CHAPTER III

EXPERIMENTAL

I. Alumina for column chromatography

In all cases Merck's aluminium oxide, active and neutral has been used for the preparation of alumina columns for chromatography.

II. Alumina for thin layer chromatography

Merck's reagent aluminium oxide G was used throughout for the preparation of thin layers of alumina.

III. Silica gel for column chromatography

Silica gel used in this work was Riedel de Hann lessthan 0,08 mm.

IV. Infra-red spectra

The infra-red spectra of all compounds have been determined as nujol mulls on a Perkin Elmer Infra-cord Model 137 E unless otherwise indicated.

V. Liebermann-Berchard test

A few drops of acetic anhydride and one drop of sulphuric acid were added to a solution of a few crystals of the compound to be tested in a small volume of chloroform. The production of a colour after a few minutes indicated the presence of steroids or triterpenoids.

VI. Thin layer chromatography

Thin layers of adsorbent were prepared in the

following manner; the thickness of each coating was not determined. A mixture of alumina (20 g.) and water (40 ml.) was stirred until it became viscous, then it was applied to glass plates (20 X 20 cm.) using a Desaga spreader. After being dried at room temperature for one hour, the plates were activated at 125° for 30 minutes, cooled and stored in a desiccator until required for use. Two lines were described on each plate, one 2 cm. from one edge and parallel to it, which we shall refer to as the base line, and the other 10 cm. above this line and parallel to it, the upper line. The compounds (about 500 µg.) to be examined in solvent were applied as small spots on the base line of a plate at 4 cm. interval and, after the solvent had evaporated, the plate was placed in a glass tank filled to a depth of 1 cm. with the eluting solvent and the tank covered with a glass cover. The eluting solvent immediately began to climb up the plate at a fast rate which gradually became slower as the distance between the solvent front and the base line increased. The time taken for the solvent front to reach the upper line varied according to the solvents used; times between 20 and 45 minutes were generally encountered. When the solvent front had reached the upper line, the plate was removed from the tank, the solvent allowed to evaporate, and the plate sprayed with a suitable reagent to reveal the compounds.

EXPERIMENTAL (Continued)

PART A. THE LEAF WAX OF THAI BANANA PLANT

I. Extraction of banana leaves

Banana leaves (930 g.), previously dried in the sun for a few days and then in an oven at 60°C, were exhaustively extracted with light petroleum in a Soxhlet extractor. A wax (51 g.) was precipitated from the petrol extract when cooled, and this was separated from the petrol soluble wax by decanting and centrifuging. Evaporation of the petrol soluble fraction in a rotatory evaporator afforded a wax (51 g.).

1. Constituents of the petrol insoluble wax

1-1. Saponification of the petrol insoluble wax

To a solution of the petrol insoluble wax (51 g.) in benzene (500 ml.) was added potassium hydroxide (25 g.) in methanol (250 ml.), and the mixture was heated under reflux on a water bath for 8 hours. The resulting solution was concentrated to a small volume in a rotatory evaporator, and then transfered to an evaporating basin heated on a water bath for the removal of the remaining solvent. A solid remained after removal of all the solvent, which was ground to a fine powder with a spatula after adding some anhydrous sodium sulphate, and transfered to a thimble

contained in a Soxhlet extractor. The solid was extracted continuously with ether until no more colour was removed. Evaporation of the ether furnished the unsaponifiable matter (5.2g.) as a wax.

1-2. Chromatography of the unsaponifiable matter

The wax (5.2g.) was adsorbed on alumina (180g.) from a solution of chloroform and benzene. Elution of the column was carried out as shown in Table I.

TABLE I

Eluant	Number of 50 ml. fractions collected	m.p. of compound °C	Liebermann-Burchard Test	Remarks
Petroleumether	16	82-83	negative	Possibly hydrocarbon (2.12g)
20 % Chloroform in Petrol	7	82-83	negative	
20 % Chloroform in Petrol	14	—	green→brown	Mainly wax with little β-sitosterol (.81g.)
20 % Chloroform in Petrol	11	—	negative	
30 % Chloroform in Petrol	18	—	negative	Unidentified (.4g.)

TABLE I (Continued)

Eluant	Number of 50 ml. fractions collected	m.p. of compound °C	Liebermann-Burchard Test	Remarks
30 % Chloroform in Petrol	7	-	green → brown	Possibly steroidal fraction (.19g.)
40 % Chloroform in Petrol	4	-	green → brown	Steroidal fraction (.2g.)
Chloroform	11	-	green → brown	Steroidal fraction (1.35g.)

Total steroidal fractions in the insoluble wax was 2.55g.

2. Constituents of the petrol soluble wax

2-1. Saponification of the petrol soluble wax

To a solution of the petrol soluble wax (51g.) in benzene (1 litre) was added potassium hydroxide (100g.) in methanol (1 litre), and the mixture was heated under

reflux on a water bath for 8 hours. The resulting solution was concentrated to a small volume in a rotatory evaporator, and then transfered to an evaporating basin heated on a water bath for the removal of the remaining solvent. A solid remained after removal of all the solvent, which was ground to a fine powder with a spatula after adding some anhydrous sodium sulphate and transfered to a thimble contained in a Soxhlet extractor. The solid was extracted continuously with ether until no more colour was removed. Evaporation of the ether furnished the unsaponifiable metter (30g.) as a wax.

2-2. Chromatography of the unsaponifiable matter.

The unsaponifiable matter (30g.) was dissolved in benzene (100 ml.) and adsorbed on silica gel (1 kg.). Elution of the column was carried out as shown in Table II.

TABLE II

Eluant	Number of fl. fractions collected	m.p. of compound °C	Libermann-Burchard Test	Remarks
Petroleum ether	1	-	-	Nothing
Petroleum ether	1	-	yellow → brown	Hydrocarbon
20 % Benzene in Petrol	1	-	yellow → brown	Hydrocarbon
50 % Benzene in Petrol	1	-	yellow → brown	Hydrocarbon
80 % Benzene in Petrol	1	-	yellow → brown	Hydrocarbon
Benzene	2	-	yellow → brown	Hydrocarbon
20 % Chloroform in Benzene	1	-	yellow → brown	Hydrocarbon
50 % Chloroform in Benzene	9	135-136 after crystallization from Chloroform and methanol	green → brown	Possibly steroidal fraction (8.21g.)

TABLE II (Continued)

Eluant	Number of 11. fractions collected	m.p. of compound °C	Libermann-Burchard Test	Remarks
75 % Chloroform in Benzene	2	103-104 after crystallization from benzene	negative	Possibly alcohol (.78g.)
Chloroform	7	103-104	negative	Alcohol

Total hydrocarbon fractions (20.13g.) was unidentified.

Each of the above fractions were subjected to thin layer chromatography on alumina using 25 % chloroform in benzene as the developing solvent and concentrated sulphuric acid as the spray reagent. Fractions having similar R_f value were combined. The compound m.p. 103° - 104° did not show a spot under these conditions.

The compound, m.p. 103° - 104° was recrystallized several times from benzene until its m.p. remained constant.

Found C=76.71%, H=12.61%

Calc. for $C_{20}H_{42}O_2$; C=76.37%, H=13.46%

The I.R. spectrum of this compound is shown in(Fig.I, P 53). Absorption peaks at 3500-3200 and 1050 cm.$^{-1}$ are

due to a hydroxyl group (polymeric association and C-O stretching), 2900,2840 and 1450 cm.$^{-1}$ to methylene stretching and bending vibrations, and at 720 and 710 cm.$^{-1}$ to the rocking mode of CH_2.

The mass-spectrum of this compound can be found in the appendix.

2-3. Preparation of the derivatives of the compound m.p. 103° - 104°

a. Acetylation

Acetic anhydride (10 drops) was added to the compound m.p. 103° - 104° (0.2g.) which was dissolved in a small amount of pyridine. The mixture was shaken, stoppered and set aside at room temperature for 24 hours. Water was added and the acetylated material was filtered off and washed with water until free from pyridine. After being dried in the air, the acetylated compound (0.16g.) when crystallized several times from chloroform and methanol gave amorphous colourless solid m.p. 60° - 61°.

Found C = 72.98%, H= 11.70%

Calc. for $C_{24}H_{46}O_4$; C = 72.56%, H=11.63%

The I.R. spectrum of this compound (Fig.II, P54) shows maximum absorption at 1725 $cm.^{-1}$ (C=O stretching), 1240 $cm.^{-1}$ (C-O-C stretching), and at 730 and 720 $cm.^{-1}$ (CH_2 rocking).

The mass spectrum of this compound can be found in the appendix.

b. Benzoylation

The compound m.p. 103° - 104° (0.2g.) in a small quantity of pyridine was treated with benzoyl chloride (a few drops) and the mixture was shaken, stoppered and set aside at room temperature for 24 hours. Water was added and the benzoylated material was filtered off, washed with water until free from pyridine and dried in the air. The benzoylated compound when crystallized several times from chloroform and methanol gave amorphous solid m.p. 60° - 61° (0.14g.).

Found	C=77.74%, H=9.86%
Calc. for $C_{34}H_{50}O_4$:	C=78.11%, H=9.42%

Strong peaks in the I.R. spectrum of this compound (see Fig. III, P 55) at 1725 $cm.^{-1}$, 1238 and 1125 $cm.^{-1}$; 718 and 685 $cm.^{-1}$; 710 and 693 $cm.^{-1}$ are due to C=O and C-O-C stretching vibrations of a benzoyl group, monosubstituted benzene and CH_2 rocking respectively.

The mass-spectrum of this compound can be found

in the appendix.

2-4. Rechromatography of the steroidal fraction.

The fractions obtained from the soluble and insoluble waxes by column chromatography, which gave a positive Liebermann - Burchard test (green→brown) were combined to give 10.76g. The mixture was adsorbed on alumina (360g.) from chloroform and benzene. Elution of the column was carried out as shown in Table III.

TABLE III

Eluant	Number of 100 ml. fractions collected	Liebermann-Burchard Test	Remarks
25% Chloroform in Benzene	4	-	Nothing
50% Chloroform in Benzene	12	green→brown	Possibly mixture
75% Chloroform in Benzene	4	green→brown	Mixture
Chloroform	5	green→brown	Mixture

The fourth and largest fraction eluted by 50 % chloroform in benzene furnished needles (0.28g.) m.p. 135° - 136° which was unchanged when mixed with β-sitosterol, after crystallized several times from chloroform and methanol.

Found C=83.70%,H=12.11%

Calc. for $C_{29}H_{50}O$;C=83.99%,H=12.15%

The I.R. spectrum of this compound shows strong absorption at 3400-3200 cm.$^{-1}$ (polymeric OH), 1050 cm.$^{-1}$ (equatorial, secondary cyclic alcohol), which are indentical in all respects with that of β-sitosterol (see Fig. IV & V, P 56 & 57).

This compound gave a blue colour which rapidly changed to green and later turned to brown in the Liebermann-Burchard test. A similar result was obtained with β-sitosterol. The melting point of this compound was undepressed when admixed with β-sitosterol.

2-5. Preparation of β-sitosteryl acetate

Acetic anhydride (10 drops) was added to the compound m.p. 135° - 136° (0.2g.) dissolved in a small quantity of pyridine. The mixture was shaken, stoppered and set aside at room temperature for 24 hours. Water was added and the acetylated material was filtered off and washed with water until free from pyridine. After being dried in the air, the acetylated compound (.15g.) was

crystallized several times from chloroform and methanol to give needles, m.p. 126° - 127°, which was undepressed on admixture with an authentic sample of β-sitosteryl acetate.

Found C=81.10%, H=11.39%

Calc. for $C_{31}H_{52}O$; C=81.52%, H=11.48%

I.R. spectrum of this compound (see Fig. VI, P 58) shows maximum absorptions at 1725 $cm.^{-1}$ (C=O stretching), and is identical with that of β-sitosteryl acetate (Fig. VII, P 59).

The remaining fractions were separated into two parts, fractions having Liebermann - Burchard test (green → brown) which shall be refered to as (A) and those showing Liebermann - Burchard test (light green → brown) which shall be refered to as (B).

2-6. Acetylation of (A)

Acetic anhydride (3ml.) was added to a solution of A (6.5 g.) in pyridine (5ml.). The mixture was shaken, stoppered and set aside at room temperature for 24 hours. Addition of water afforded a solid which was filtered off and washed with water until free from pyridine. After being dried in the air, the mixture of acetate (5.9g.) was adsorbed on alumina (180g.) from petroleum ether and benzene. Elution of the column wae carried out as shown in Table IV.

TABLE IV

Eluant	Number of 100ml. fractions collected	Liebermann-Burchard test	Remarks
60% Benzene in Pet. ether	5	-	Nothing
70% Benzene in Pet. ether	9	green → brown	When crystallized several times from chloroform and methanol a small quantity of β-sitosteryl acetate m.p. 126°- 127° was obtained together with mixture which could not be separated by crystallizations.
80% Benzene in Pet. ether	18	green → brown	
90% Benzene in Pet. ether	10	green → brown	
Benzene	12	green → brown	

2-7. Acetylation of (B)

To a solution of B (1.6 g.) in small quantity of pyridine, acetic anhydride (1 ml.) was added, then the mixture was shaken, stoppered and set aside at room temperature for 24 hours. Water was added and the acetylated material was filtered off and washed with water until free

from pyridine. After being dried in the air, the acetylated compound (1.1g.) was crystallized several times from chloroform and methanol. This failed to give a single compound.

PART B. THE SKIN WAX OF THAI BANANA PLANT

II. Extraction of banana skins

Banana skins (1926g.), previously dried in the sun for a few days and then in an oven at 60°C, were extracted with petroleum ether (b.p. 55° - 70°) in a Soxhlet extractor. Evaporation of petroleum ether in a rotatory evaporator afforded a wax (115g.).

1. Constituents of the wax

Methanol (200 ml.) was gradually added to a solution of the wax (115g.) in hot chloroform (150 ml.), and the contents of the flask were allowed to cool to room temperature. The powdery white solid (10g.) m.p. 75° - 78°, which separated was filtered off and the mother liquor was evaporated to dryness. This afforded an oil (100g.).

1-1. Chromatography of the solid

The solid (10g.), m.p. 75° - 78°, was adsorbed an alumina (300g.) from a solution of peteroleum ether and benzene. Elution of the column was carried out as shown in Table V.

TABLE V

Eluant	Number of fractions collected	Liebermann-Burchard test	Remarks
Pet. ether	5	negative	These fractions afforded the ester (6g.)
25% Benzene in Pet. ether	3	negative	
50% Benzene in Pet. ether	5	negative	
75% Benzene in Pet. ether	3	negative	
75% Benzene in Pet. ether	3	yellow → brown	These fractions were not investigated. They consisted of a mixture of steroids which would not crystallized.
Benzene	5	yellow → brown	

Crystallization of tbe first sixteen fractions from methanol and chloroform furnished the ester as glistening plate, m.p. 79° - 80° (6g.).

Found C=81.84%,H=13.72%

Calc. for $C_{52}H_{104}O_2$; C=82.03%,H=13.77%

The I.R. spectrum of this ester (see Fig. VIII, P 60) shows ν max at 1730 $cm.^{-1}$ are due to C=O stretching, at 735 and 720 $cm.^{-1}$ to the methylene rocking mode.

The mass spectrum of this compound can be found in the appendix.

1-2. Hydrolysis of the ester

A solution of 10 % ethanolic KOH (40 ml.) was added to the ester (2g.), and the mixture was heated under reflux on a water bath for 4 hours. Evaporation of the ethanol gave a solid, which was transfered to a thimble contained in a Soxhlet extractor and extracted continuously with ether for 24 hours. Evaporation of the ether furnished an alcohol (1.15g.), which when crystallized several times from benzene afforded an amorphous solid m.p. 79° - 80°.

Found C=81.59%, H=14.19%

Calc. for $C_{26}H_{54}O$; C=81.59%, H=14.22%

Strong peaks in the I.R. spectrum of this compound (see Fig. IX, P61) at 3400-3300 and 1050 $cm.^{-1}$; 2940, 1464 and 1455 $cm.^{-1}$; 725 and 715 $cm.^{-1}$; are due to O-H and C-O stretching, stretching and bending vibrations of CH_2 and C-CH_3 grouping, and $(CH_2)_n$ rocking respectively.

Acidification of the solid which remained in the thimble after the extraction of the alcohol afforded an impure acid (0.30g.). Crystallization of this acid several times from chloroform and methanol furnished an amorphous solid, m.p. 79° - 80°.

Found C=78.69%,H=12.83%

Calc. for $C_{26}H_{52}O_2$; C=78.72%,H=13.21%

The I.R. spectrum of this acid (see Fig. XI, P 63) shows maximum absorption at 3400, 2700 and 1690 $cm.^{-1}$, (OH and CO stretching of a CO_2H); 2900 and 2835 $cm.^{-1}$, 1460 $cm.^{-1}$, (CH_2 stretching and bending vibrations); 1420 $cm.^{-1}$ (CH_2 adjacent to CO_2H); 920 $cm.^{-1}$ (OH out of plane bending of acid dimer); 720 and 710 $cm.^{-1}$ (CH_2 rocking).

1-3. Acetylation of the alcohol

Acetic anhydride (10 drops) was added to the alcohol (0.2g.) in pyridine, the mixture was shaken, stoppered and set aside at room temperature for 24 hours. Addition of water afforded a solid which was filtered off and washed with water until free from pyridine. After being dried in the air, the acetate (0.15g.) was crystallized several times from petroleum ether to give an amorphous solid m.p. 60° - 61°.

Found C=79.51%,H=13.20%

Calc. for $C_{28}H_{56}O_2$; C=79.18%,H=13.28%

The I.R. spectrum of this compound is shown in (Fig. X,P 62). The absorption peaks at 1725 $cm.^{-1}$, 1238 $cm.^{-1}$ are due to C=O and C-O-C asymmetic stretching of acetate, at 732 and 722 $cm.^{-1}$ to methylene rocking.

1-4. Preparation of the amide

Redistilled thionyl chloride (1 ml.) was added to the acid (0.2g.), the mixture was shaken and then heated under reflux on a water bath for 3 hours. The excess of thionyl chloride was removed by distillation, cold ammonia solution was added and the mixture was warmed on a water bath. The amide (0.12g.) produced was filtered off and crystallized several times from dilute alcohol to give an amorphous solid m.p. 108° - 109°.

Found C=79.00%, H=13.63%, N=3.73%

Calc. for $C_{26}H_{53}ON$; C=78.92%, H=13.50%, N=3.54%

The I.R. spectrum of the amide shows strong peaks at 3380 and 3180 $cm.^{-1}$ (free NH stretching vibrations), 2900 and 2840 $cm.^{-1}$ (CH_2 stretching vibrations), 1633 $cm.^{-1}$ (C=O stretching associated primary amide), 1460 and 1410 $cm.^{-1}$ (CH_2 bending adjacent to amide), and 710 $cm.^{-1}$ (CH_2 rocking), (see Fig. XII, P 64).

2. Constituents of the oil

2-1. Chromatography of the oil

The oil (100g.) was adsorbed on silica gel (1½ kg.) from a solution of petroleum ether (500 ml.). Elution of the column was carried out as shown in Table VI.

TABLE VI

Eluant	Number of 500ml. fractions collected	Liebermann-Burchard test	Remarks
40% Benzene in Pet. ether	6	-	nothing
60% Benzene in Pet. ether	6	yellowish green fluorescence → yellow → brown	These fractions should be refered to below as x (29g.)
80% Benzene in Pet. ether	4	yellow → brown	Not investigated further (20.5 g.)
Benzene	6	yellow → brown	Not investigated further (4.5g.)
20% Chloroform in Benzene	6	yellow → brown	Not investigated (13.6g.)
40% Chloroform in Benzene	6	slightly green → yellow → brown	Not investigated (2.5g.)
60% Chloroform in Benzene	6	yellow → brown	Not investigated (1.8g.)
80% Chloroform in Benzene	6	yellow → brown	Not investigated (1.4g.)
Chloroform	8	yellow → brown	Not investigated (10.5g.)
Methanol	8	yellow → brown	Not investigated (11.2g.)

2-2. Saponification of the mixture X

Mixture X (29g.) was saponified using potassium hydroxide (30g.) in methanol (300ml.). The reaction mixture was heated under reflux on a water bath for 5 hours, and the methanol removed by evaporating to a small volume in a rotatory evaporator followed by heating in an evaporating basin on a water bath for the removal of all the remaining solvent. A solid remained after removal of all the solvent, which was ground to a fine powder with a spatula after adding some anhydrous sodium sulphate, and transfered to a thimble contained in a Soxhlet extractor. The solid was continuously extracted with ether until no more colour was removed. Evaporation of the ether furnished the unsaponifiable matter (13g.) as a wax.

2-3. Acetylation of the unsaponifiable matter

Acetic anhydride (10ml.) was added to the wax (13g.) in pyridine (30ml.), and mixture was heated under reflux on a water bath for 6 hours, water added and the mixture cooled. The mixture was transfered to a separatory funnel (1l.), sodium hydroxide solution was added to decompose the excess of acetic anhydride and to neutralize the acetic acid produced, and the resulting mixture extracted with ether. The ethereal solution was washed several times with hydrochloric acid to remove

the pyridine which remained, then with brine and dried over anhydrous sodium sulphate. Evaporation of the ether furnished a wax (10.8g.).

2-4. Chromatography of the steroidal acetate

The mixture of acetate (10.8g.) were adsorbed on alumina (350g.) from a solution of 30 % benzene in petrol (100ml.). Elution of the column was carried out as shown in Table VII.

TABLE VII

Eluent	Number of 500ml. fractions collected	Liebermann-Burchard test	Remarks
30% Benzene in Petrol	2	yellowish green fluorescence → yellow → brown	Shall be refered to as Y (5.2g.)
50% Benzene in Petrol	2	yellow → brown	Not investigated any further
75% Benzene in Petrol	4	yellow → brown	
Benzene	6	yellow → brown	

2-5. Rechromatography of Y

Mixture Y (5.2g.) was adsorbed on alumina (200g.) from a solution of benzene and petroleum ether. Elution of the column was carried out as shown in Table VIII.

TABLE VIII

Eluant	Number of 50ml. fractions collected	Liebermann-Burchard test	Remarks
20% Benzene in Petrol...	9	yellowish green fluorescence → yellow → brown	Crystallization from chloroform and methanol afforded 24-methylenecycloartanyl acetate.
20% Benzene in Petrol...	4	yellow → brown	Wax which would not crystallize
25% Benzene in Petrol...	4	yellow → brown	
30% Benzene in Petrol...	8	yellow → brown	

The fourth, fifth and sixth fractions eluted by 20% benzene in petroleum ether furnished 24-methylene-cycloartanyl acetate as needles, (.25g.) m.p. 115° - 116° when crystallized several times from chloroform and methanol.

Found C=82.00%, H=11.22%

Calc. for $C_{33}H_{54}O_2$: C=82.03%, H=11.22%

The I.R. spectrum of this compound showed maximum absorption at 1725 cm.$^{-1}$ (C=O stretching), 1640 and 885 cm.$^{-1}$ (C=C stretching and CH out of plane bending of a vinylidene group), 1238 cm.$^{-1}$ (C-O-C asymmetric stretching of acetate), and is identical with that of 24-methylenecycloartanyl acetate (see Fig. XIII and XIV p 65 and 66).

2-6. Hydrolysis of 24-methylenecycloartanyl acetate

A solution of 10 % methanolic KOH (5 ml.) was added to the acetate (.2g.) The mixture was heated under reflux on a water bath for 4 hours. Evaporation of the methanol gave a solid which was extracted several times with ether. The ethereal solution was washed several times with brine. Evaporation of the ether gave a solid, which when crystallised several time from chloroform and methanol furnished 24-methylenecycloartanol as needles (.10g.), m.p. 122° - 125°.

Found C=84.79%, H=12.07%

Calc. for $C_{31}H_{52}O$: C=84.48%, H=11.89%

Strong absorption in the infra-red spectrum of this compound (Fig. XV, p 67) is shown at 3640 cm.$^{-1}$ (OH stretching), 1640 and 890 cm.$^{-1}$ (C=C stretching and out of plane bending of vinylidene group), 1030 cm.$^{-1}$ (secondary alcohol).

Preparative layer chromatography of 24-methylenecycloartanyl acetate

Thick layers of adsorbent were prepared in the following manner; the thickness of eech coating was adjusted to 2 mm. A mixture of Merck standardized aluminium oxide (160g.), aluminium oxide G (40g.) and water (160 ml.) was shaken in a stoppered bottle until it was thoroughly mixed. The slurry was poured into the trough of a Cameg spreader, and glass plates (20 X 20 cm.) were passed slowly under the trough until they were coated with adsorbent. After being dried for one hour at room temperature, the plates were activated for 3 hours at 110° - 120°, cooled and stored in a desiccator until required for usc.

Two lines were described on each plate, one 2 cm. from one edge and parallel to it, which we shall refer to as the bese line, and the other (the upper line) 15 cm. above this line and parallel to it. The mixture of acetate obtained from the aluminium oxide column (about 250 mg.) dissolved in chloroform (2 ml.) were applied as a streak on the base line of the plate and, after the solvent had evaporated, the plated was placed in a glass tank filled to a depth of 1 cm. with benzene / light petroleum (1:5), and the tank covered with a glass cover. When the solvent front had reached the upper line, about 45 minutes was required for this operation, the plate was removed

from the tank, the solvent allowed to evaporate, and the plate sprayed with morin. Several bands were observed when the plate was viewed by U.V. light at wavelength 254 or 350 mμ. However, complete separation of the band was not achieved after only one run, and so the plate was subjected to two further runs. Five bands, including one on the base line, were observed when the plate was viewed under U.V. light of wavelength 254 or 350 mμ. These bands were removed separately from the plate, ground to a fine powder, poured into a chromatographic column and eluted quickly with ether / methanol (1:1). Evaporation of each fraction under reduced pressure yielded a solid, which was dissolved in a small quantity of chloroform, and one drop of thie solution applied as a spot to a thin layer of aluminium oxide. Elution of the layer with benzene / light petroleum (1:5) followed by detection of the spots with morin showed that only the upper band was nearly pure. Crystallization of the solid from this band from chloroform and methanol furnished a very small quantity of 24-methylenecycloartanyl acetate m.p. 115° - 116°. Rechromatography and recrystallization of the solids from the other bands did not afford pure material.

Thin layer chromatography of β-sitosterol

β-sitosterol obtained from banana leaf wax was dissolved in a small quantity of chloroform and applied as a spot to a thin layer of Merck silica gel G. The plate was developed

four times with chloroform/light petroleum (1:1), the solvent being allowed to evaporate from the layer between each elution. Five spots were observed on the plate after it had been sprayed with 2,7-dichlorofluorescene, the most intense spot being due to β-sitosterol, which was the second spot from the top, as shown by comparison with commercial β-sitosterol.

Preparative layer chromatography of β-sitosterol

A mixture of Merck silica gel lessthan 0.08 mm. (200g.) and water (350ml.) was shaken in a stoppered bottle until it was thoroughly mixed, and the resulting slurry was poured into the trough of a Desaga spreader. The gate of the spreader was adjusted to 2 mm., and a glass plate (20 X 20 X 0.3 cm.) was coated with the slurry by passing the spreader over it. The resulting layer was allowed to dry overnight and then activated by heating it in an oven for one hour at 110°. After being cooled, a solution of the β-sitosterol (20mg.) in chloroform (1ml.) was applied as a streak on the base line of the plate. Development of the layer 5 times with petroleum/chloroform (1:1) resulted in the seperation of the mixture into several bands, as shown by spraying the layer with 2,7-dichlorofluorescene and viewing it under U.V. light. The second band, which contained β-sitosterol, was removed from the plate, ground to a fine powder, poured into a chromatographic column and eluted quickly with ether. Evaporation of this fraction afforded β-sitosterol m.p. 135° - 136° $[\alpha]_D^{20}$ - 17.6° (c=1.78; $CHCl_3$) when crystallized several times from chloroform and methanol.