carbonyl, which is shielded compared with the external carbonyl carbons, posses slightly lower chemical shifts. Glycerol backbone shows signals of central carbon near 69 ppm and external carbons near 63 ppm.

Proton noise decoupled spectrum of *S. foetida* seed oil (see Table 4 and Appendix A) displayed peak at unusual high field, 7.42 ppm., which was splitted into triplet in an off-resonance spectrum (see Appendix A) ensured an assignment for methylene carbon of the cyclopropene moiety.

The signal at 109.12 and 109.45 ppm. which were unchanged in an off-resonance spectrum was assigned to the two olefinic carbons of cyclopropene group.

Table 4. Interpretation of ^{13}C NMR Proton Noise Decoupled Spectrum of *Sterculia foetida* Seed Oil

δ ppm	Assignment
7.42	methylene carbon of cyclopropene group
14.09	methyl carbon
22.65	methylene carbon next to methyl group
24.81	methylene carbon next to $-\text{CH}_2\text{COOR}$
26.01, 27.36, 29.04, 29.37	central methylene carbons
31.80	methylene carbon next to $-\text{CH}_2\text{CH}_3$
33.92, 34.08	methylene carbons next to $-\text{COOR}$
61.98, 68.86	glycerol backbone
109.12, 109.45	olefinic carbons of cyclopropene group
127.87, 128.03, 129.76, 130.03	olefinic carbons
172.40, 172.98	carbonyl carbons of ester groups

Proton noise decoupled spectrum of *S. lychnophora* oil (see Table 5 and Appendix A) shows very weak signals at both 7.42, 109.12 and 109.45 ppm. indicating the presence of small amount of CPFA.

S. lychnophora oil, in turn, displayed medium intensity signals at 127.87, 128.63, 129.60, 129.82 and 130.03 ppm., assigned to double bond moieties, while these signals were very weak in the spectrum of *S. foetida* seed oil indicating that *S. lychnophora* oil contained more of other unsaturated fatty acids than *S. foetida* seed oil. This result is in good agreement with GLC data.

Both *S. lychnophora* and *S. foetida* oils displayed the signals of glycerol backbone (61.98, 68.92 and 61.93, 68.86 respectively) and carbonyl carbons (172.45, 172.89 and 172.40, 172.78 ppm. respectively), proving that the fatty acids are present in both oils as triglycerides.

The signal of carboxyl group at 179.77 ppm. was very informative to reveal that some fatty acids of *S. lychnophora* oil were present as free acids, probably due to the hydrolysis of glycerol esters.

In ^1H NMR spectrum of *S. lychnophora* oil, there was no peak of methylene protons of cyclopropene ring at 0.78 ppm. (Jevans and Hopkins. 1968 : 2168) while the spectrum of *S. foetida* oil had a weak singlet at this region.

Cyclopropene moiety gives rise to characteristic absorption bands in IR at 1010 and 1871 cm^{-1} (Berry. 1979 : 226 ; Kaimal and Lak Shminarayana. 1970 : 2225 - 2229 ; Rinchart, Tarimu and Culbertson. 1959 : 5007 - 5008).

IR spectra of *S. foetida* seed oil, known to contain around 50% of CPFA (Schnieder, Loke and Hopkins. 1968 : 585 - 590), showed weak absorption at these characteristic regions.

Table 5 Interpretation of ^{13}C NMR Proton Noise Decoupled Spectrum of *Sterculia lychnophora* Oil

δ ppm	Assignment
7.42	methylene carbon of cyclopropene group
14.09	methyl carbon
22.65	methylene carbon next to methyl group
24.65	methylene carbon next to $-\text{CH}_2\text{COOR}$
24.81	methylene carbon next to $-\text{CH}_2\text{COOH}$
25.63, 27.14, 29.09, 29.31, 29.69	central methylene carbons
31.53, 31.91	methylene carbons next to $-\text{CH}_2\text{CH}_3$
34.03	methylene carbon next to $-\text{COOH}$ or $-\text{COOR}$
61.98, 68.92	glycerol backbone
109.12, 109.45	olefinic carbons of cyclopropene group
127.89, 128.03, 129.60, 129.82,	
130.03	olefinic carbons
172.45, 172.89	carbonyl carbons of ester groups
179.77	carboxyl carbon

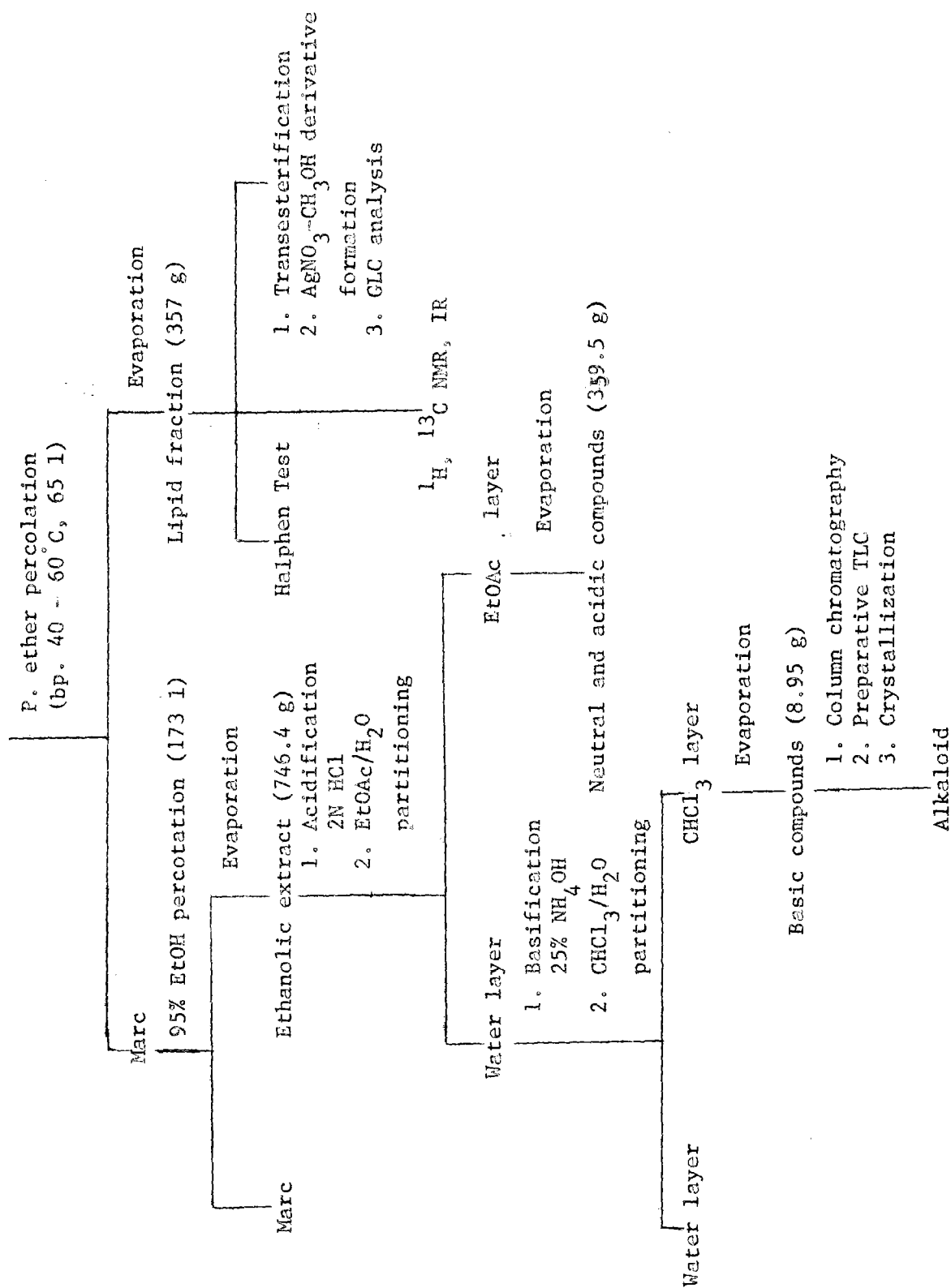
In IR spectrum of *S. lychnophora* oil, there is no absorption at these characteristic frequencies, probably due to the low content of CPFA.

Presented results indicate that IR and ^1H NMR techniques are not sensitive enough to detect the presence of CPFA at low concentration, while the gas chromatographic analysis is the method of choice for qualitative and quantitative determination. ^{13}C NMR spectra are proved to be useful in qualitative analysis of lipid fraction.

Chemical Investigation of Ethanolic Extract

The ethanolic extract, positive to alkaloid test, was fractionated into basic and neutral and acidic fractions (8.95 g, 0.12% yield and 359.5 g, 4.74% yield respectively). The fractionation procedure is shown in Figure 3. The basic compounds (2.5 g) were further fractionated by silica gel column chromatography (3.5×44 cm), eluted with CHCl_3 and CHCl_3 - CH_3OH mixtures of increasing polarity. 30 ml fractions were collected. The fractions were monitored by thin-layer chromatography using CHCl_3 - CH_3OH (9 : 1) as the solvent system and visualised by UV light (254 and 366 nm), spraying with 5% sulphuric acid in methanol and Dragendorff's spray reagent. Fractions were combined on the basis of their similarities in thin-layer chromatograms.

Although several fractions gave positive Dragendorff's test for alkaloid, only the CHCl_3 - CH_3OH (9 : 1) eluate (fractions 155 - 161 and 162 - 166, 384 and 209 mg respectively) afforded crystalline products.

Figure 3 Fractionation Scheme of *Sterculia lychnophora* Fruits

These fractions were composed of more than one compound but the major component was alkaloid (Rf 0.33 in CHCl_3 - CH_3OH , 9:1). The combined fractions were crystallized from CHCl_3 - CH_3OH mixture yielding white crystals, mp. $256 - 257.5^\circ \text{C}$ (15 mg), which were slightly contaminated with some more polar compounds.

Further purification was carried out by mean of preparative TLC using CHCl_3 - CH_3OH (9:1) as the solvent system. The resultant white solid (7.3 mg) which give yellow spot (Rf 0.33) under UV light (366 nm), was recrystallized from CHCl_3 - CH_3OH mixture yielding white crystals (1.5 mg) mp. 254°C , positive to Dragendorff's spray reagent.

As the amount of isolated alkaloid was very limited, only IR and UV spectra were obtained. The IR spectrum in KBr pallet (see Appendix A) exhibited broad band at $3600 - 3200 \text{ cm}^{-1}$ due to moisture which may obscure some other bands ; absorptions at 2955, 2920 and 2850 cm^{-1} correspond to methyl and methylene groups respectively ; 1738 and 1725 cm^{-1} can be assigned to carbonyl groups; few bands from $1630 - 1690 \text{ cm}^{-1}$ indicate the presence of amide groups; $1510 - 1550 \text{ cm}^{-1}$ corresponds to $-\text{NH}-$; and $1258, 1020 \text{ cm}^{-1}$ is due to aryl-alkyl ether.

The UV spectrum, in methanol, showed absorption at λ_{max} 215 nm ($E_{\text{cm}}^{1\%}$ 12.5), 256 nm (5) tentatively assigned to aromatic function.

The isolated alkaloid might be cyclopeptide alkaloid but the confirmation of this hypothesis requires further studies including analysis of ^1H and ^{13}C NMR and MS spectra, which the latter is particularly informative (Fehlhaber. 1968 : 19487k).

Chapter III

EXPERIMENTAL PART

Apparatus and Material

Gas-liquid chromatographic analysis was carried out on a JEOL JR-251 A gas chromatograph equipped with hydrogen flame ionization detector. The analysis was performed using a stainless steel column (3m × 3mm) packed with 20% diethylene glycol succinate adsorbed on 80 - 100 mesh chromosorb WAW, operated at 190 °C with carrier gas nitrogen at a flow rate of 40 ml/min. The injection port temperature was maintained at 230 °C.

A recorder was used for graphic presentation of the data and a Takeda Riken TR-2215 A digital integrator was used to obtained peak areas.

All melting points were determined on a Gallenham melting point apparatus and are uncorrected. UV spectra were determined in MeOH on a Beckman DB-G spectrophotometer. IR spectra of the alkaloid were registered in KBr pallets with Jasco IRA-1 spectrophotometer. IR spectra of *S. foetida* and *S. lychnophora* oil were determined on a UNICAM SP. 200 G spectrophotometer.

The ^1H and ^{13}C NMR spectra were recorded on a JEOL FX 90 Q Spectrometer operating at 89.55 and 22.50 MHz respectively. The spectra were obtained in CDCl_3 - CCl_4 (1:1) solution with TMS as an internal standard.

Thin-layer chromatography was carried on $200 \times 200 \times 0.25$ mm layers of kieselgel 60 GF 254 (Merk), activated at 105°C for 1 hour, and protectively stored in a desiccator.

Preparative TLC was carried on $200 \times 200 \times 0.75$ mm layers of kieselgel 60 GF 254 (Merk) activated at 105°C for 1 hour.

Silica gel 50 (70 - 230 mesh, Fluka) was used for column chromatography.

Plant Material

Sterculia lychnophora fruits were collected by Mr. Arun Wattanaphun, Chief of Pong Rad Rubber Plantation Station, in the forest in Chantaburi province in July 1981. The plant was authenticated under the direction of Dr. Chawalit Niyomdham, the Forest Herbarium officer, Royal Forest Department.

Sterculia foetida seeds were obtained from Miss Prateungsri Sinchaisri, Oil Seed Laboratory of Agricultural Chemistry Division of Department of Agriculture.

Phytochemical Screening of *Sterculia lychnophora* Fruits

Screening for alkaloids was done according to the phytochemical screening techniques (Phytochemical Screening. 2521 : chem 3 - 37 ; คู่มือปฏิบัติการทางพฤกษเคมี 2523 : 151 - 186). *S. lychnophora* dried ground fruits (50 g) was extracted with 80% EtOH (120 ml) by boiling on a water-bath for 1 hour. The extract was evaporated on a rotary evaporator under reduced pressure, yielding a syrupy residue which was then acidified

with 2 N HCl (15 ml), stirring on water-bath for 10 minutes. The acidic solution was filtered through filter paper and divided into two portions, 2 and 20 ml respectively.

Preliminary Alkaloid Test

The first portion (2 ml) was divided into 4 aliquots. Few drops of Dragendorff's, Mayer's, Valser's and Wagner's reagents were added to each aliquot.

Dragendorff's and Wagner's reagents gave orange and brown precipitates respectively, while Mayer's and Valser's reagents resulted white precipitates, indicating the presence of alkaloids in the examined sample.

Confirmed Alkaloid Test

The remaining filtrate (20 ml) was added to 25% NH_4OH to render the solution alkaline to litmus paper and then extracted with CHCl_3 (3×10 ml). The combined chloroform extracts were evaporated on water-bath. The obtained residue was dissolved in 2 N HCl (3 ml), stirring on water-bath for 3 minutes.

The acidic solution was divided into 4 aliquots, few drops of Dragendorff's, Mayer's, Valser's and Wagner's reagents were added to each aliquot respectively. The solution was clearly positive to these alkaloid tests.

Extraction Procedure

S. lychnophora fruits were oven dried at 50°C for 20 hours then ground to fine powder. The ground material (7.58 kg) was extracted by percolation with petroleum ether (bp. 40 - 60°C, 65 l) and then 95% ethanol (173 l). In practice the plant material was divided into three portions and separately extracted.

The yields of extractions are shown in Table 6. Solvents were removed on a rotary evaporator under reduced pressure at 40°C. Brown oil was obtained from the petroleum ether extract and dark brown syrupy residue from the ethanolic extract.

Table 6 Extraction Yields

Solvent	1.4 kg plant material		3.0 kg plant material		3.18 kg plant material		Total	
	Volume of solvent (l)	Yield (g)	Volume of solvent (l)	Yield (g)	Volume of solvent (l)	Yield (g)	Volume of solvent (l)	Yield (g)
Petroleum ether	15	76.7	26	114	24	166.3	65	357
95% ethanol	21.1	107.9	84.4	310.5	67.5	328	173	746.4

Plant material (460 g) left after extraction with 95% ethanol was moistened with 10% NH_4OH (250 ml) and 1% Na_2CO_3 (250 ml), then dried under sunlight and extracted with chloroform in Soxhlet extractor for 16 hours.

The extract was evaporated on a rotary evaporator under reduced pressure yielding brown residue (1.9 g), which was negative to alkaloid tests (Dragendorff's, Mayer's and Valser's reagents).

Extraction of *Sterculia foetida* Seeds

S. foetida seeds were oven dried at 50°C for 24 hours and cracked to obtain kernel, which was then ground finely. The ground material (23.2 g) was extracted in a Soxhlet extractor with petroleum ether (bp. 40 - 60°C) for 25 hours. Solvent removal on a rotary evaporator under reduced pressure at 40°C left a yellow oil (11.8 g).

Fractionation Procedure

Crude ethanolic extract of *S. lychnophora* fruits and extracted with ethyl acetate to remove neutral and acidic compounds. The aqueous layer was then basified with 25% NH_4OH to pH 9 - 10 and extracted with chloroform until extract was negative to alkaloid tests.

Thus crude ethanolic extract (310.5 g) was divided into two portions (155 and 155.5 g) and each portion was suspended in 2 M HCl (600 ml) and extracted with ethyl acetate (11 × 600 ml). The aqueous layer was then basified with 25% NH_4OH to pH 9 - 10 and extracted with chloroform (11 × 600 ml) until the extract was negative to alkaloid tests.

The combined ethyl acetate extracts containing neutral and acidic compounds were evaporated under reduced pressure at 40°C yielding dark green syrupy residue (149.5 g). Chloroform extract yielded red syrupy residue (3.72 g).

In total, extraction and fractionation of 7.58 kg of plant material gave 359.5 g of neutral and acidic compounds and 8.95 g of basic compounds respectively.

Halphen Test

Reagent (5 ml), prepared by mixing 1% S in CS₂ (30 ml) with amyl alcohol (30 ml), was added to *S. lychnophora* oil (5 ml) and heated on a water-bath (70 - 80 °C) until the foam disappeared (15 min). Then the reaction mixture was heated on an oil bath (112 °C) for 10 minutes.

Crude cotton seed oil (5 ml) which is known to contain 1% of cyclopropenoid fatty acids was examined paralelly.

S. lychnophora oil and crude cotton seed oil gave similar dark red colour developments under the reaction conditions.

Preparation of the Methyl Esters of *Sterculia lychnophora* Oil

Methyl esters were prepared by refluxing the *S. lychnophora* oil (970.2 mg) with 1% solution of sodium methoxide in methanol (50 ml) for 20 minutes. Then the solution was cooled, diluted with distilled water (150 ml) and methyl esters were extracted twice with petroleum ether (2 × 100 ml, bp. 40 - 60 °C). The combined extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure at 30 °C

Silver Nitrate Derivatives Formation

The crude methyl esters were allowed to react with saturated solution of silver nitrate in anhydrous methanol (150 ml) for 20 hours at room temperature. Then water (150 ml) was added to the reaction mixture and the unchanged saturated methyl esters and the reaction products were extracted three times with petroleum ether (3 × 50 ml, bp. 40 - 60°C). The combined petroleum ether extracts were dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure at 30°C. The resulted residue was further analyzed by GLC.

Thus the residue was dissolved in petroleum ether (0.5 ml, bp. 40 - 60°C), and the aliquots (0.2 µl) of this solution were injected into the gas-liquid chromatograph.

Preparation of the Methyl Esters and Silver Nitrate Derivatives of

Sterculia foetida Seed Oil

The methyl esters and silver nitrate derivatives of *S. foetida* seed oil (637.6 mg) were prepared in the same manner as described previously.

The obtained residue was dissolved in petroleum ether (0.5 ml) and the aliquots (1 µl) were injected into the gas-liquid chromatograph.

Column Chromatography of the Basic Fraction

Basic fraction (2.5 g) was chromatographed on a silica gel column (55 × 3.5 cm). The sample was dissolved in minimal amount of chloroform-methanol mixture, silica gel (5 g) was added and solvent removed on a rotary evaporator under reduced pressure at 40°C.

The sample, adsorbed on silica gel, was transferred on the top of the column which was eluted with chloroform (2,350 ml) then with chloroform-methanol mixture of increasing polarity (2% MeOH in CHCl_3 , 1,500 ml ; 5% MeOH in CHCl_3 , 1,250 ml ; 10% MeOH in CHCl_3 , 1,100 ml ; 15% MeOH in CHCl_3 , 1,000 ml) followed by absolute methanol (2,000 ml), 30 ml fractions were collected. The fractions were monitored by thin-layer chromatography using chloroform-methanol (9:1) as the solvent system and visualised by UV light (254 and 366 nm), spraying with 5% sulphuric acid in methanol and Dragendorff's spray reagent. Fractions were combined on the basis of their similarities in thin-layer chromatography as presented in Table 7.

The combined fractions number 5, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18 were positive to Dragendorff's spray reagent.

Fractions 155 - 161 and 162 - 166 (384 and 209 mg respectively) were crystallized from chloroform-methanol mixture yielding white crystals, mp. 256 - 257.5 °C (15 mg). TLC examination of these crystals showed that they were slightly contaminated with some more polar compounds. Further purification of this compound (15 mg) was done by preparative TLC in chloroform-methanol (9:1). The band corresponding to R_f 0.33 (positive to Dragendorff's spray reagent) was scraped off from the plate. The alkaloid was eluted with 30% methanol in chloroform, filtered, and the solvent was removed on rotary evaporator under reduced pressure at 40 °C. The resultant white solid (7.3 mg) was recrystallized from chloroform-methanol mixture, yielding white crystals (1.5 mg) of mp. 254 °C ; UV max (MeOH) 215 nm ($E_{1\%}^{1\text{cm}}$ 12.5), 256 nm (5) ; IR ν max 3,600 - 3,200 cm^{-1}

(moisture) ; 2955, 2920 cm^{-1} ($-\text{CH}_3$) ; 2850 cm^{-1} ($-\text{CH}_2-$) ; 1738, 1725 cm^{-1} ($-\text{CO}-$) ; 1690 - 1630 cm^{-1} ($-\text{CONH}-$) ; 1550 - 1510 cm^{-1} ($-\text{NH}-$) ; 1258, 1020 cm^{-1} (C-O-C).

Table 7 Results of Column Chromatography of Basic Fraction

No.	Fraction	Weight (mg)	Rf value
1.	1 - 15	58	0.88
2.	16 - 27	14	0.88, 0.67
3.	80 - 105	27	0.49
4.	106 - 118	77	0.49, 0.42
5.	119 - 127	12	0.42
6.	128 - 132	25	0.42, 0.36 (yellow under UV light at 366 nm)
7.	133 - 136	10	0.42, 0.36 (blue under UV light at 366 nm)
8.	137 - 141	12	0.36
9.	142 - 150	42	0.36, 0.33 (blue under UV light at 366 nm)
10.	155 - 161	384	0.42, 0.36, 0.33 (yellow under UV light at 366 nm)
11.	162 - 166	209	0.33, 0.27, 0.24
12.	167 - 184	244	0.33, 0.27, 0.24, 0.18
13.	185 - 188	10	0.33, 0.27, 0.24, 0.18
14.	189 - 194	29	0.24, 0.18, 0.15
15.	195 - 198	21	0.24, 0.18, 0.15
16.	199 - 208	105	0.24, 0.18, 0.15
17.	209 - 218	69	0.15, 0.12
18.	219 - 284	745	0.15, 0.12, 0.09, 0.06, 0.03 and on the base line

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- เต็ม สมิติพันธ์ ชื่อพรรณไม้แห่งประเทศไทย กรมป่าไม้ 2523, 379 หน้า
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APPENDIX

APPENDIX A

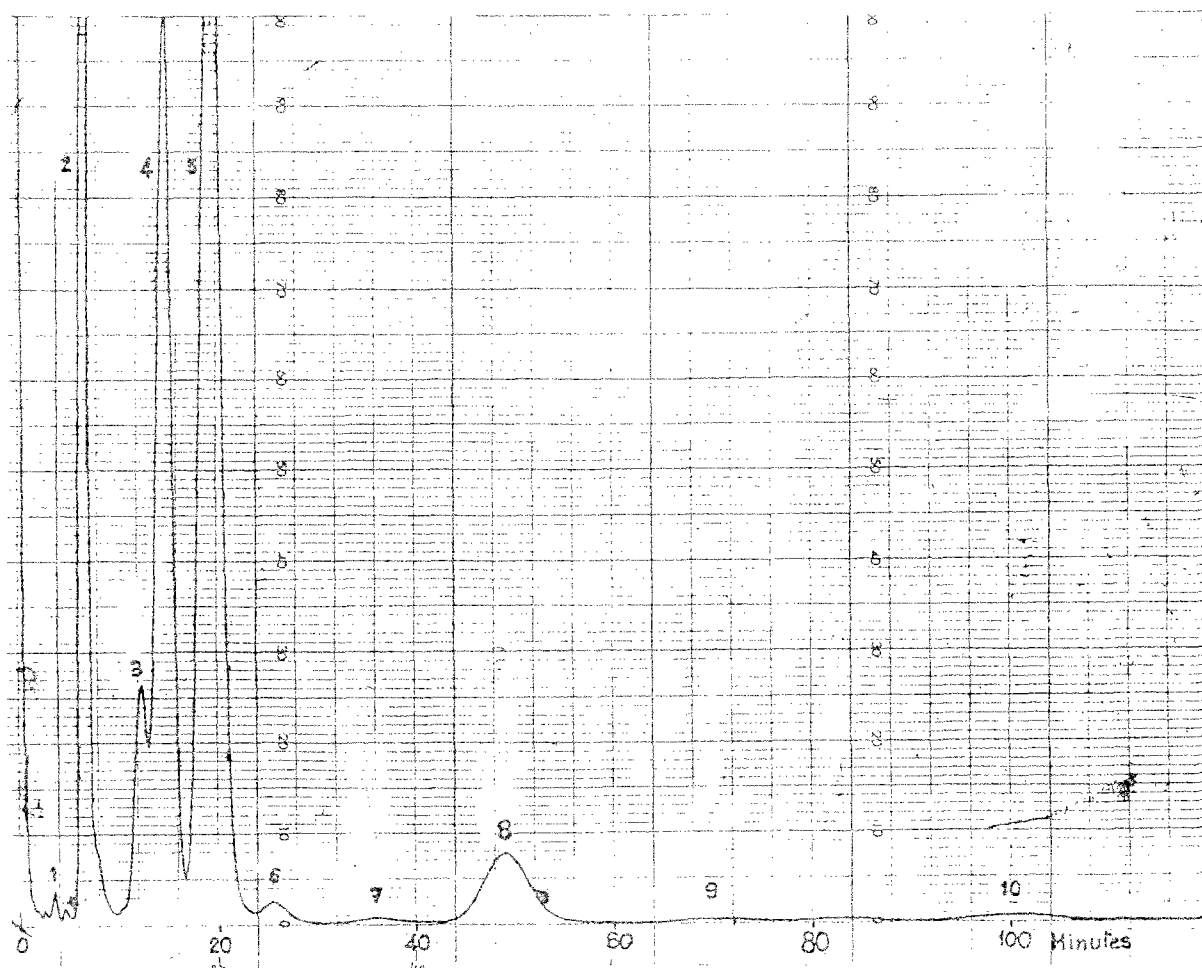


Figure 4. Gas-Liquid Chromatogram of $\text{AgNO}_3\text{-CH}_3\text{OH}$ Derivatives of Methyl Esters of *Sterculia lychnophora* Oil. Peaks are listed as follows:

1-methyl myristate; 2-methyl palmitate; 3-methyl stearate; 4-methyl oleate; 5-methyl linoleate; 6-methyl linolenate; 7-ether derivative of methyl malvalate; 8-ether derivative of methyl stercolate; 9-ketone derivative of methyl malvalate; 10-ketone derivative of methyl stercolate.

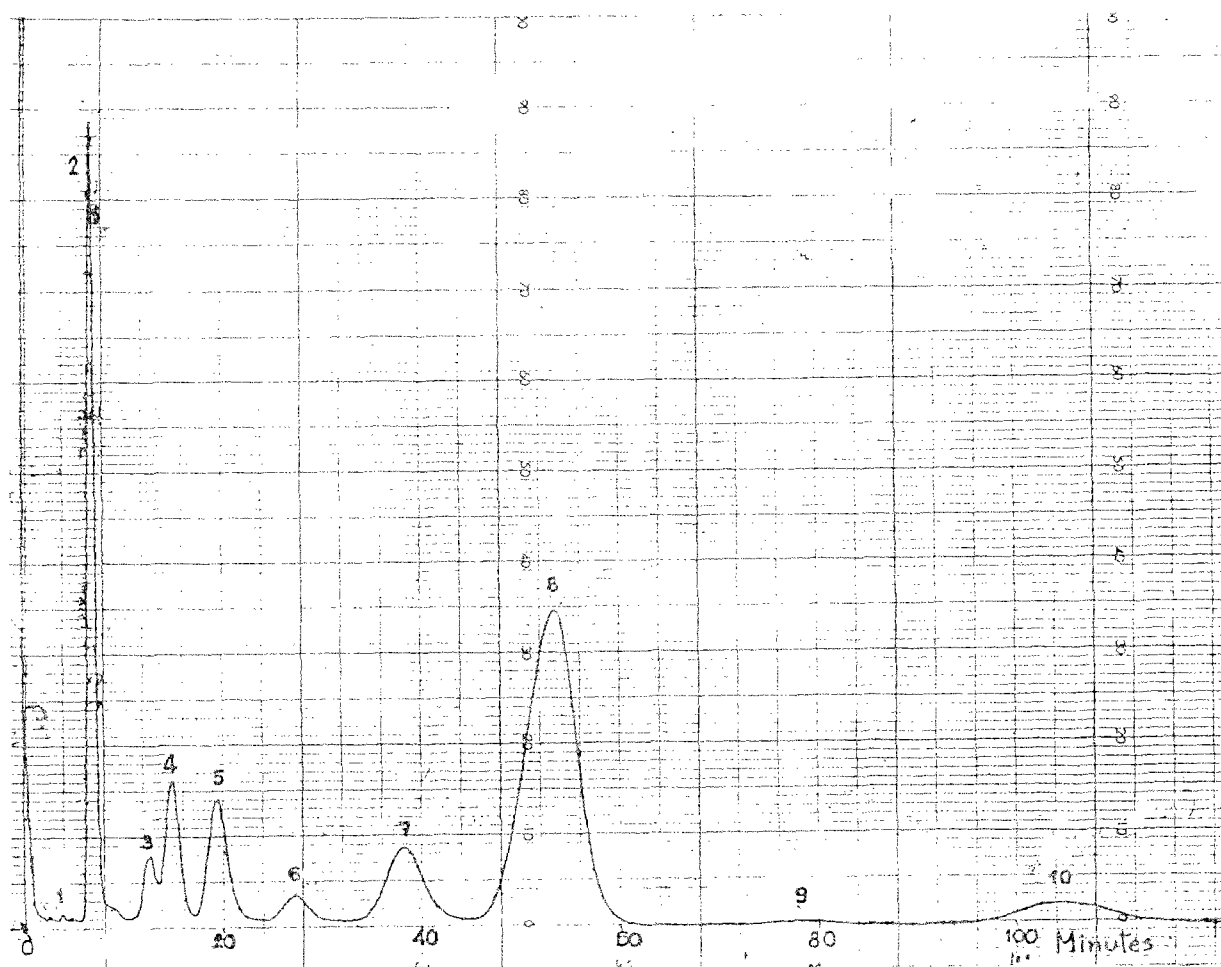


Figure 5. Gas-Liquid Chromatogram of $\text{AgNO}_3\text{-CH}_3\text{OH}$ Derivatives of Methyl Ester of *Sterculia foetida* Seed Oil. Peaks are listed as follows:
 1-methyl myristate; 2-methyl palmetate; 3-methyl stearate; 4-methyl oleate; 5-methyl linoleate; 6-methyl linolenate; 7-ether derivative of methyl malvalate; 8-ether derivative of methyl stercolate; 9-ketone derivative of methyl malvalate; 10-ketone derivative of methyl stercolate.

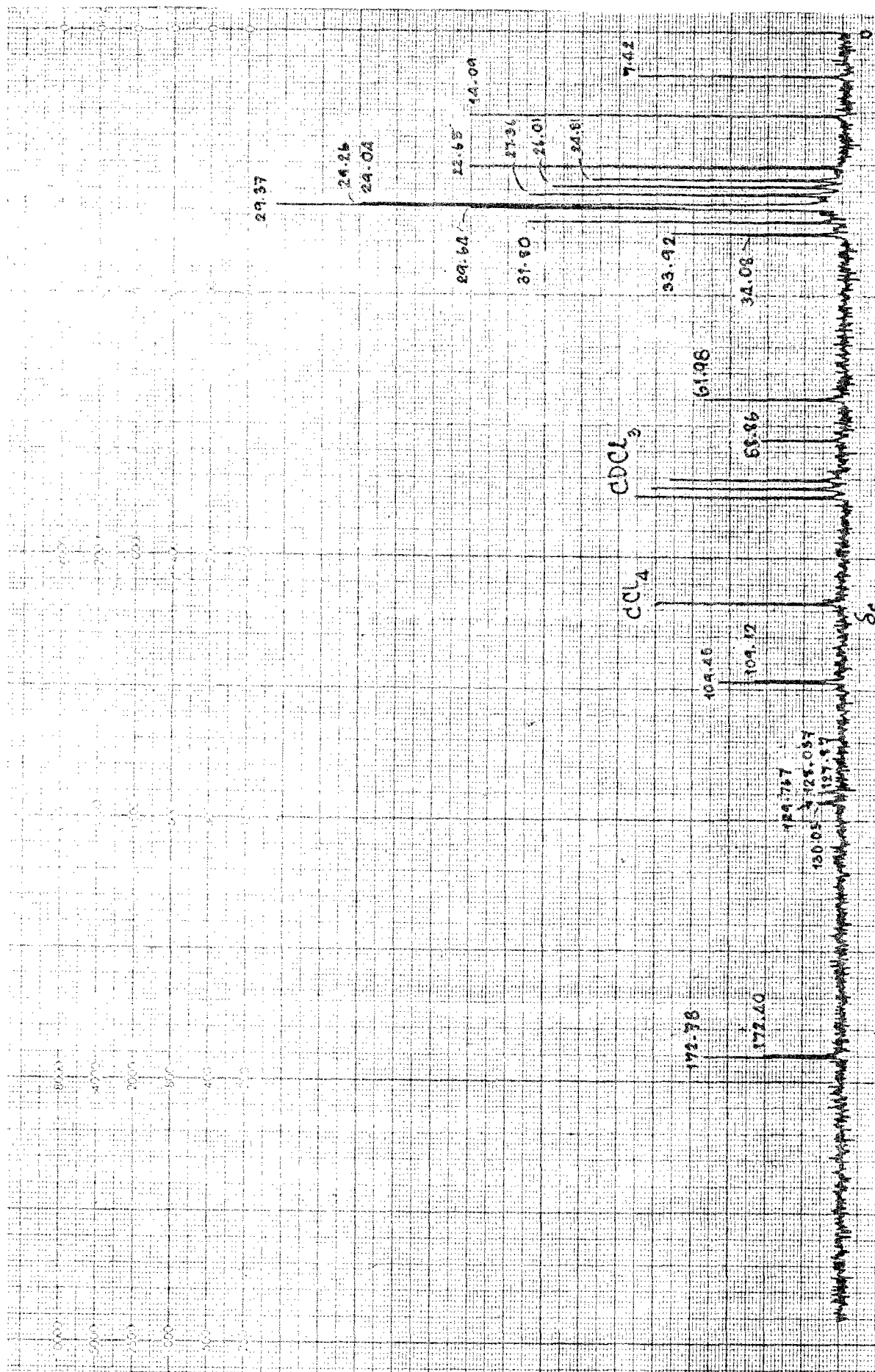


Figure 6. ^{13}C NMR Proton Noise Decoupled Spectrum of Sterculia foetida Seed Oil in $\text{CDCl}_3\text{-CCl}_4$ (1:1) Solution, Using TMS as an Internal Reference.

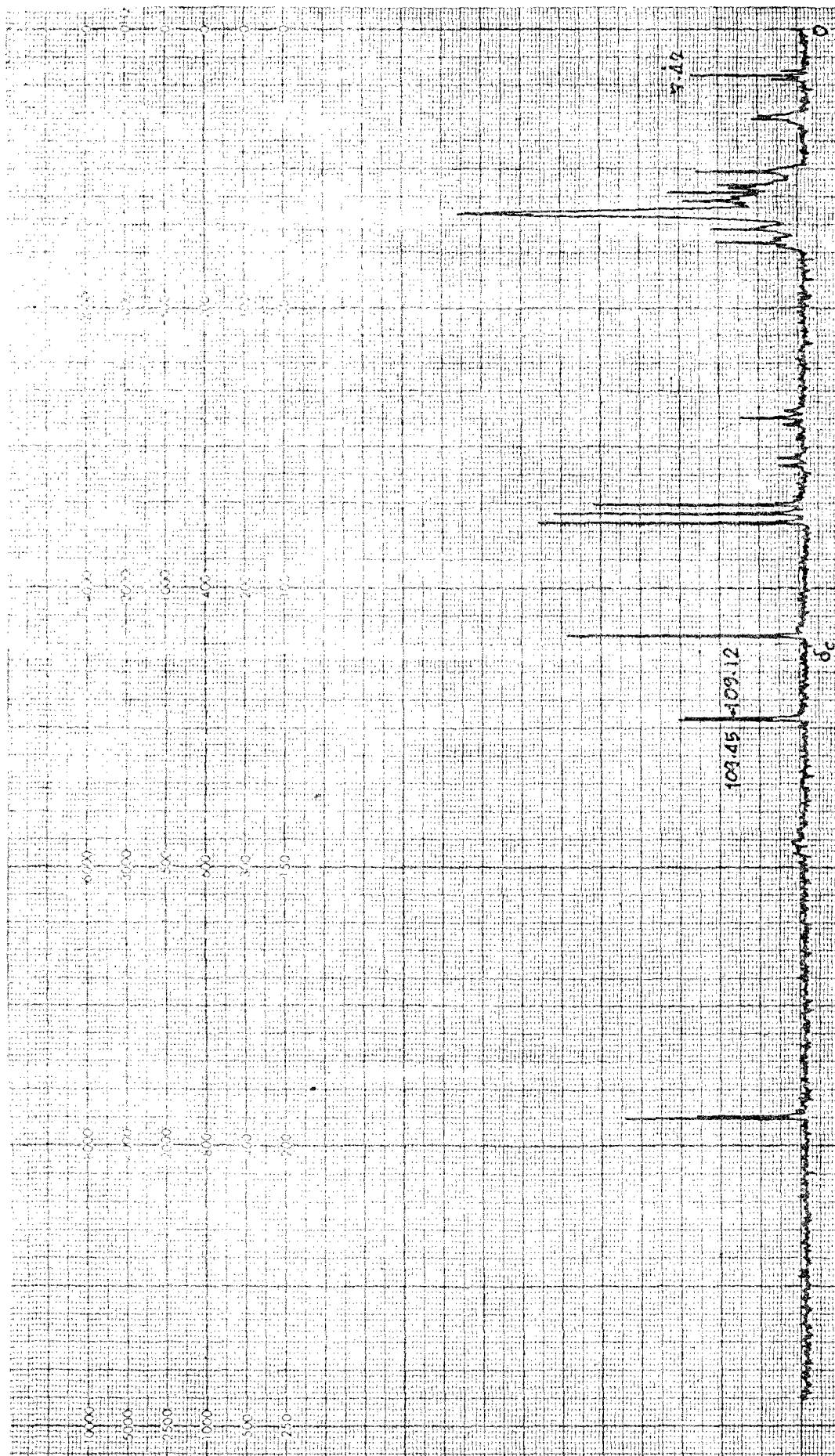


Figure 7. ^{13}C NMR Off-Resonance Decoupled Spectrum of *Sterculia foetida* Seed Oil in $\text{CDCl}_3\text{-CCl}_4$ (1:1) Solution,

Using TMS as an Internal Reference.

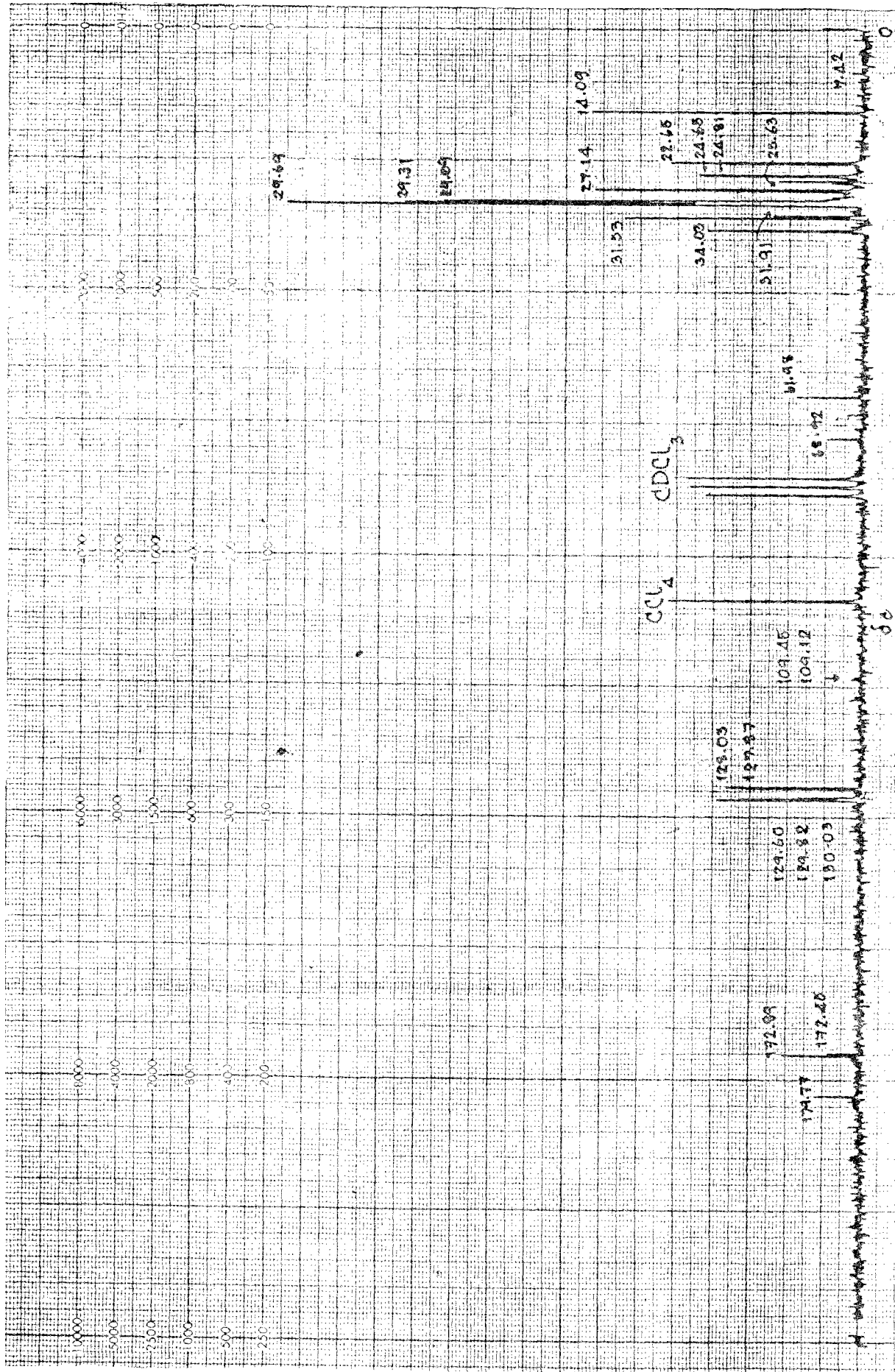


Figure 8. ^{13}C NMR Proton Noise Decoupled Spectrum of Sterculia lychrophora Oil in CDCl_3 - CCl_4 (1:1) Solution, Using TMS as an Internal Reference.

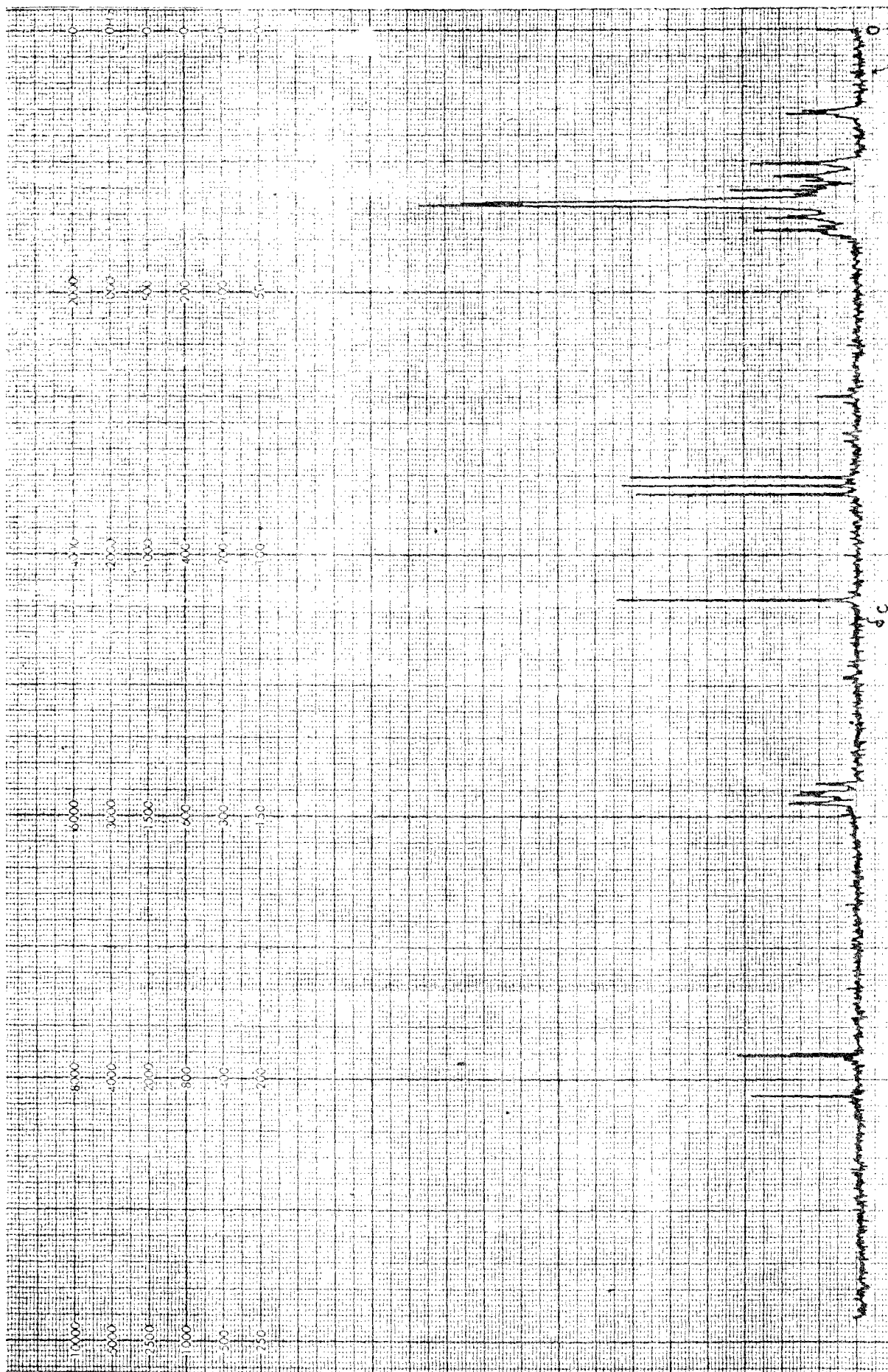


Figure 9. ^{13}C NMR Off-Resonance Decoupled Spectrum of Sterculia lychnophora Oil in $\text{CDCl}_3\text{-CCl}_4$ (1:1) Solution, Using TMS as an Internal Reference.

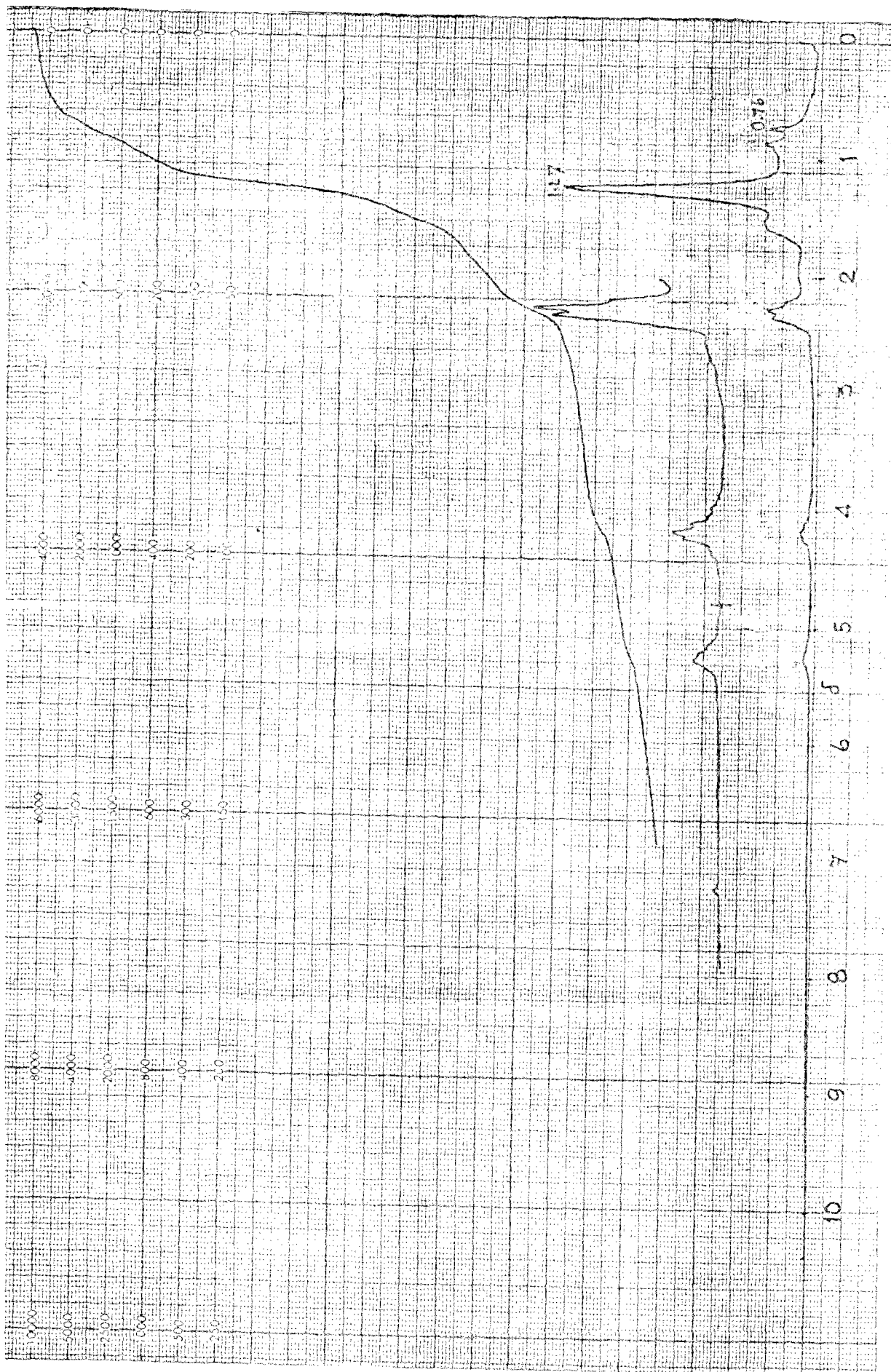
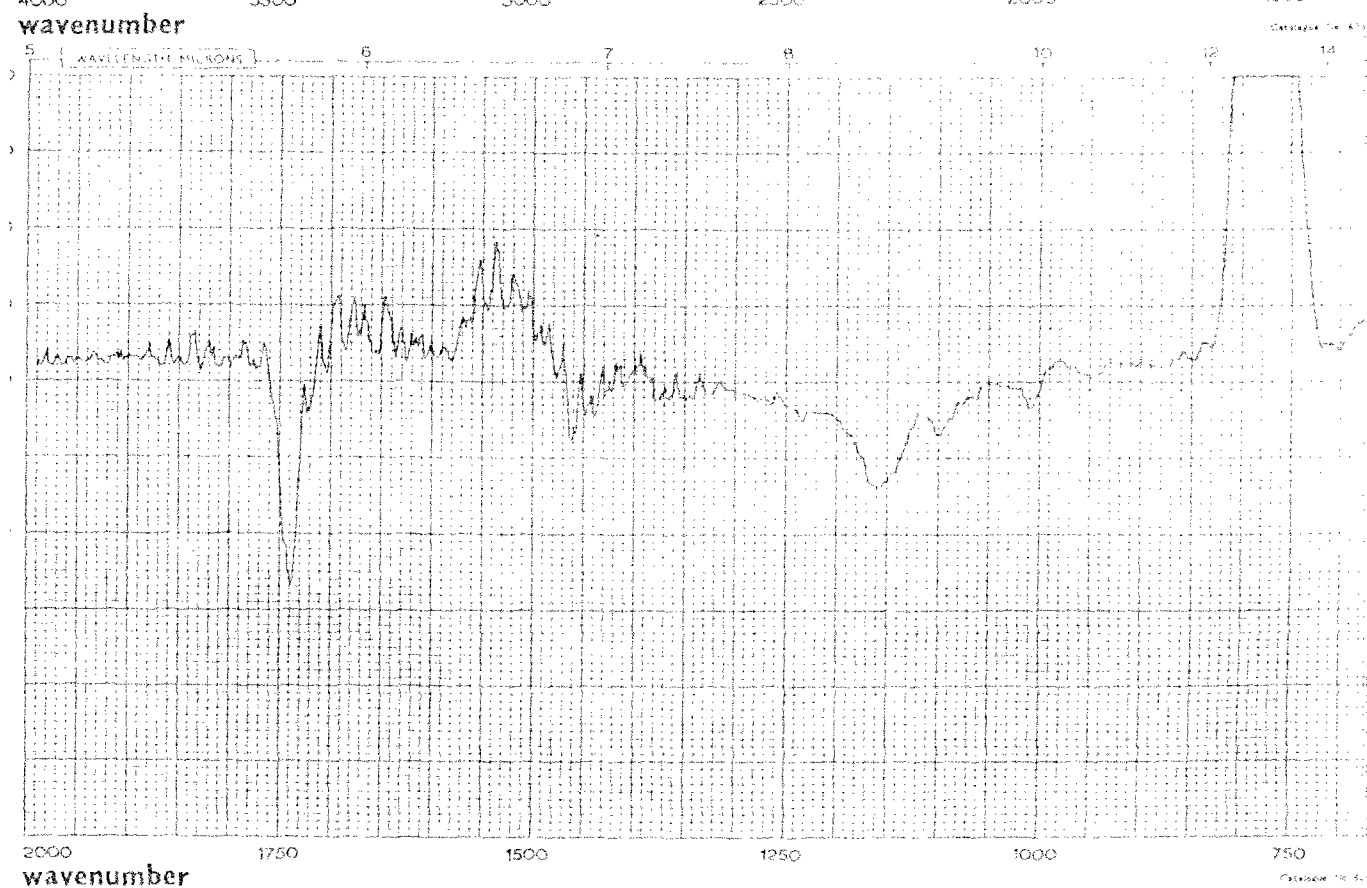


Figure 11. ^1H NMR Spectrum of *Sterculia foetida* Seed Oil in $\text{CDCl}_3\text{-CCl}_4$ (1:1) Solution, Using TMS as an Internal Reference.



e 12. IR Spectrum, Obtained in CCl_4 Solution, of *Sterculia foetida* Seed Oil.



Catalogue No. 60604

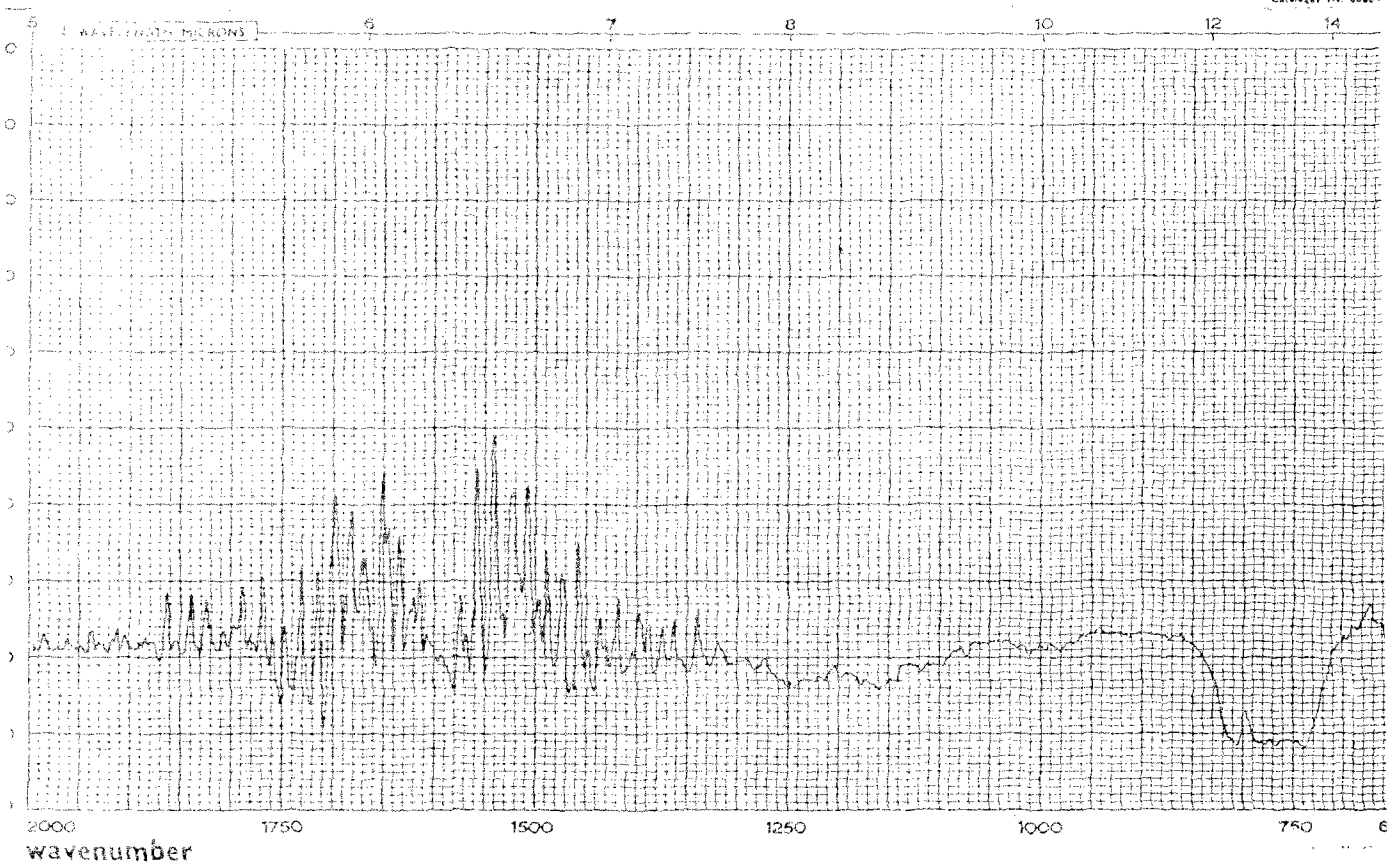


Fig. 13. IR Spectrum, Obtained in CCl_4 Solution, of *Sterculia lychnophora* Oil.

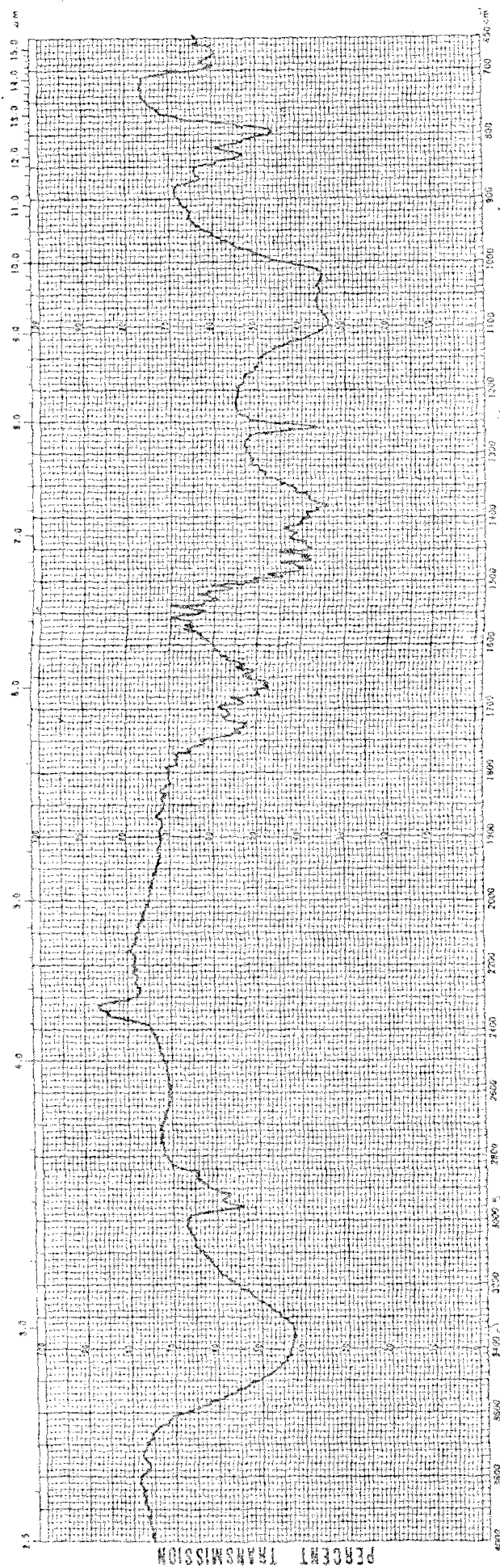


Figure 14. IR Spectrum, Obtained in KBr Pallet, of the Isolated Alkaloid.

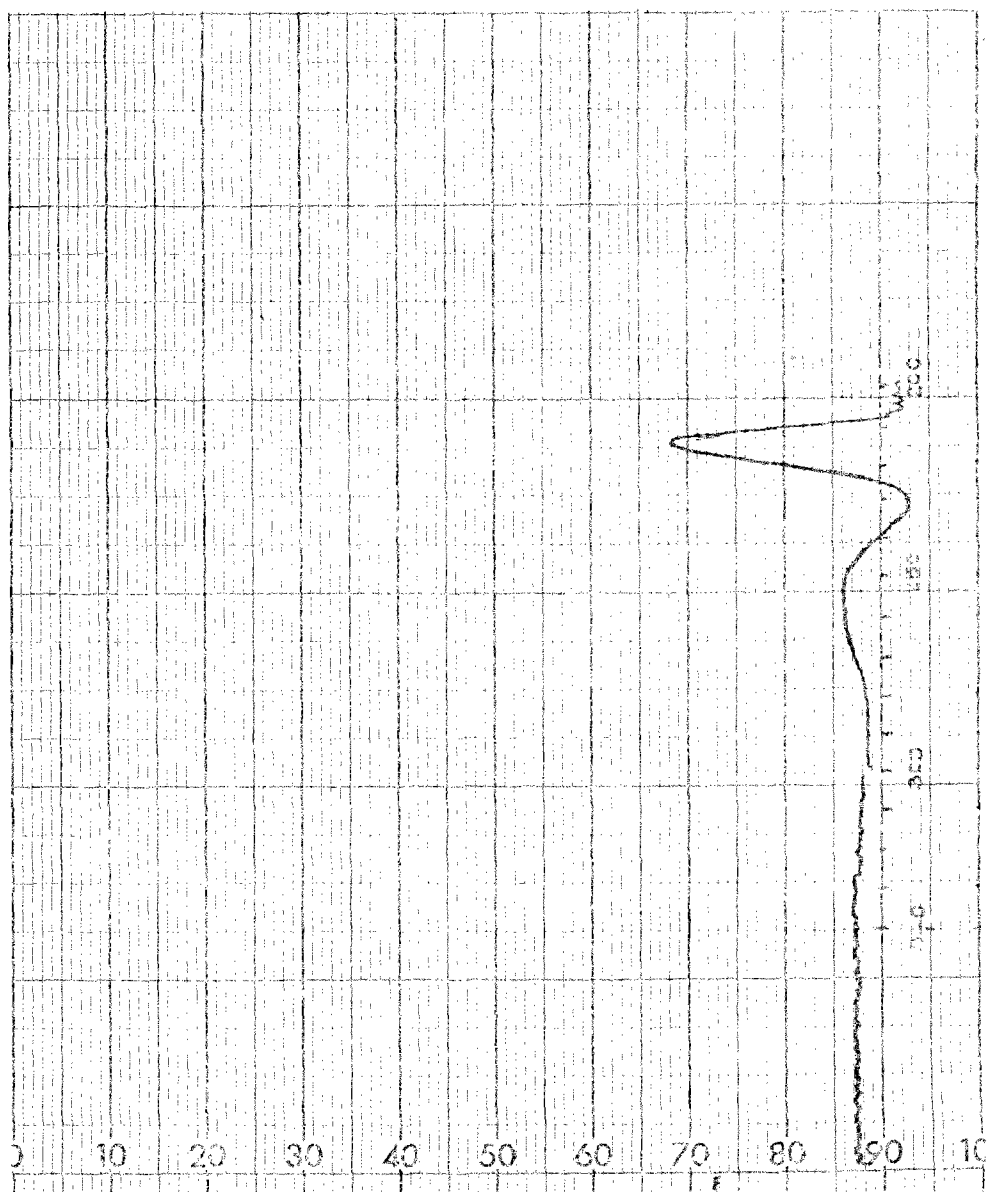


Figure 15. UV Spectrum, in MeOH, of the Isolated Alkaloid.

APPENDIX B

Reagents for Alkaloid Test

1. Dragendorff's

Bismuth nitrate 8 g HNO_3 (30% w/v) 12 ml

Potassium iodide 27.2 g Distilled water

Dissolve $\text{Bi}(\text{NO}_3)_3 \cdot 5 \text{H}_2\text{O}$ in HNO_3 , Then add with KI solution (in 50 ml H_2O). Make the volume to 100 ml with water.

2. Dragendorff's spray

Solution A : Dissolve 0.35 g of basic bismuth nitrate in the mixture of acetic acid : H_2O (10:40).

Solution B : Dissolve 8 g of KI in 20 ml H_2O

Mix 1 ml of each solution together, then add with 4 ml acetic acid and 20 ml H_2O prior to use. Protect the solution from light.
(The solution has to be prepared 1 week before use.)

3. Mayer's

Mercuric chloride 1.36 g Potassium iodide 5 g

Distilled water

Dissolve HgCl_2 in 60 ml water, then add with KI solution (in 10 ml H_2O). Add water to the solution to make 100 ml.

4. Valser's

Potassium iodide 10 g Red mercuric iodide 14 g

Distilled water

Dissolve KI in 80 ml water. Make the volume to 100 ml with water.
Add HgI_2 to the solution, stir until HgI_2 is nearly completely dissolved.

5. Wagner's

Iodine 1.27 g

Potassium iodide 2 g

Distilled water

Dissolve I_2 in KI solution (in water). Add water to the solution
to make 100 ml.

CHEMICAL INVESTIGATION OF *STERCULIA LYCHNOPHORA* HANCE FRUITS

AN ABSTRACT

BY

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This work is a part of the *Sterculia lychnophora* (Phungtha-lai) project under the Programme "Development in Quality Improving and Controlling for Medicinal Plants for Export" of Thailand Institute of Scientific and Technological Research.

We have undertaken the research on *Sterculia lychnophora* fruits because they are commonly used for medicinal purposes but the plant has never been investigated and reported in scientific literature. Phytochemical screening of the ethanolic extract revealed the presence of alkaloids. Lipid fraction, obtained by preliminary extraction of the ground fruits with petroleum ether, gave positive Halphen test, characteristic for cyclopropenoid fatty acids (CPFA).

Quantitative analysis of lipids was done by gas-liquid chromatography of $\text{AgNO}_3\text{-CH}_3\text{OH}$ derivatives of crude oil which was found to comprise 6% of CPFA and 65% of other unsaturated fatty acids. The occurrence of CPFA was also supported by the analysis of ^{13}C NMR spectra.

Fractionation of ethanolic extract afforded basic fraction (4.74% yield) containing several alkaloids. Column chromatography of this fraction followed by preparative TLC resulted an alkaloid (7.3 mg) which was further crystallized yielding crystals (1.5 mg) mp. 254°C .

As the amount of the isolated compound was very limited, only IR and UV spectra were obtained. IR spectrum exhibited bands corresponding to peptide linkage and aryl-alkyl ether. The UV spectrum showed the presence of aromatic function.

Structure elucidation of the alkaloid requires further research, including ^1H , ^{13}C NMR and MS studies.

การศึกษาองค์ประกอบทางเคมีในผลพวงทะเลาย

บทคัดย่อ

ของ

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เพื่อเป็นส่วนหนึ่งของการศึกษาตามหลักสูตร

ปริญญาการศึกษามหาบัณฑิต

กรกฎาคม 2525

งานวิจัยนี้เป็นส่วนหนึ่งของงานวิจัยผลพวงทะเลาย ซึ่งอยู่ในโครงการ "การพัฒนาด้านการปรับปรุงและควบคุมคุณภาพของพืชสมุนไพรเพื่อการส่งออกขายต่างประเทศ" ของสถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ผู้วิจัยเลือกทำวิจัยเรื่องนี้เนื่องจากผลพวงทะเลายใช้เป็นสมุนไพร แต่ยังไม่เคยมีการศึกษาองค์ประกอบทางเคมีมาก่อน จากการทดสอบสิ่งที่สกัดได้ในเอทานอลของผลพวงทะเลายนี้อย่างคร่าว ๆ พบว่ามีสารประกอบประเภทอัลคาลอยด์ นอกจากนี้ส่วนที่เป็นไลปิด ซึ่งได้จากการสกัดโดยใช้อีเทอร์เลียมฮีเธอร์ พบว่ามีกรดไขมันไฮโดรโปรีนอยด์ โดยทดสอบด้วยวิธีทดสอบของ ฮาลเพน

จากการวิเคราะห์ปริมาณกรดไขมัน ในรูปอนุพันธ์ของซิลเวอร์ไนเตรต-เมทานอล ด้วยวิธีก๊าซลิควิดโครมาโตกราฟี พบว่าส่วนที่เป็นไลปิดประกอบด้วยกรดไขมันไฮโดรโปรีนอยด์ ร้อยละ 6 และกรดไขมันชนิดไม่อิ่มตัว ร้อยละ 65 นอกจากนี้ในการวิเคราะห์ส่วนที่เป็นไลปิดด้วย ^{13}C NMR พบว่า มีกรดไขมันไฮโดรโปรีนอยด์เช่นกัน

สิ่งที่สกัดได้ในเอทานอล มีส่วนที่มีสมบัติเป็นเบสคิดเป็นปริมาณร้อยละ 0.12 ซึ่งประกอบด้วยสารประกอบประเภทอัลคาลอยด์ จากการแยกด้วยคอลัมน์โครมาโตกราฟี และเพอร์พาราทีฟรินเลเยอร์โครมาโตกราฟี ได้อัลคาลอยด์ 7.3 มิลลิกรัม หลังจากตกผลึกแล้วได้ผลึกอัลคาลอยด์ 1.5 มิลลิกรัม มีจุดหลอมเหลว 254°C

เนื่องจากปริมาณของอัลคาลอยด์ที่แยกได้มีจำกัด จึงทำให้ศึกษาได้แต่เพียง IR และ UV สเปกตรัมเท่านั้น ใน IR สเปกตรัมพบว่ามีหมู่ที่เกาะกันด้วยพันธะเปปไทด์ และหมู่เออร์ล-อัลคิลฮีเธอร์ ส่วนใน UV สเปกตรัม พบว่าโมเลกุลของอัลคาลอยด์มีส่วนที่เป็นอะโรมาติก

การที่จะศึกษาโครงสร้างของอัลคาลอยด์ให้เสร็จสมบูรณ์นั้น ยังต้องอาศัยข้อมูลจาก ^1H , ^{13}C NMR และ MS