A Study of the Neutral Fraction of Ox-bile.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Thesis presented to

The University of Glasgow

in fulfilment of the requirements for

the degree of

Doctor of Philosophy

by

R. R. Wilson.

December, 1951.

The author wishes to record his sincere appreciation of the guidance given during the course of this investigation by Professor F. S. Spring.

He would also thank Dr. J. McLean and Dr. G. T. Newbold for their continued interest and advice.

Thanks are also due to the Department of Scientific and Industrial Research for financial provision and to Messrs. T. and H. Smith (Edinburgh) for assistance in carrying out the large scale extraction of ox-bile.

CONTENTS.

HISTORICAL.

INTRODUCTION

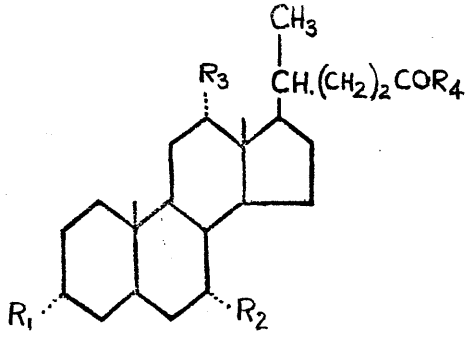
In this section, a review is given of the various known constituents of ox-bile together with an account of the methods used for isolation of both neutral and acidic components.

As the chemistry of the bile acids and of cholesterol has been extensively reviewed in the literature (1 to 4) it is not made a subject for further discussion.

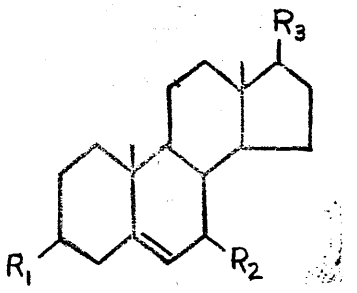
CONSTITUENTS OF OX-BILE.

Ox-bile is a golden brown liquid having an alkaline reaction (pH 7 to 9) and containing inorganic salts, bile salts (sodium salts of conjugated bile acids) and cholesterol, lecithin and the bile pigments.

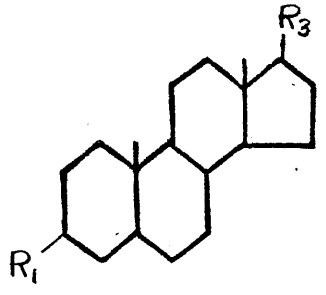
A detailed summary of the known constituents of ox-bile is set out in the accompanying table (Table I). The heterocyclic components of ox-bile (pigments) are not included in the table (see note (e)). Fatty acids (stearic, palmitic, oleic) are also omitted (see note (f)).



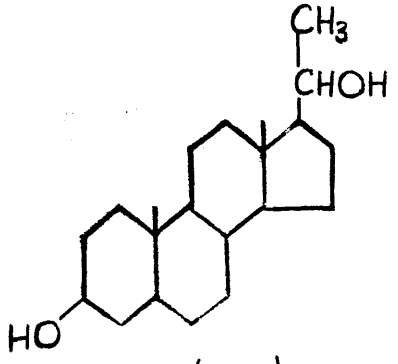
(I)



(II)



(III)



(IV)

TABLE I

COMPOSITION OF OX-BILE.

COMPOUND	MOLECULAR FORMULA	STRUCTURAL FORMULA	m.p.	$([\alpha]_D)$	REF.	ACETATE			REF.
						MOLECULAR FORMULA	m.p.	$([\alpha]_D)$	
CHOLIC ACID	$C_{24}H_{40}O_5$	I., $R_1=R_2=R_3=R_4=OH$	198°	+ 37° (alcohol)	5,6	$C_{31}H_{48}O_8$ (methyl ester)	94°	+78° (methanol)	7
GLYCOCHOLIC ACID	$C_{26}H_{43}O_6N$	I., $R_1=R_2=R_3=OH$ $R_4=-NH.CH_2.COOH$	130° (preheated bath)	+32.5° (alcohol)	8,9.	--	--	--	--
TAUROCHOLIC ACID	$C_{26}H_{45}O_7NS$	I., $R_1=R_2=R_3=OH$ $R_4=-NH.(CH_2)_2SO_3H$.	ca.125°d.	+ 38.8° (dil.alcohol)	10.	--	--	--	--
DESOXYCHOLIC ACID	$C_{24}H_{40}O_4$	I., $R_1=R_2=R_4=OH$ $R_2=H$	172-3°	+ 57° (alcohol)	6,11 12,13.	$C_{29}H_{46}O_6$ (methyl ester)	117°	--	7
GLYCODESOXYCHOLIC ACID	$C_{26}H_{43}O_5N$	I., $R_1=R_3=OH$ $R_2=H$ $R_4=-NH.CH_2.COOH$.	186-7°	+48.0° (alcohol)	14	--	--	--	--
TAURODESOXYCHOLIC ACID	$C_{26}H_{45}O_6NS$	I., $R_1=R_3=OH$ $R_2=H$ $R_4=-NH.(CH_2)_2SO_3H$.	ca.200°	+33° (water)	14	--	--	--	--
CHENODESOXYCHOLIC ACID (a)	$C_{24}H_{40}O_4$	I., $R_1=R_2=R_4=OH$ $R_3=H$	140°	+11.1° (alcohol)	15,16, 17,18.	--	--	--	--
LITHOCHOLIC ACID (a)	$C_{24}H_{40}O_3$	I., $R_1=R_4=OH$ $R_2=R_3=H$.	184-6°	+32° (alcohol)	19,20.	$C_{20}H_{42}O_4$	169°	--	--

Continued ...

TABLE I (Continued)

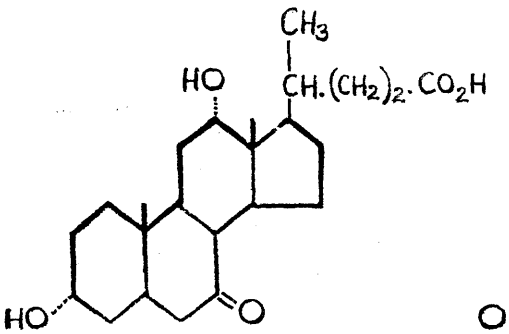
3 -HYDROXY-12-KETOCHOLANIC ACID (d)	$C_{24}H_{38}O_4$	I., $R_1 = R_4 = OH$, $R_2 = H$, $R_3 =$ "=O"	164-5°	+ 110.2° (alcohol)	17.	$C_{26}H_{40}O_5$	197°	--	--
STEROCHOLIC ACID	$C_{28}H_{46}O_4$	unknown	256°	--	17.	--	--	--	--
CHOLESTEROL	$C_{27}H_{46}O$	II., $R_1 = OH$, $R_2 = H$, R_3=-CH(Me).$(CH_2)_3$.CH(Me)$_2$	148.5°	- 39.5° (chloroform)	21,22, 23,24.	$C_{29}H_{48}O_2$	115-6°	- 47.4° (chloroform)	--
7-HYDROXYCHOLESTEROL (b)	$C_{27}H_{46}O_2$	II., $R_1 = R_2 = OH$, R_3=-CH(Me)$(CH_2)_3$.CH(Me)$_2$	--	--	25.	--	--	--	--
CHOLESTANOL	$C_{27}H_{48}O$	III., $R_1 = OH$, R_3=-CH(Me)$(CH_2)_3$.CH(Me)$_2$	142°	+ 24° (chloroform)	26,27, 28	$C_{29}H_{50}O_2$	110°	+ 13.3°	26,27,28.
COMPOUND A	$C_{27}H_{40}O_3$	unknown	300°	--	29.	$C_{29}H_{42}O_4$	216-7°	--	29
ALLOPREGNANEDIOL (COMPOUND B)	$C_{21}H_{36}O_2$	IV	192-3°	--	29,30.	$C_{25}H_{40}O_4$	142-3°	--	29
COMPOUND C	$C_{25/6}H_{40/2}O_4$	unknown	255-7°	--	29.	$C_{27/8}H_{42/4}O_5$	187°	--	29,30.
COMPOUND D	$C_{24}H_{40}O_3$	unknown	232-3°	--	29.	$C_{26}H_{42}O_5$	111°	--	29
COMPOUND E	$C_{24}H_{42}O_4$	unknown	202°	--	29.	$C_{28}H_{46}O_6$	142.5°	--	29

NOTES (a). The acids occur in bile as the sodium salts of the corresponding glyco- and tauro-acids. The conjugated acids are difficult to isolate (1).

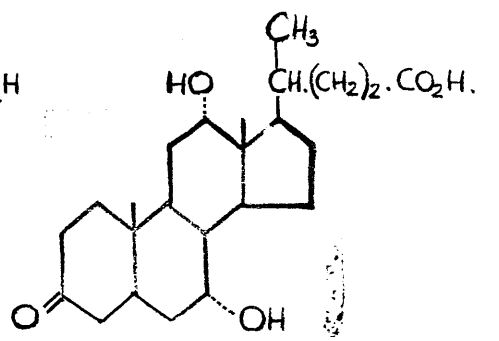
(b) It has been shown by Blix (31) that cholesterol, in colloidal aqueous solution is very readily attacked by molecular oxygen at room temperature. A detailed study of the reaction has been made by Bergström and Wintersteiner (32, 33, 34) who prepared the colloid in a phosphate buffer solution containing sodium stearate as a stabiliser. A trace of heavy metal catalyst seems to be essential. They were able to identify 7 - ketocholesterol, 7α - hydroxycholesterol and 7β - hydroxy-cholesterol in the reaction products. This throws considerable doubts on the significance of the isolation of 7 - hydroxy-cholesterol from bile (25).

(c). The molecular formulae advanced for Compounds A, C, D and E are tentative (29).

(d). Haslewood (25) has isolated, in minute amounts from cow bile, two keto acids as their ethyl esters, shown to be



(V)



(VI)

3 : 12 - dihydroxy - 7 - ketocholanic acid (V) and 7 : 12 - dihydroxy - 3 - ketocholanic acid (VI). The form in which they occur in bile is unknown.

(e). Bilirubin was isolated first from the gallstones of cattle (35) and subsequently from those of man (36). The formula has been established as $C_{33}H_{36}O_6N_4$ (37). Bilirubin forms a sodium salt (38). The chemistry of the bile pigments has been reviewed (37).

(f). The fatty acids form barium salts which are insoluble in ether (39 to 44). For the method of assay of fatty acids in bile, see reference (45).

Composition of Ox-bile

Table II has an approximate analysis of ox-bile

Table II - Analysis of Ox-bile

Compound	Concentration (g/litre)	Reference
Cholic acid	50	20
Desoxycholic acid	6	20
Lithocholic acid	0.02	20
Chenodesoxycholic acid	0.02	20
3 -Hydroxy-12-ketocholanic acid	less than 0.01	17
Sodium Stearate) Sodium Palmitate) Sodium Oleate)	1.65	45
Bilirubin	0.10	45
Biliverdin	2.5	46
Taurine	0.05	47
Glycine	0.14	47
Lecithin	0.10	47
Cholesterol	0.75	25
Cholestanol	0.08	25
7-Hydroxycholesterol	0.02	25
Pearlman's Compounds A,B,C,D and E	less than 0.005	29

It will be observed that the total amount of neutral material falls short of 1g/litre, approximately 90% of which is made up of cholesterol, cholestanol and 7-Hydroxy-cholesterol. This leaves a residue (10%) of neutral material, the composition of which, with the exception of Pearlman's investigation (29, 30) remains doubtful.

Methods of Separation of Acidic and Neutral Components of Bile

The classical method of isolation of the neutral and acidic components consisted in saponification of the bile with methanolic sodium hydroxide, removal of neutral material by extraction with ether followed by acidification of the aqueous phase and filtration or ether extraction of the acidic fraction (20, 25, 5, 6, 7, 8, 15, 16, 17, 18, 29, 30).

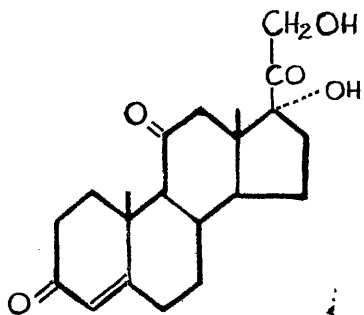
Nakagawa and Fujikawa (48) developed a method for extraction of the bile acids. Their procedure consisted in extraction of the bile acids with alcohol after the medium had been rendered strongly alkaline with ammonia (pH above 10), with subsequent absorption of the bile pigments on animal charcoal. The method had the disadvantage of high alkaline conditions with a low degree of separation.

During the course of his investigations on the "Oestrus Hormone Activity" of the bile of various animals (including man), Gsell - Büsse (49) was able to isolate active preparations from bile by extraction with ether after the medium had been rendered neutral. The ethereal extract was concentrated and the concentrate assayed for hormone activity in the usual manner.

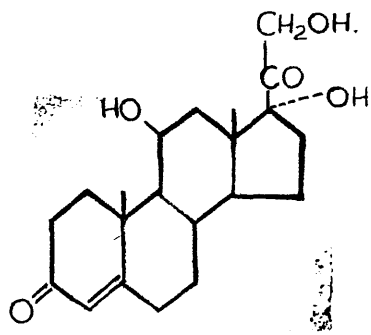
THEORETICAL.

INTRODUCTION.

The recent discovery by Kendall and Hensch (60, 61) of the use of 17∝-Hydroxy-11-dehydrocorticosterone (cortisone, VII) and 17∝-Hydroxycorticosterone (VIII) as a temporary relief for rheumatoid arthritis has



(VII)



(VIII)

stimulated a search for compounds of similar structural and physiological characteristics and of more ready availability than cortisone (VII) itself.

Clinical evidence has drawn attention to the fact that considerable alleviation of symptoms occur in patients suffering from rheumatoid arthritis, should an attack of jaundice ensue. The same alleviation is observed in the case of arthritic women during pregnancy.

As jaundice is a condition characterised by the presence of bile in the blood it seemed reasonable to assume that bile itself contains a compound or number of

compounds capable of exerting a direct or indirect (after possible chemical modification) action *in vivo* on the arthritic condition.

Biological experiments employing heavy isotopes have confirmed this assumption in so far as they have demonstrated that the bile acids are synthesised *in vivo* from cholesterol (50). The ability of the animal body to synthesise cholesterol was clearly shown by Channon (51), by Randles and Knudson (52), by Eckstein and Treadwell (53) and by Schoenheimer and Breusch (54). Schoenheimer (53) proved that the synthesis of cholesterol involved the condensation of a large number of small molecules and deduced that about half of the hydrogen atoms in the cholesterol molecule were obtained from water molecules. Later, Bloch and Rittenberg (56, 57, 58) demonstrated that acetic acid was a precursor of cholesterol in animals and established the liver as the site of synthesis (59).

From consideration of the evidence presented above and in the previous section it was considered desirable to undertake a chemical examination of the neutral fraction of ox-bile. The following sections describe the isolation of a neutral fraction from ox-bile and its resolution, by various chemical techniques, into eleven separate compounds.

The Theoretical part of this thesis has been divided into two sections. Section A deals with the isolation and extraction of a neutral fraction from ox-bile and its resolution into eleven separate compounds, whilst Section B is devoted to a discussion on the chemistry of one of these compounds, Compound A.

Section A.

Isolation and Examination of the Neutral Fraction of Ox-Bile

The method used initially was based on the fact that the alkali salts of the bile pigments, bile acids and fatty acids are insoluble in dry ether, while the conjugated bile acids (present in bile as the sodium salts) are insoluble in absolute ethanol.

Summer-beef ox-bile was completely dehydrated by freeze drying and subsequent desiccation of the material over phosphorus pentoxide and calcium chloride. The time required for drying 1.77 kg., bile to constant weight (170g. ; 9.6%) by this method was five weeks. Trituration of the dry solid with ethanol gave the conjugated bile acids as their sodium salts, together with a solution of the non-conjugated bile acids in ethanol. Freudweiler has shown that bile contains approximately 54% by weight of bile acids expressed as cholanic acid (62).

Accepting this value, the calculated amount of potassium hydroxide required for neutralisation of the remaining acidic material in the ethanolic solution was added. The potassium salts were precipitated by addition of ether but they proved to be hygroscopic, a property ascribed to the presence of potassium hydroxide. The ether/alcoholic filtrate was freed from alkali by treatment with carbon dioxide and filtration of the precipitated potassium carbonate. The neutral fraction so obtained amounted to 3% by weight of the original dried bile. Acetylation of this neutral material at 0^{o} gave a gum which was chromatographed on alumina. Twelve solid fractions were obtained, six of which, after purification by crystallisation, were shown to be either cholesterol or cholesteryl acetate; the formation of the former showed that the acetylation of the neutral fraction had not been complete.

In the second trial (A(ii), see Experimental) the dried bile was subjected to treatment similar to that out outlined above with the exception that barium oxide was used instead of potassium hydroxide for neutralisation. The precipitates obtained were not hygroscopic and the process was more easily manipulated. Excess barium ion was readily removed with carbon dioxide.

The neutral fraction obtained (0.76%) was acetylated and divided into ketonic and non-ketonic fractions using Girard's reagent (63). The non-ketonic fraction was cholesterol. The ketonic fraction (70mg.; 10% based on weight of neutral fraction) was obtained as a gum and could not be crystallised from methanol.

The low yield of neutral fraction obtained on a small scale, together with the high vacuum required for freeze-drying (less than 1 mm.) rendered this method unsuitable for application on a large scale. The modifications entailed are discussed below.

As the barium salts of the bile acids, fatty acids (39, 44) and bile pigments (38) are insoluble in water (barium cholate is an exception) it was felt that addition of excess barium ion (as barium hydroxide octahydrate) to ox-bile would result in the precipitation of practically all the acidic constituents in a form which could readily be removed by filtration. Subsequent extraction of the filtrate with ether would remove the neutral material.

Experiment A (iii) describes in detail the method for working such on a large scale. Fresh ox-bile (not dried) was treated with baryta and the barium salts removed by filtration. The filtrate was continuously extracted with ether to yield a neutral fraction as a

greasy yellow solid (which represented a yield of 11% based on weight (20kg.) bile used). The neutral fraction was partially soluble in ethyl acetate. The soluble part readily crystallised and after acetylation and chromatography gave cholesterol. Extraction of the ethyl acetate soluble fraction with light petroleum gave an insoluble solid and a solution. From the latter, cholesterol was readily obtained. The petrol insoluble solid (solid A) was a dry yellow/grey powder and showed an absorption in the ultraviolet. The solid A was chromatographed on alumina and each of the fractions obtained was examined for selective absorption in the ultraviolet. Sixty-seven fractions were obtained, seventeen of which showed selective absorption. As the fractions were obtained as gums, no estimate of intensity could be made.

An analysis of the spectroscopic data showed that the results could be grouped as follows:-

1. Fractions showing absorption between 210mu and 230mu with an absorption band of lower intensity at 250mu.
2. Fractions showing an absorption band between 220mu and 230mu with a band of lower intensity between 270mu and 290mu.
3. Fractions showing an absorption at 258mu.
4. Fractions showing an absorption maxima at or between 280mu and 300mu.

The fractions were combined to form seventeen different groups and attempts were made to crystallise the materials. No success was achieved, due probably to the very small quantity of material available, even after grouping.

In Experiment A(iv) the neutral fraction (8g.) obtained from eight litres bile by the method previously outlined A(iii) was divided into a petroleum insoluble fraction (1.88g.) and a petroleum soluble fraction (6.17g.). The petroleum insoluble fraction was acetylated and chromatographed on alumina. Sixty fractions were obtained, forty-one of which showed selective absorption in the ultraviolet between 210 mu and 400 mu. Analysis of the spectroscopic data revealed five groups:-

1. Fractions showing an absorption band between 255mu and 259mu.
2. Fractions showing an absorption band at 360mu.
3. Fractions showing a maximum between 210mu and 217mu with a band of lower intensity between 245mu and 258mu.
4. Fractions showing a maximum between 210mu and 230mu with two maxima in the regions 248-250mu and 280-300mu.
5. One fraction (fraction 23) showed four maxima - at 215mu, 250mu, 258mu and 300mu.

The fractions were not further investigated as amount of material in individual fractions was small and bulking on basis of spectroscopic data, uncertain.

The petrol soluble fraction was examined by fractional crystallisation and each fraction was tested for light absorption in the ultraviolet (220mu to 400mu). None of the fractions showed selective absorption in this region. A small quantity of cholic acid was obtained, and this was the sole occasion on which a bile acid was found. The remaining fractions were shown to be cholesterol.

To summarise, a method was obtained which allowed of cold extraction of the neutral fraction of ox-bile with the minimum of processing and under mild conditions. The neutral fraction so obtained could easily be divided into a ketonic and a non-ketonic fraction with light petroleum. Spectroscopic analysis indicated that the unsaturated compounds were present exclusively in the petroleum insoluble (or ketonic) fraction. Before a large scale extraction could be undertaken, the process was modified with the introduction of chloroform in place of ether as the solvent for extraction of the neutral fraction. Experiment (A,v) was designed for this purpose. Bile was treated with baryta in the usual manner, filtered and the filtrate extracted with chloroform.

The material extracted by chloroform was extracted with ether and the solid so obtained divided into a petroleum insoluble fraction and a petroleum soluble fraction. A brief assay of the ketonic (petroleum insoluble) fraction using Girard's reagent showed a concentration of ketonic material (ca 10%) similar to that obtained previously (Exp., A,ii). The petrol soluble fraction was rejected.

The method of extraction was now established and consisted of the following stages:-

(a) Treatment of fresh ox-bile with baryta with stirring over a period of several days.

(b) Removal of the precipitated barium salts by filtration and washing the residue with water.

(c) Extraction of the combined washings and filtrate with chloroform (Five to eight extractions per batch).

(d) Concentration of the chloroform extracts under reduced pressure, the temperature being kept below 50^{o}.

The remaining operations were carried out in the laboratory and involved the following steps:-

(e) Extraction of the dried chloroform extracts with ether and subsequent concentration to yield the neutral fraction.

(f) Resolution of the neutral fraction with light petreoleum, the petroleum soluble fraction was rejected.

(g) Chromatographic analysis of the petroleum insoluble fraction and examination of the fractions obtained by usual methods.

This procedure was carried out on a total of 240 gallons of bile from which the neutral fraction obtained (90lg.) afforded a petroleum insoluble part (76g.) and a petroleum soluble part (58lg.) The latter fraction consisted mainly of cholesterol. The petroleum insoluble fraction was chromatographed on alumina. Two hundred and ten fractions were obtained and these fractions were further grouped into twelve large fractions (I to XII) based on inspection and subfractional mixed melting points. Examination of the fractions so obtained resulted in the isolation of eleven separate compounds. The methods of resolution employed are tabulated below (Table IV)

TABLE IV

Fraction	Yield (g.)	m.p.	PROCEDURE				COMPOUND	Yield(g.)
I	35.13	135 - 157°	Crystallisation and chromatography				Cholesterol	29.470
							Amorph.prod.	0.580
II	1.24	158 - 174°	Acetone	Acetone sol.	Chromatographed		Compound F	0.226
							Compound A	0.137
							Compound I	0.027
				Acetone insol.	x Petrol.	Petrol.insol.	Compound I	0.010
						Petrol. sol.	Compound A	0.072
III	2.89	185 - 208°	Petrol.	Petrol. sol.	cryst. from petrol.		Compound A	1.210
				Petrol.insol.	cryst. from toluene		Compound B	0.085
IV	1.25	185 - 215°	Petrol.	Petrol. sol.	cryst. from petrol.		Compound A	0.820
				Petrol. insol.	See under V		----------	0.200
V	1.19	178 - 185°	Petrol.	Petrol. sol.	cryst. from petrol.		Compound A	0.920
				Petrol.insol.	Solvent partitioning crystallisation		Compound K	0.089
							Compound I	0.036
VI	1.74	154 - 161°	Cold EtOAc	Insoluble	cryst. from EtOH		Compound I	0.015
				Soluble	Concentrate	Insoluble	Compound A	0.120
						Soluble	Compound A	0.011
							Amorphous	0.550
VII	12.63	150 - 160°	chromatography(alumina)				amorphous	12.63
VIII	1.73	155 - 176°	chromatography(alumina)				amorphous	1.73
IX	2.02	171 - 185°	Acetone	Soluble			Compound J	0.011
				Insoluble			amorphous	1.500
X	2.69	180 - 202°	Chromatography (alumina)				Compound C	.027
							Compound D	.015
							Compound E	.017
							amorphous	1.081
XI	1.67	150 - 8°	Chromatography (alumina)				Compound C	0.010
							Compound G	0.030
							Compound H	0.027
							amorphous	0.876
XII	2.68	167 - 205°	Chromatography (alumina)				amorphous	2.68

x Petroleum b.p. 60/80° was used throughout.

Table V summarises the constants obtained for each compound. No acetates were obtained for Compounds C, D, E, G, H and J and the molecular formula assigned to these compounds is tentative. Derivatives were obtained for Compounds A, B, F, I and K. For these Compounds the molecular formulae were checked against analyses of acetates and (in the case of Compounds A and F) on % Acetyl.

TABLE V

Compound	m.p.	$[\alpha]_D$ (in chl.)	Molecular Formula	Analysis		Crystalline Form (Solvent)	Source
				Found	Calcd.		
A	186-7°	-22.6°	$C_{22}H_{36}O_3$	C=75.5% H=10.5. C=75.3% H=10.5%	C=75.8% H=10.4%	NEEDLES (petrol. 60/80)	II, III, IV, V VI
(1) A DIACETATE	173-4°	-43.1°	$C_{26}H_{40}O_5$	C=72.7% H= 9.5% C=72.5% H= 9.3%	C=72.2% H= 9.3%	NEEDLES (Methanol)	
B	260-1°	-58.3°	$C_{19}H_{32}O_3$	C=74.4% H=10.0% C=74.04% H=10.3%	C=74.03% H=10.3%	NEEDLES (Toluene)	III
B ACETATE	223-5°	-50.7°	$C_{21}H_{34}O_4$	C=71.9% H= 9.8%	C=71.9% H=10.0%	NEEDLES (Methanol)	
C	191-4°	+24.3°	$C_{24}H_{42}O_4$	C=72.9% H=10.6% C=72.8% H=10.7%	C=73.1% H=10.2%	PRISMS (Acetone)	X, XI
D	202-4°	+23.5°	$C_{22}H_{37}O_4$	C=72.5% H=10.0% C=72.5% H=10.5%	C=72.3% H=10.2%	CUBES (Acetone)	X

(1) : % Acetyl, Found: 21.8%; Calculated: 21%

Cont'd.....

TABLE V (Cont'd.)

Compound	m.p.	$[\alpha]_D$ (in chl.)	Molecular Formula	Analysis		Crystalline Form (Solvent)	Source
				Found	Calcd.		
E	214-216°	* +32.9°	$C_{21}H_{38}O_5$	C=68.4% H=10.0% C=67.8% H=10.6%	C=68.1% H=10.3%	PLATES (Acetone)	X
F	191-193°	-32.3°	$C_{20}H_{34}O_3$	C=74.4% H=10.6%	C=74.5% H=10.6%	PLATES (Acetone)	II
(2) F DIACETATE	171-2°	-16°	$C_{24}H_{38}O_5$	C=71.2% H= 8.9%	C=71.0% H= 9.3%	NEEDLES (Methanol)	
G	148-150°	+28.7°	$C_{22}H_{40}O_5$	C=69.1% H=10.2% C=69.4% H=10.4%	C=68.8% H=10.4%	PRISMS (Petrol./ acetone)	XI
H	181-3°	+32.8°	$C_{22}H_{44}O_5$	C=69.5% H=10.6% C=69.5% H=10.6%	C=69.9% H=10.4%	PRISMS (Petrol./ acetone)	XI

(2): % Acetyl, Found: 21% ; Calculated 21.3% * in ethanol

Cont'd.....

TABLE V (Cont'd.)

Compound	m.p.	$[\alpha]_D$ (in Chl.)	Molecular Formula	Analysis		Crystalline Form (Solvent)	Source
				Found	Calcd.		
I	246-8°	-38.8°	$C_{23}H_{40}O_4$	C=72.9% H= 9.9%	C=72.7% H=10.5%	NEEDLES (Ethanol)	II, V, VI
I TRIACETATE	218-220°	-43.9°	$C_{29}H_{46}O_7$	C=68.8% H= 9.5%	C=68.9% H= 9.1%	NEEDLES (Ethanol)	
J	201-3°	+39.9°	$C_{21}H_{37}O_4$	C=71.9% H=10.4% C=71.7% H=10.5%	C=71.4% H=10.5%	GRAINS (petrol./ benzene)	IX
K	250-2°	-71.6° ** -47.8°	$C_{21}H_{36}O_3$	C=75.4% H=10.7%	C=75.5% H=10.6%	NEEDLES (t-amyl alcohol)	V
K ACETATE	220-4°	-50°	$C_{25}H_{40}O_5$	C=71.8% H= 9.7%	C=71.6% H= 9.5%	NEEDLES (Methanol)	

** in pyridine

It is not proposed to discuss in detail the isolation of each compound in this section. The methods used are well known - fractional crystallisation, Soxhlet extraction and chromatography. Unfortunately these techniques provide no information as to the nature of the functional groups in the molecule and in the case of Compounds C, D, E, G, H and J no definite information is available. The crude material obtained during purification of Compounds C, D and E was acetylated. The acetylated material was amorphous and could not be purified by either crystallisation or chromatography. Scarcity of pure material, in case of Compounds G, H and J prevented an attempt to prepare an acetate, the crude material being rejected as a possible source of a derivative in view of the results obtained with Compounds C, D and E.

Compound G was the only compound isolated which gave a yellow colouration with tetranitromethane. None of the compounds showed any selective absorption in the ultraviolet (220 to 400mu) a rather surprising fact in view of the results previously obtained in the laboratory experiments prior to the large scale extraction.

The ready solubility of Compound A in petrol was of great value in the purification of the individual fractions

as this compound is the only petroleum soluble compound obtained (of Experimental, Fractions II, III, IV, V). Another factor of some importance lay in the solubility of accompanying amorphous material in solvents such as acetone (Compound C, D, E, H), alcohol (Compound G), benzene (Compound J) thus enabling pure material to be relatively easily removed by repeated precipitation with petroleum. It was found that after six to eight precipitations from petrol./acetone or petrol./alcohol, a substance of constant melting point could be obtained. These purified fractions crystallised very slowly from acetone or better acetone/petrol. (C, D, E, G, H).

The amorphous fractions (VII, VIII, XII) did not respond to crystallisation or to chromatography of the free alcohols or of the acetates. The acetylated material was amorphous. None of these fractions showed absorption in the ultraviolet (200 to 400mu).

Assuming the compounds to be steroids they may be classified as follows:-

Table VI

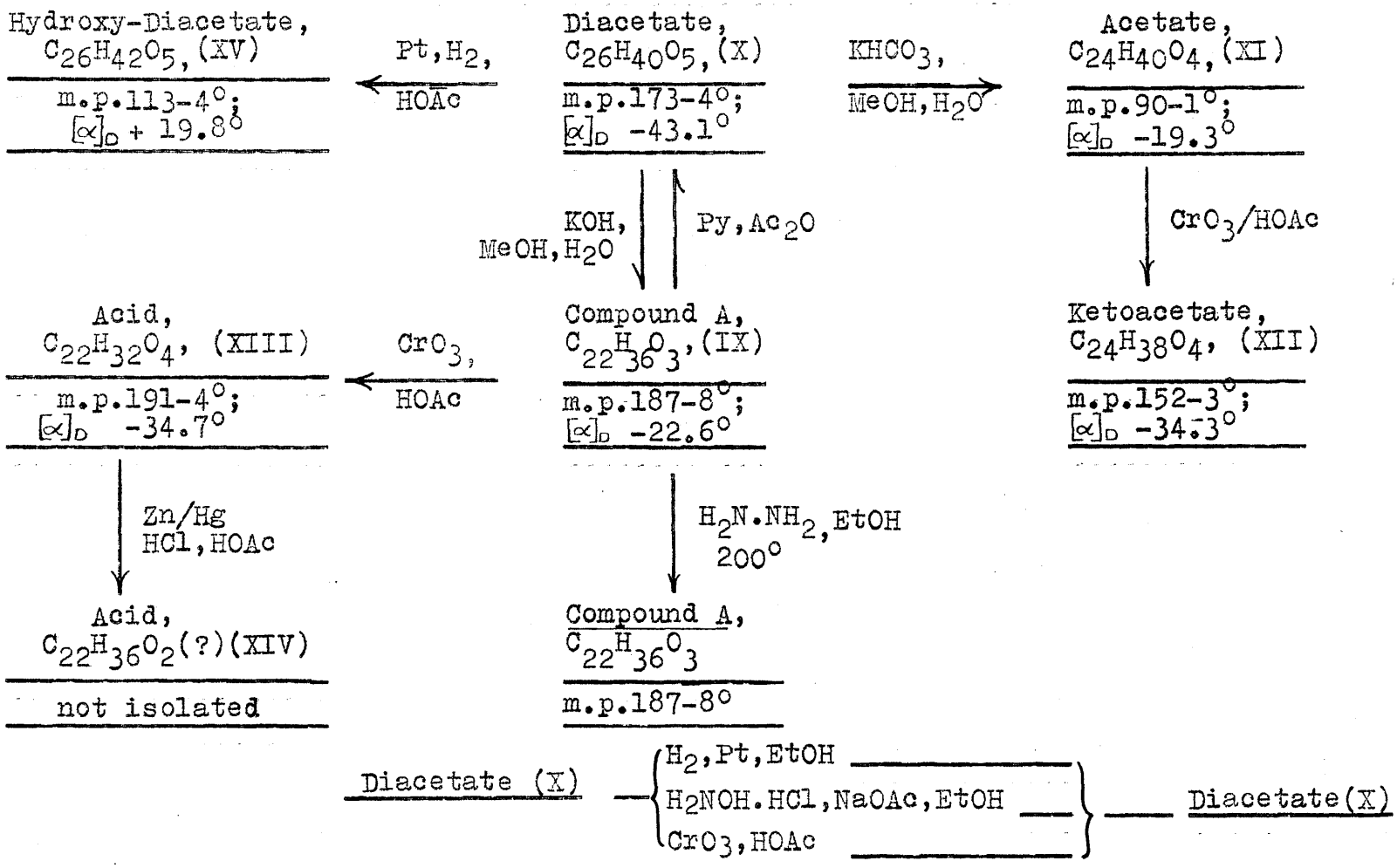
Class	Molecular Formula	Compound	Molecular Formula
ANDROSTANE	$C_{19}H_{32}$	B	$C_{19}H_{32}O_3$
NORPREGNANE (HOMOANDROSTANE)	$C_{20}H_{34}$	F	$C_{20}H_{34}O_3$
PREGNANE	$C_{21}H_{36}$	E	$C_{21}H_{38}O_5$
		J	$C_{21}H_{37}O_5$
		K	$C_{21}H_{36}O_3$
BISNORCHOLANE	$C_{22}H_{38}$	A	$C_{22}H_{36}O_3$
		D	$C_{22}H_{37}O_4$
		G	$C_{22}H_{40}O_5$
		H	$C_{22}H_{44}O_5$
NORCHOLANE	$C_{23}H_{40}$	I	$C_{23}H_{40}O_4$
CHOLANE	$C_{24}H_{42}$	C	$C_{24}H_{42}O_4$

No attempt is made to assign structures to these compounds at this stage. A note on the chemistry of Compound A is given in the next section (Section B)

Section B.

The reactions of Compound A are shown in the accompanying table (Table VI)

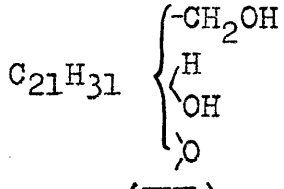
TABLE VI - Reactions of Compound A.



The formation of a diacetate (X) showed that compound A contained two hydroxyl groups and oxidation of the alcohol (IX) to an acid (XIII) having the same number of carbon atoms proved that one of the hydroxyl groups was primary: the other hydroxyl group must be secondary and be contained in a cyclic structure. The inert nature of the third oxygen function was demonstrated by the following reactions:-

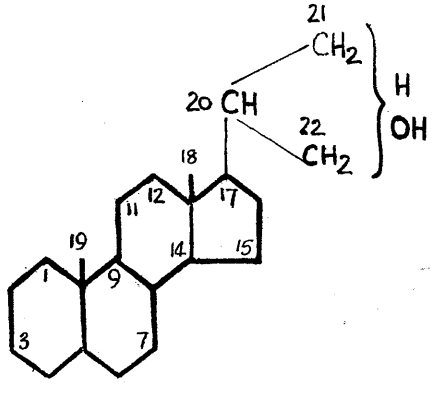
1. Compound A was recovered unchanged after hydrolysis of the diacetate with methanolic potassium hydroxide.
2. The diacetate (X) contained no active hydrogen atoms (Zerewitinoff) and was recovered unchanged after treatment with hydroxylamine hydrochloride in ethanol.
3. The diacetate was stable to oxidation and was recovered unchanged after attempted reduction in neutral solution. Hydrogenation of the inert oxygen was effected slowly in glacial acetic acid at room temperature.
4. Compound A was recovered unchanged after treatment with hydrazine at 200°.

Compound A may be formulated as (XVI)



(XVI)

The formula, $C_{21}H_{31}(O)(OH)(CH_2OH)$, (XVl), corresponds to a hydrocarbon $C_{21}H_{36}$, proving that Compound A is tetracyclic. The history of compound A favours interpretation of this tetracyclic structure as a steroid nucleus having an iso-propyl side chain at position C17, with a hydroxyl group at either C21 or C22 (XVll).



(XVll).

The inert nature of the third oxygen function is best explained by assuming a keto group at position C,ll as no other position in the steroid nucleus has this unique stability towards ketonic reagents (64 to 74).

A characteristic reaction of an 11-keto steroid is exhibited by Compound A diacetate (X) in its stability towards reduction in neutral solution and its relatively sluggish hydrogenation in an acid medium (64). The stability of compound A diacetate (X) towards alkaline hydrolysis, to reduction in neutral solution and to hydrazine, render the possibility of interpretation of the third oxygen function as an oxide linkage unlikely. An oxide linkage in the nucleus at position (5;6, 6;7, 7;8, 9;11, 11;12, 16;17) would rearrange under the influence of acid or alkali (75 to 80) and would be reduced in neutral solution (81, 82, 83).

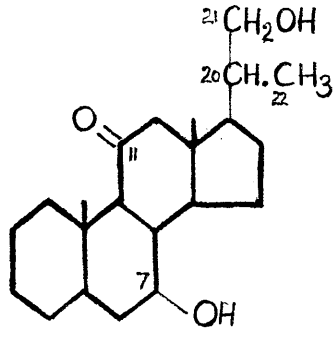
The position occupied by the secondary hydroxyl group may be located in either ring A or ring B. As neither acid (XIII) nor the keto acetate (XII) showed selective absorption in the ultraviolet (220 to 400mu), the location of a keto group at C3 is improbable, a deduction tentatively proved by comparison of the molecular rotation of acid (XIII) with the molecular rotation of 3 ; 11-diketobisnorcholanic acid and of 3 : 11-diketobisnorallocholanic acid (Table VII).

TABLE V11

Compound	M_D	Ref.
3:11-diketobisnorcholanic acid	+180° *	80
3:11-diketobisnorallocholanic acid	+235° *	92
acid X111	-118°	This Thesis.

(* Calculated from data for methyl ester. Bile acids and their methyl esters show no significant molecular rotation difference (84).)

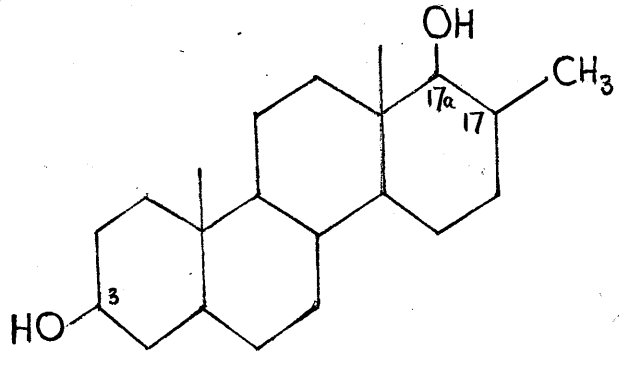
The fact that the secondary hydroxyl group was acetylated under mild conditions and the acetate was readily hydrolysed points to the presence of an unhindered hydroxyl group. Position C7 is the most probable, as all compounds previously obtained from bile are oxygenated at either C3, C7 or C12. A hydroxyl group at C12 cannot be acetylated at room temperature (79, 80), hence compound A was tentatively assumed to be a 7:21(22?)-dihydroxy-11-ketobisnorcholane (XV111)



(XV111).

This formula explains the reactions of Compound A as given in Table VI.

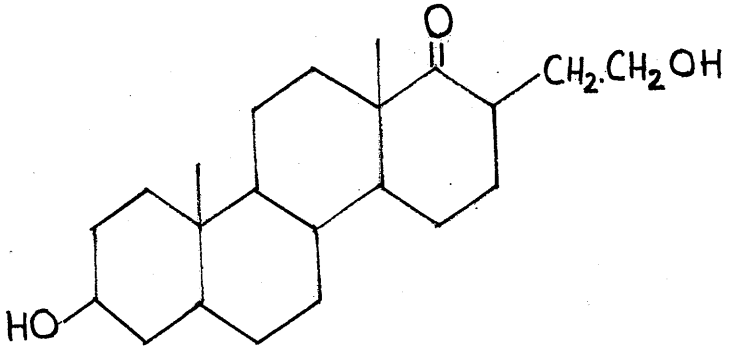
The failure to obtain acid (XIV) by Clemensen reduction of acid (XIII) rendered comparison with a steroid of known structure (bisnorcholanic acid), impossible. The assumption of a steroid nucleus as the only possibility, is doubtful in view of the isolation by Marker et.al. (85 to 88) of a uranediol, C H O , from the urine of pregnant mares, subsequently proved by Klyne (89) to be 17 methyl-D-homoandrostane-3 :17a-diol (XIX).



(XIX)

Assuming compound A to be a D-homosteroid, structure (XX), with a 17-(w-hydroxyethyl) side chain must be

postulated, as the 17a keto group, without a substituent



(XX).

at C17 is easily reduced in neutral solution and forms a semicarbazone (90). This structure for Compound A does not seriously contradict the experimental evidence but it is doubtful if the 17a keto group could withstand treatment with hydrazine at 200°.

In conclusion, the structure (XVlll) explains all the reactions of compound A and affords the most satisfactory explanation of the unreactivity of the non-acylable oxygen function. On the other hand, compound A and its degradation products (except the hydroxydiacetate (XV)) are laevorotatory, a fact easily explained on assumption of a D-homoandrostane nucleus (91).

E X P E R I M E N T A L.

EXPERIMENTAL.

All m.p.s. are uncorrected. Micro-analyses are by Drs. Weiler and Strauss, Oxford and Mr. Wm. McCorkindale, The Royal Technical College.

SECTION A.

Experiment A(i)

Dehydration of Ox-bile.

Summer-beef ox-bile (1.77kg.; untreated with preservative) was freeze-dried (vac., 0.01mm.) over a period of several days to yield partially dried bile as a red semi-viscous mass.

Yield = 300g. (16.9%).

The partially dried product was transferred to a number of Petri dishes, each dish containing a layer of bile 2mm. to 3mm. in depth and 762mm. in diameter. The material was dried in a vacuum desiccator over a period of fourteen days (vac., 2mm., desiccant used was a mixture of P_2O_5 and $CaCl_2$) and afforded anhydrous bile as a dark red friable solid, easily powdered and strongly hygroscopic.

Yield = 170g. (9.6% based on weight of dried bile).

Removal of Conjugated Bile Acids.

Dried bile (50g.) was shaken for 12 hours with anhydrous ethanol (175 c.c.). The bile slowly dissolved

forming a dark red solution bearing a yellow suspension. After standing for 2 hours, the mixture was quickly filtered and the residue washed with absolute ethanol (280 c.c.) until the washings were no longer coloured. The residue was dried in vacuo for 12 hours to give a pale yellow crystalline solid (5.12g.; 10.2%) containing nitrogen and sulphur (Lassaigne) and having no definite m.p. The material was assumed to be a mixture of the sodium salts of glycholic and taurscholic acids. This solid was not further examined. The combined filtrate and washings (450 c.c.) obtained above were treated as described below.

Removal of Free Acids.

To the solution (450 c.c.) was added ethanolic potassium hydroxide solution (3.72g. KOH in 42.1c.c. ethanol) and the mixture was agitated mechanically under anhydrous conditions over a period of 12 hours. To this mixture (bearing a slight yellow precipitate) was added dry ether (500 c.c.) when a white flocculent solid, rapidly reverting to a white pasty mass, was deposited. After cooling for 24 hours at 0°, the mixture was filtered and the residue washed with ether. The residue was not further examined. The combined washings and filtrate (1 litre) were concentrated to a

volume of 300 c.c. (vac., 10mm.,bath temp., 25-30°) and dry ether (500 c.c.) was added, the mixture cooled and the residue filtered and washed with ether. This procedure was repeated four times in all, when all the ether insoluble material was precipitated. The concentrate finally obtained (200 c.c.) was cooled to -5° and dry ether (200 c.c.) was added and a stream of carbon dioxide was passed through the cooled mixture until precipitation of potassium carbonate was complete (time, 6 hours). The mixture was filtered and the filtrate was concentrated in vacuo (0.05mm., bath temp.,30°), to yield the neutral fraction as a pale yellow gum (1.5g., 3% based on weight of dried bile). Attempts made to crystallise the neutral material from benzene, ethanol, acetone, ethanol/acetone and ethyl acetate were unsuccessful. The dried neutral fraction (1.4g., gum) was acetylated at 0° over a period of 24 hours with pyridine (3 c.c.) and acetic anhydride (8 c.c.). The acetylation mixture was decomposed with water, and the gummy acetates were taken up in ether (100 c.c.) and the ethereal solution successively washed with water (4 x 100 c.c.), hydrochloric acid (2N; 3 x 75 c.c.), sodium carbonate solution (10%; 4 x 80 c.c.) and water (4 x 100 c.c.). The ethereal solution was dried (Na_2SO_4), the ether removed by distillation and the residue (1.06g.) dissolved in light petroleum (100 c.c.) and the solution was chromatographed

on alumina (Spence Type H, Grade II, 20g.)

Eluent	Volume (c.c.)	Fraction No.	Eluate (Yield, mg.)
Petrol	50	1	-
"	50	2	50
"	50	3	10
"	50	4	20
"	50	5	140
"	50	6	90
"	50	7	130
"	50	8	10
"	50	9	30
"	50	10	60
"	50	11	15
"	50	12	-
Petrol 4/benzene 1	50	13	-
" / "	50	14	250 (gum)
" / "	150	15	-
Petrol 1/benzene 1	100	16	-
Benzene	150	17	170 (gum)

Fraction 2.

After four crystallisations from methanol this fraction gave cholesteryl acetate, m.p. 112-114° (11mg.), undepressed on admixture with an authentic specimen (m.p. 114-115°). Fractions 4, 5, 6 and 7 were similar to fraction 2 in giving cholesteryl acetate (yields:- 12mg., 80mg., 50mg. and 90mg. respectively) and were not further examined.

Fraction 14.

After several crystallisations from ethanol this fraction gave cholesterol, m.p. 144-5°, $[\alpha]_D = -36°$ ($l = 1$, c = 1.15 in chloroform) undepressed on admixture with an authentic specimen (m.p. 148-148.5°, $[\alpha]_D = -39°$ ($l = 1$, c = .521 in chloroform)) Yield = 200 mg.

Fraction 17 afforded cholesterol (105mg., m.p.144-5°, $[\alpha]_D = -37°$ ($l = 1$, c = 0.711 in chloroform) undepressed on admixture with an authentic specimen.

Fractions 3, 8, 9, 10 and 11 could not be resolved by crystallisation from ethanol or methanol and were not further examined.

Experiment A(ii)

Dried bile (100g.) was shaken with ethanol (400 c.c.) over a period of 24 hours. The precipitated conjugated acids (12.8g.; 12.8%) were filtered, washed with absolute ethanol (200 c.c.) and the combined washings and filtrate (600 c.c.) treated with barium oxide (5.1g.) The mixture was agitated mechanically for 24 hours, the precipitate filtered, washed with ethanol (100 c.c.) and the combined washings and filtrate (700 c.c.) treated with dry ether several times in the manner previously described. The filtrate obtained after removal of all ether insoluble material gave only a slight turbidity with carbon dioxide. The cleared, barium free solution was evaporated to dryness under reduced pressure (vac., 2mm., bath temp. 25°) and afforded the neutral fraction as a yellowish-red gum. Yield = 0.76g.; 0.76%).

Treatment of the Neutral Fraction with Girard's Reagent.

To the neutral fraction (0.76g.) was added absolute

ethanol (90g., 114 c.c.) and glacial acetic acid (10g., 9.5 c.c.) followed by Girard's reagent P (3.0g.) and the mixture refluxed for one hour on the water bath and then allowed to cool to room temperature over a period of two hours. The cooled solution was decomposed with ice and sodium carbonate solution containing sufficient alkali to neutralise nine-tenths of the acetic acid (7.95g. Na_2CO_3). The mixture (500 c.c.) was extracted with ether (10 x 50 c.c.), and the ethereal extracts combined and dried (Na_2SO_4), to give the non-ketonic fraction.

The aqueous residue (476.5 c.c.) was made 0.5N with concentrated hydrochloric acid (19.8 c.c.), the mixture allowed to stand at room temperature for one hour and then extracted with ether (8 x 50 c.c.). The ethereal extracts were combined and dried (Na_2SO_4) to give the ketonic fraction.

(a) NON-KETONIC FRACTION.

The solvent was removed under reduced pressure (60mm., bath temp., 30°) and the residue evaporated to dryness in vacuo (0.1mm.). The residue obtained (640mg., gum) after three crystallisations from ethanol yielded 204mg. plates, m.p. 144-5°, the melting point of which was undepressed on admixture with an authentic specimen of cholesterol, m.p. 148°.

No crystalline material could be obtained from the mother liquors. These fractions were not further examined.

(b) KETONIC FRACTION.

Concentration of the ethereal extracts followed by evaporation of the residue under high vacuum yielded the mixed ketones (70mg.) as a pale yellow gum. Repeated crystallisation from ethanol failed to effect further purification.

Spectroscopic examination of the mixture revealed an absorption maxima at 230 mu (intensity 0.45). This fraction was not further examined.

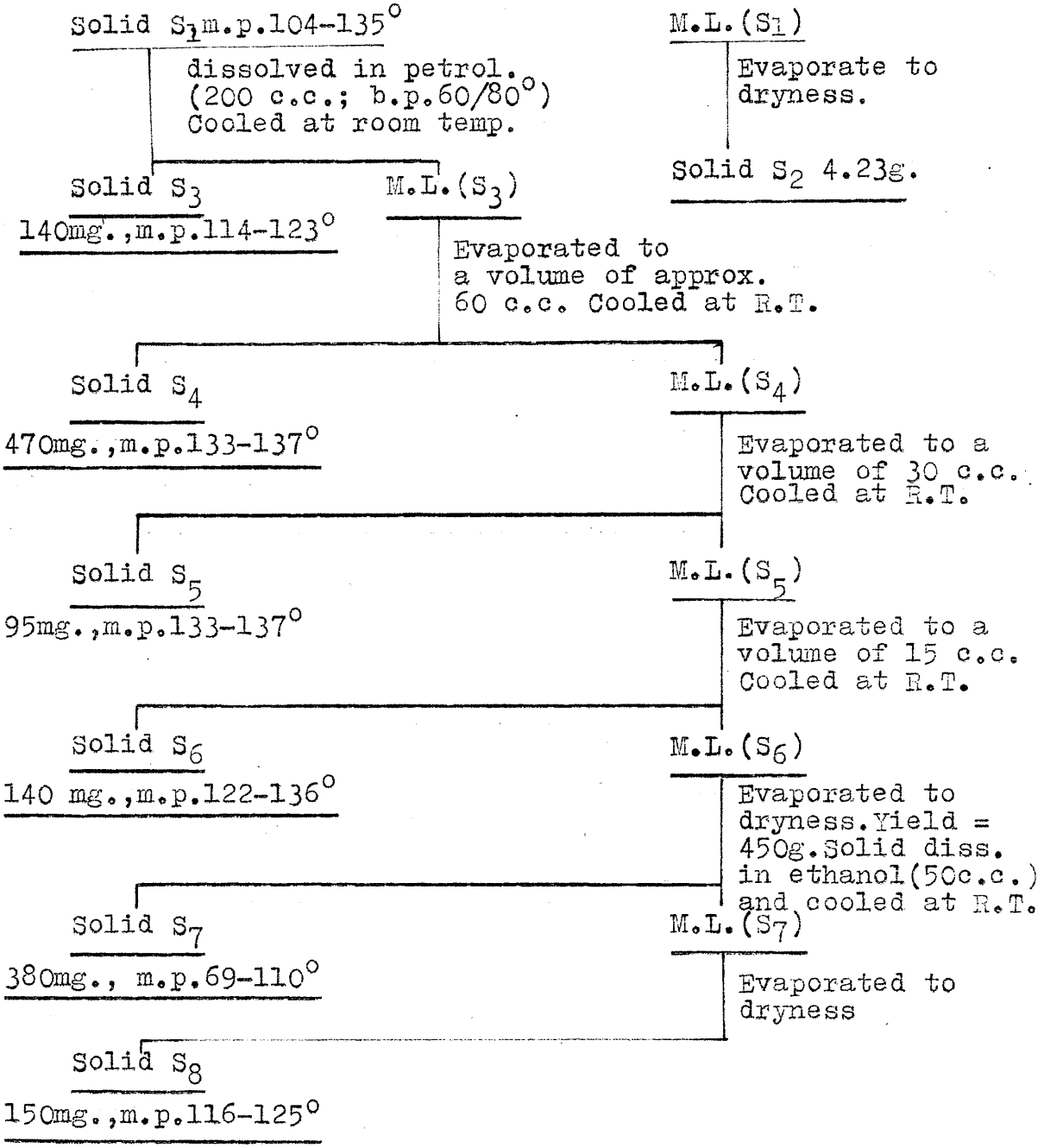
Experiment A(iii)

To fresh ox-bile (20 litres) was added barium hydroxide (800 gm.) and the mixture was stirred vigorously for two days at room temperature, allowed to stand for 8 hours, the supernatant liquor decanted and the residue washed with water until the washings were no longer coloured. The combined washings and filtrate (22 litres), contained in a large continuous ether extraction unit (fitted with a stirrer), were extracted with ether over a period of 10 days. The ethereal extract (4.2[illegible].) was collected, the solvent removed under reduced pressure and the yellow, pasty residue (32g.) dried in vacuo for 36 hours to yield the neutral fraction (25g.) as a greasy, yellow solid which had no definite melting point.

To the neutral fraction (13g.) was added ethyl acetate

(100 c.c.) and the yellow solution obtained after warming and concentration in vacuo to a volume of 50 c.c. (Solution S) was treated as described below.

Solution S.



The solid fractions obtained (S_1, S_3 to S_8) were acetylated in the usual manner with pyridine/acetic anhydrede at room temp. (24 hours). The results are tabulated below.

Fraction.	m.p.	ACETATE m.p.	Yield (mg.) ACETATE
S_1	104 - 135°	-	-
S_3	114 - 123°	-	-
S_4	134 - 140°	105 - 107°	480
S_5	133 - 137°	106 - 110°	90
S_6	122 - 136°	90 - 95°	132
S_7	69 - 110°	95 - 104°	275
S_8	116 - 125°	82 over 360°	120

Solid S_3

After four recrystallisations from ethanol yielded 78mg. crystals (plates), m.p. 146-8°, a specimen of which was undepressed on admixture with a specimen of cholesterol, m.p. 146-8°.

Solid S_4

The acetylated material, m.p. 105-107°(480mg.) was dissolved in dry distilled benzene (200 c.c.) and chromatographed on alumina (Spence Type H, Grade II, 25g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	246mg.,m.p.108-109°
"	100 c.c.	2	247mg.,m.p.110-111°
"	400 c.c.	3	12mg., gum.
Ether	200 c.c.	4	9mg., gum.

Fraction 1.

After one crystallisation from ethanol yielded 204mg. needles, m.p.111-112°, $[\alpha]_D = -40°$ ($l = 1$, C = 0.55 in chloroform) a specimen of which was undepressed on admixture with a specimen of cholesteryl acetate m.p. 114°.

Fraction 2.

After one crystallisation from ethanol yielded 195mg. needles, m.p. 109-111°, $[\alpha]_D = -45°$ ($l = 1$, C = 0.71 in chloroform), the melting point of which was undepressed on admixture with an authentic specimen of cholesteryl acetate.

Fractions 3/4.

These fractions could not be resolved by crystallisation and were not further examined.

Solid S_5

The material (90mg., m.p.106-110°) was dissolved in dry benzene (100 c.c.) and chromatographed on alumina (Spence Type H, Grade II, 10g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	50mg.,m.p.110-111°
"	100 c.c.	2	27mg.,m.p.112-113°
"	200 c.c.	3	6mg.,m.p.114°
Ether	300 c.c.	4	4mg., (gum).

Fractions 1, 2 and 3 showed no depression on admixture with a specimen of pure cholesteryl acetate, m.p. 112-114°. Fraction 4 was not further examined.

Solid S_6

The material (132mg.,m.p. 90-95°) was dissolved in dry benzene (100 c.c.) and chromatographed on alumina (Grade II, 15g.)

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	66mg., m.p.109-110°
"	100 c.c.	2	33mg., m.p.111-113°
"	100 c.c.	3	23mg., m.p.112-114°
"	100 c.c.	4	6mg., Gum.
"	200 c.c.	5	5mg., Gum.

Fractions 1, 2 and 3 were shown to be cholesteryl acetate (mixed m.p. Liebermann Reaction).

Fractions 4/5 were not further examined.

Solid S_7

The fraction (275mg., m.p.95-104°) was dissolved in dry benzene (175 c.c.) and chromatographed on alumina (Grade II, 25g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	169mg., m.p.107-111°
"	100 c.c.	2	47mg., m.p.109-110°
"	100 c.c.	3	10mg., m.p.111-112°
"	400 c.c.	4	6mg., m.p.112-113°
Ether	100c.c.	5	10mg., Gum.
"	300 c.c.	6	23mg., Gum.

Fraction 1.

After two crystallisations from ethanol yielded 131mg. needles, m.p.112-114° a specimen of which was undepressed on admixture with a specimen of pure cholesteryl acetate, m.p. 112-114°.

Fractions 2, 3 and 4.

These were shown to be cholesteryl acetate (mixed m.p.)

Fractions 5/6.

These could not be resolved by crystallisation and were not further examined.

Solid S_8

The material (120mg., m.p.82 above 360°) was dissolved in dry benzene (90 c.c.) and the suspension chromatographed on alumina (15g., Grade II).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	85mg., m.p.108-109°
"	100 c.c.	2	25mg., m.p.111-112°
"	200 c.c.	3	3mg., m.p.112°
Ether	200 c.c.	4	Nil

Fraction 1.

After one crystallisation from ethanol yielded 74mg. needles, m.p. 112-114° a specimen of which was undepressed on admixture with cholesteryl acetate m.p.114°.

Fractions 2/3.

These were shown to be cholesteryl acetate (mixed m.p.).

Solid S_2

The dark red solid (4.23g.) was continuously extracted with light petroleum (400 c.c.) over a period of 8 hours in a Soxhlet apparatus. The petroleum extracts were collected, combined evaporated to dryness to yield a solid S_2(a)., m.p. 84-135°.

The petrol. insoluble fraction (1.05 g., m.p.110-130°) could not be resolved by crystallisation. The material showed no selective absorption in the U.V. (220 to 400mu), a flat curve, extending from 300mu (intensity zero) to 220mu (intensity = 0.4) being obtained.

The solid (petrol. insoluble, 983mg., m.p.110-130°) was dissolved in dry benzene (550 c.c.) and chromatographed on alumina (Grade II, 30g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	17mg., solid.
"	100 c.c.	2	18mg., solid.
"	100 c.c.	3	Trace
"	100 c.c.	4	6mg., solid.
"	200 c.c.	5	10mg.,solid.
"	100 c.c.	6	5mg.,solid.
"	100 c.c.	7	6mg.,solid.
"	100 c.c.	8	11mg.,solid.
"	100 c.c.	9	8mg.,solid.
"	200 c.c.	10	15mg.,solid.
"	100 c.c.	11	3mg.,solid.
"	400 c.c.	12	4mg.,solid.
"	100 c.c.	13	5mg.,(brown gum)
"	100 c.c.	14	3mg.,(brown gum)
"	300 c.c.	15	5mg.,(brown gum)
Benz./ether (1%)	300 c.c.	16	22mg.,(brown gum)
" "	100 c.c.	17	10mg.,(brown gum)
" "	100 c.c.	18	8mg.,(brown gum)

Cont'd.....

Brought forward:

Eluent.	Volume.	Fraction No.	Eluate.
Benz./ether (1%)	100 c.c.	19	30mg., (yellow solid)
" "	100 c.c.	20	6mg., (yellow solid)
" "	300 c.c.	21	5mg., (yellow solid)
" "	400 c.c.	22	10mg., (white solid)
" "	300 c.c.	23	14mg., (brown gum)
" "	200 c.c.	24	27mg., (brown gum)
" "	500 c.c.	25	10mg., (brown gum)
" "	100 c.c.	26	10mg., (brown gum)
" "	100 c.c.	27	9mg., (brown gum)
" "	300 c.c.	28	5mg., (brown gum)
" "	200 c.c.	29	6mg., (clear gum)
" "	100 c.c.	30	5mg., (clear gum)
Benz./ether(2%)	100 c.c.	31	3mg., (brown gum)
" "	300 c.c.	32	5mg., (brown gum)
" "	100 c.c.	33	7mg., (brown gum)
" "	100 c.c.	34	4mg., (brown gum)
" "	100 c.c.	35	4mg., (brown gum)
" "	100 c.c.	36	8mg., (brown gum)
" "	100 c.c.	37	5mg., (brown gum)
" "	100 c.c.	38	Trace
" "	100 c.c.	39	3mg., (brown gum)
Benz./ether(2%)	300 c.c.	40	7mg., (brown gum)
Benz./ether(4%)	100 c.c.	41	5mg., (brown gum)
" "	100 c.c.	42	10mg., (brown gum)
" "	100 c.c.	43	4mg., (brown gum)
" "	100 c.c.	44	13mg., (brown gum)
" "	100 c.c.	45	3mg., (brown gum)
Benz./ether(8%)	100 c.c.	46	6mg., (brown gum)
" "	100 c.c.	47	10mg., (brown gum)
" "	100 c.c.	48	12mg., (brown gum)
" "	100 c.c.	49	18mg., (brown gum)
" "	100 c.c.	50	15mg., (brown gum)
" "	100 c.c.	51	10mg., (brown gum)
" "	100 c.c.	52	13mg., (brown gum)
" "	100 c.c.	53	8mg., (brown gum)
Benz./ether(8%)	300 c.c.	54	14mg., (brown gum)
" "	300 c.c.	55	14mg., (brown gum)
Benz./ether(20%)	300 c.c.	56	6mg., (clear gum)
" "	100 c.c.	57	8mg., (clear gum)
Benz./ether(40%)	100 c.c.	58	8mg., (clear gum)
" "	100 c.c.	59	8mg., (clear gum)
" "	100 c.c.	60	12mg., (clear gum)
" "	100 c.c.	61	8mg., (clear gum)
" "	100 c.c.	62	8mg., (clear gum)
" "	400 c.c.	63	7mg., (clear gum)
Benz./ether(80%)	100 c.c.	64	8mg., (clear gum)
" "	100 c.c.	65	5mg., (clear gum)
" "	100 c.c.	66	6mg., (clear gum)
" "	100 c.c.	67	4mg., (clear gum)

Cont'd.....

Brought forward:

Eluent.	Volume.	Fraction No.	Eluate.
Benz./ether(80%)	100 c.c.	68	10mg., (clear gum)
" "	100 c.c.	69	9mg., (clear gum)
" "	100 c.c.	70	8mg., (clear gum)
Ether (100%)	400 c.c.	71	19mg., (clear gum)
Ethanol	100 c.c.	72	322mg., (yellow solid)
Ethanol	100 c.c.	73	20mg., (brown gum)
			967mg.

The fractions were dried for 24 hours at room temp. under high vacuum and examined for absorption in U.V. (200 to 400 mu).

Fraction No.	Maxima (density)	Fraction No.	Maxima (density)
4	252mu (0.28)	13	280mu (0.49)
5	255mu (0.83)	14	290mu (0.50)
6	255mu (0.94)	15	238mu(0.64); 292mu(0.37)
7	225mu (0.60); 290mu(0.26)	16	240mu (1.00)
12	280mu (0.36)	32	315mu (0.14)
33	208mu (1.33)	37	222mu(0.62); 258mu(0.39)
38	255mu (0.42)	39	258mu (0.34)
53	288mu (0.20)	57	258mu (0.52)
63	258mu (0.40)	-	-

The materials were not weighed and densities quoted are approximate. The remaining fractions showed no selective absorption.

The fractions were combined into 17 different groups based on order of elution from the column and on spectroscopic data.

Group	Component Fractions	Yield (m.g.)	Nature.
(a)	4, 5, 6.	15	gum
(b)	7, 8, 9, 10.	35	gum
(c)	11,12,13,14.	10	gum
(d)	15,16.	18	gum
(e)	17,18,19.	40	gum
(f)	20,21,22,23,24.	55	gum
(g)	25,26,27.	22	gum
(h)	28,29,30,31,32,33.	26	gum
(i)	34,35.	5 .	gum
(j)	36,37.	9	gum
(k)	38,39,40,41,42,43, 44,45,46,48,50,51.	80	gum
(l)	47,49,52,53.	32	gum
(m)	54 to 62 inc.	73	gum
(n)	63.	7	gum
(o)	64,65,66,67,69,70.	40	gum
(p)	71,72.	35	gum
(q)	73.	320	solid

The foregoing fractions could not be resolved by crystallisation and were not further examined.

Experiment A(iv).

A further quantity of neutral material (8.0g.) was obtained from ox-bile (8 litres) by treatment with barium hydroxide (320g.) with stirring over a period of 2 days. The suspension was filtered and washed with water (3 litres) and the combined washings and filtrate (11 litres) extracted continuously with ether over a period of 5 weeks. The ether extracts yielded 8g. neutral material after removal of solvent. The residue yielded a petroleum insoluble fraction (1.83g.) after extraction with light petroleum over a period of 24 hours.

The petroleum insoluble fraction (183g., m.p.160-165°) was dissolved in pyridine (6 c.c.) and acetic anhydride (4 c.c.) and the mixture allowed to stand at room temperature for 12 hours, then warmed on the water bath for 3 hours (bath temp. 60°). Excess solvent was removed by distillation under reduced pressure (2mm.) and the residue (1.85g., gum) dissolved in ether (100 c.c.). The ethereal solution was washed with water (300 c.c.), dilute hydrochloric acid (2N; 150 c.c.), dilute sodium carbonate solution (1%; 200 c.c.) and finally with water (150 c.c.). The ether solution was dried (Na_2SO_4) and excess solvent removed on the water bath. The residue (1.49g., gum)

could not be crystallised from organic solvents and was evaporated to dryness several times with dry benzene (100 c.c. portions) and the residue dissolved in dry benzene (200 c.c.) and chromatographed on alumina (Grade II).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	390mg., (brown gum)
"	100 c.c.	2	384mg., " "
"	100 c.c.	3	64mg., " "
"	100 c.c.	4	80mg., (clear gum)
"	100 c.c.	5	34mg., " "
"	100 c.c.	6	18mg., " "
"	100 c.c.	7	22mg., " "
"	100 c.c.	8	12mg., " "
"	100 c.c.	9	5mg., " "
"	100 c.c.	10	3mg., " "
"	100 c.c.	11	4mg., " "
"	100 c.c.	12	Trace
"	100 c.c.	13	4mg., (clear gum)
"	100 c.c.	14	3mg., " "
"	100 c.c.	15	12mg., " "
"	100 c.c.	16	2mg., (brown gum)
"	100 c.c.	17	2mg., " "
"	100 c.c.	18	2mg., " "
"	100 c.c.	19	2mg., " "
"	200 c.c.	20	3mg., " "
Benz./ether(1%)	100 c.c.	21	12mg., " "
" "	100 c.c.	22	2mg., " "
" "	100 c.c.	23	5mg., " "
" "	100 c.c.	24	4mg., (clear gum)
" "	100 c.c.	25	2mg., " "
" "	100 c.c.	26	2mg., " "
" "	100 c.c.	27	3mg., " "
" "	200 c.c.	28	15mg., " "
" "	200 c.c.	29	2mg., " "
Benz./ether(2%)	100 c.c.	30	2mg., " "
" "	100 c.c.	31	3mg., " "
" "	100 c.c.	32	3mg., " "
" "	100 c.c.	33	7.5mg., (brown gum)
" "	400 c.c.	34	6mg., " "
Benz./ether(4%)	100 c.c.	35	8mg., (clear gum)
" "	200 c.c.	36	10mg., " "
" "	100 c.c.	37	2mg., (brown gum)
" "	100 c.c.	38	8mg., " "

Cont'd.....

Eluent.	Volume.	Fraction No.	Eluate.
Benz./ether(4%)	100 c.c.	39	9mg., (brown gum)
Benz./ether(8%)	300 c.c.	40	16mg., " "
" "	100 c.c.	41	12mg., " "
" "	100 c.c.	42	6mg., " "
" "	300 c.c.	43	9mg., " "
Benz./ether(12%)	100 c.c.	44	11mg., " "
" "	100 c.c.	45	6mg., " "
" "	200 c.c.	46	15mg., " "
" "	100 c.c.	47	10mg., " "
" "	100 c.c.	48	10mg., " "
" "	100 c.c.	49	3mg., (clear gum)
Bez./ether(20%)	100 c.c.	50	3mg., " "
" "	100 c.c.	51	5mg., " "
" "	100 c.c.	52	5mg., " "
Benz./ether(40%)	300 c.c.	53	12mg., " "
Benz./ether(80%)	300 c.c.	54	8mg., " "
Ether	100 c.c.	55	10mg., " "
"	100 c.c.	56	10mg., " "
"	100 c.c.	57	2mg., " "
Ethanol	100 c.c.	58	117mg., (brown gum)
"	100 c.c.	59	10mg., " "
"	100 c.c.	60	20mg., " "
		Total	1353mg.

The fractions were dried in vacuo for 24 hours and examined for light absorption in the ultraviolet. The results are appended below.

Fr. No.	Abs.Max.(density)*	Fr. No.	Abs.Max.(density)*
3	275mu (1.48)	16	258mu (0.45)
4	215 mu(0.52) 245mu (0.16)	17	250mu (0.88) 258mu (0.86)
6	285mu (0.96)	18	230mu (1.60) 258mu (0.70) 248mu (0.47)
8	215mu (1.50) 250mu (0.48)	19	258mu (0.52) 300mu (0.20)

Cont'd.....

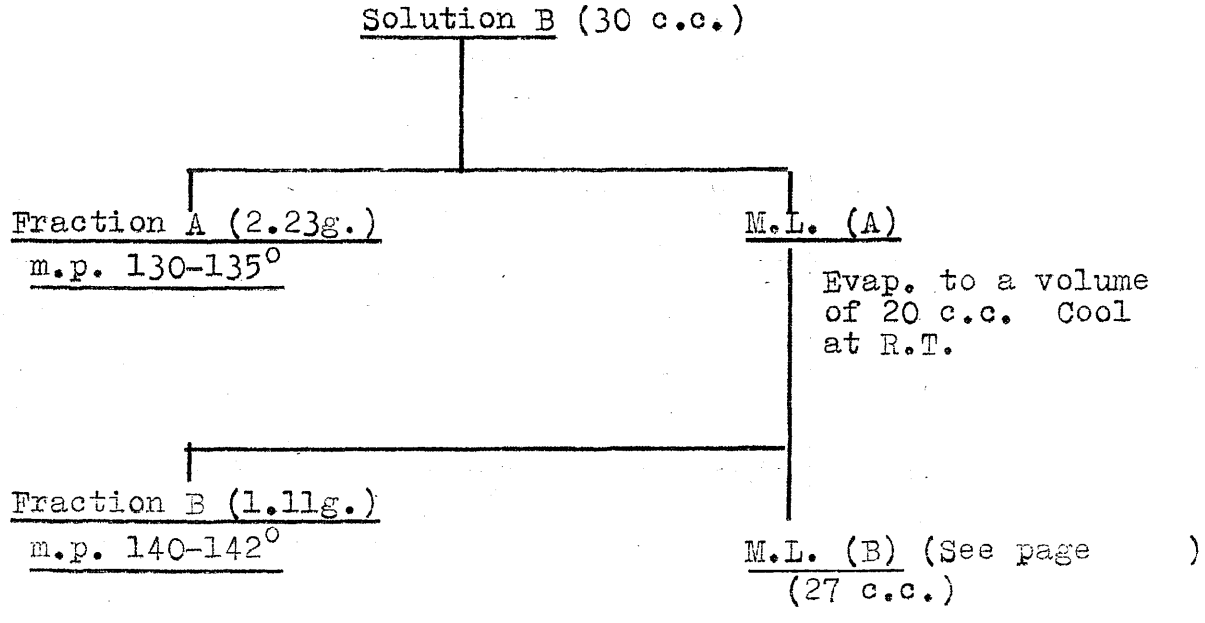
Fr.No.	Abs.Max.(density)*	Fr.No.	Abs.Max.(density)*
10	210mu (1.29) 250mu (0.76)	20	215mu (1.48) 258mu (0.84)
11	217mu (0.82) 250mu (0.34) 282mu (0.18)	21	220mu (1.22) 258mu (0.78)
12	258mu (0.46)	22	258mu (0.48)
13	225mu (1.11) 257mu (0.92)	23	215mu (1.48),250mu (0.96),258mu (1.00), 300mu (0.34)
14	258mu (0.38)	25	215mu (1.12),250mu (0.96),258mu(1.00)
15	215mu (1.76) 258mu (1.46) 300mu (0.36)	26	222mu (0.49), 258mu (0.34)
28	258mu (0.60)	43	258mu (1.30)
29	258mu (0.62)	44	360mu (0.11)
30	248mu (0.54) 258mu (0.56)	45	255mu (0.69)
31	258mu (1.00)	46	258mu (1.15)
32	250mu (0.47)	47	258mu (0.73)
33	258mu (0.84)	49	258mu (0.53)
34	258mu (0.64)	50	257mu (0.77)
35	360mu (0.13)	52	259mu (0.59)
37	360mu (0.13)	53	258mu (0.43)
39	220mu (0.95) 258mu (0.48)	55	258mu (0.84)
40	258mu (0.81)		

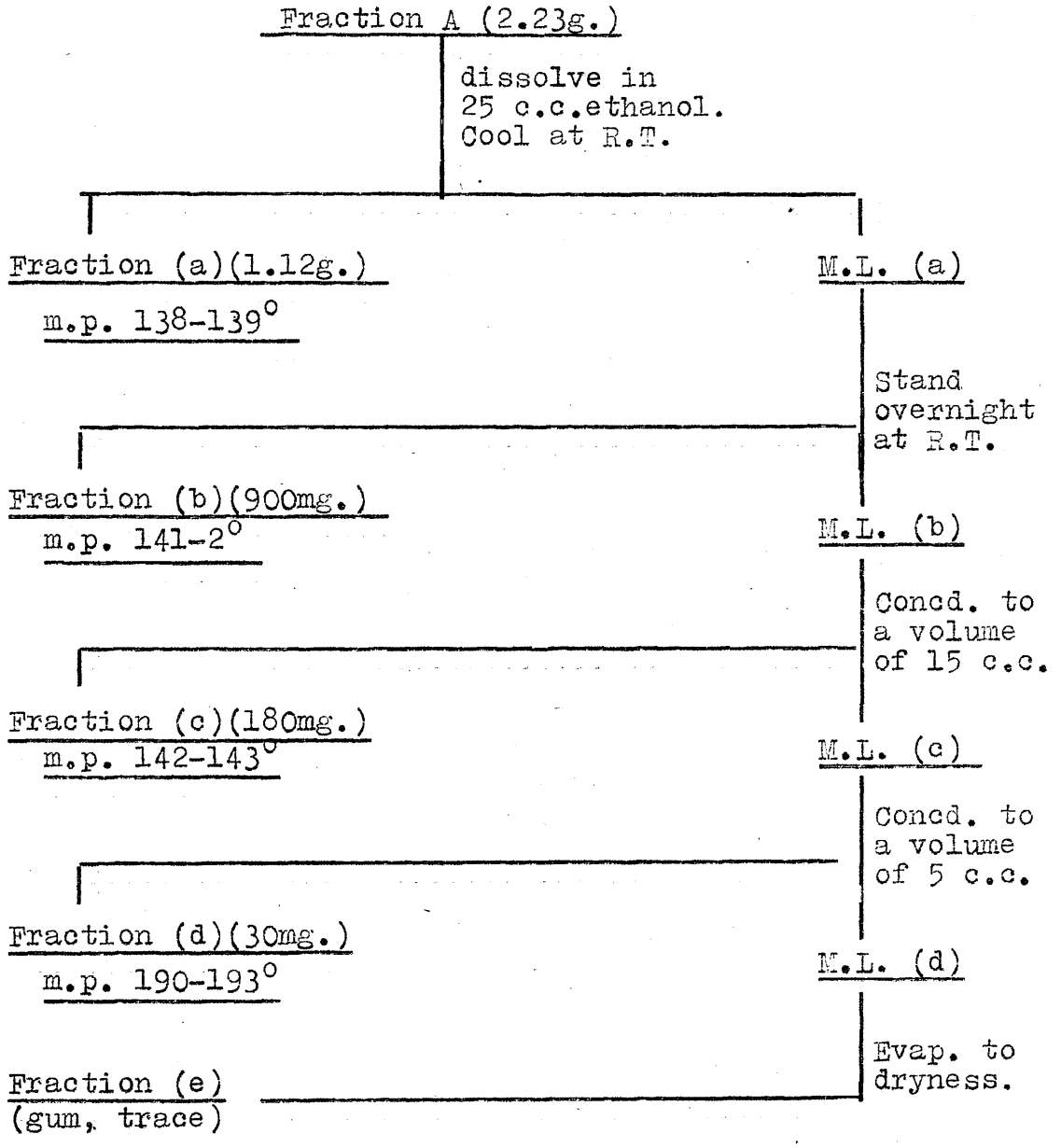
*Fractions were obtained as gums. The density figures are included to show relative values only. Concentration is approx. the same in all cases and may be taken as 0.01g./litre

The remaining fractions showed no light absorption. These fractions were not further examined.

The petroleum soluble fraction (350 c.c.) (see page 51) was concentrated to a volume of 50 c.c. and allowed to stand at 0° for 15 minutes. A mass of crystalline material m.p.136-140° (570mg.) was obtained. This material yielded 323mg. pure cholesterol after several recrystallisations from ethanol.

The petroleum mother liquors and washings were combined and evaporated to dryness. The residue was dissolved in ethanol (30 c.c.) and the solution (Solution B) was allowed to stand at room temperature for 10 minutes.



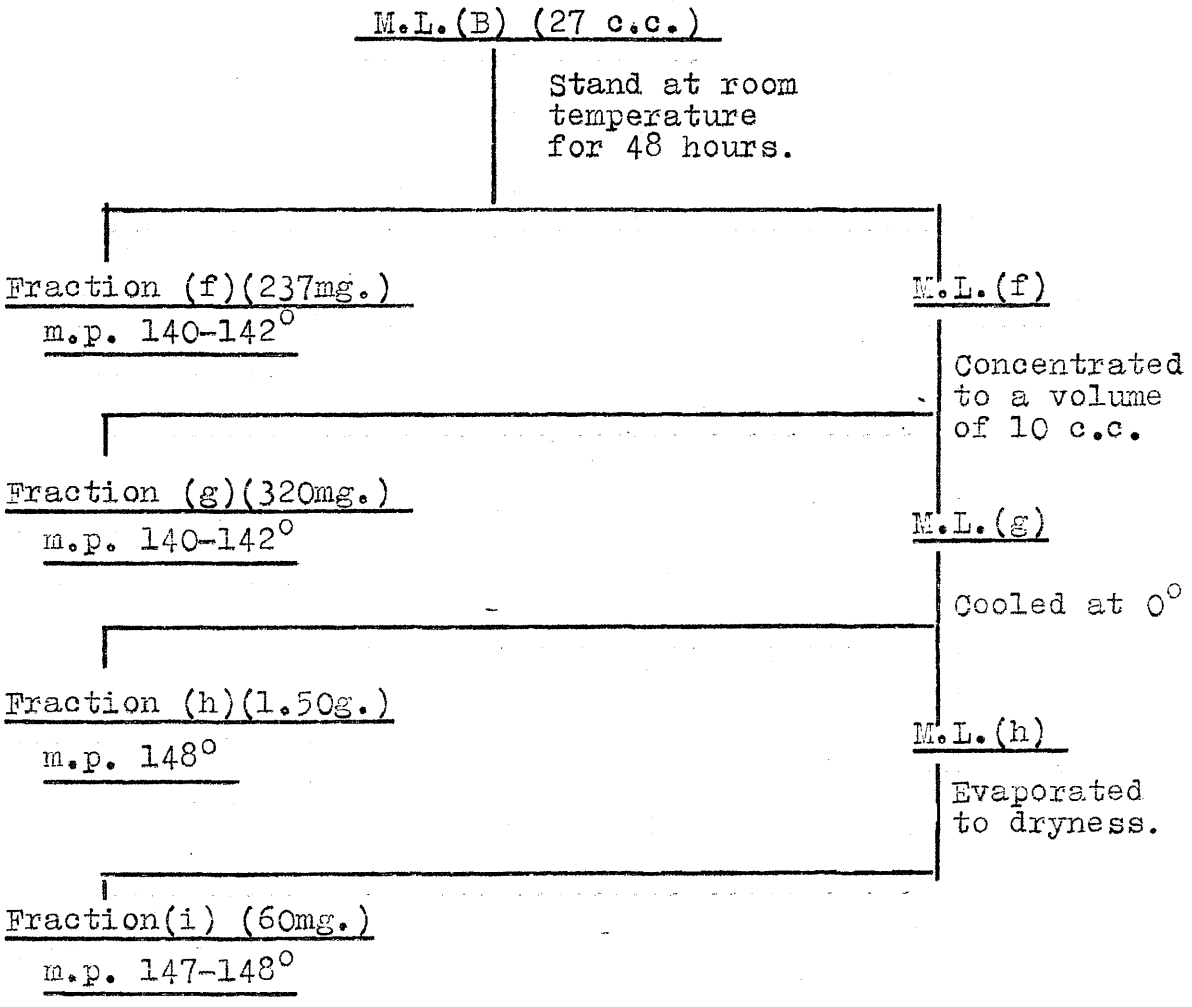


Fractions a, b, c, and d showed no selective absorption in the ultraviolet (220mu --400mu).

Fractions a, b, c, were shown to be cholesterol (mixed m.p.)

Fraction e

After two crystallisations from ethanol yielded a compound, m.p. 196-7° (11mg.) the melting point of which was undepressed on admixture with an authentic specimen of cholic acid, m.p. 196-7°



Fractions f, g, h, i showed no selective absorption in the ultraviolet in the region 220-400mu. A specimen of each fraction (in the case of fractions f and g after one crystallisation from ethanol) showed no depression on admixture

with an authentic specimen of cholesterol, m.p. 147-8°.

These fractions were not further examined.

Experiment A(v).

To fresh ox-bile (2 gals.) was added barium hydroxide octahydrate (360g.) in water (2 litres) and the mixture was stirred vigorously for 4 hours in 20 litre aspirator bottle fitted with a stopcock at the bottom to facilitate the removal of chloroform. After standing for two hours the mixture was filtered and washed with water (3 litres) and the combined washings and filtrate (12 litres) were extracted with chloroform as described below.

Each run was carried out at room temperature and the volume of chloroform used per run was 2½ litres. The mixture was subjected to vigorous mechanical stirring throughout the extraction period (4 hours and 16 hours alternatively). The chloroform layer was run off via the tap at the bottom of the aspirator bottle and was evaporated to dryness under reduced pressure (water bath, temperature 30-40°), the residue dried and weighed and extracted with ether until the extracts were no longer coloured. The ethereal extracts were concentrated and the residue dried and weighed. This residue was then extracted with light petroleum and the petroleum extracts evaporated to dryness to yield the petroleum soluble fraction. The residue

(petroleum insoluble fraction) and petroleum soluble fraction were dried and weighed. The results are tabulated below.

Run No.	Time of extrn. (hrs.)	Vol.chl run off (litres)	Weight residue from D (g.)	Weight neutral material from D (g.)(E)	Petrol. soluble from (E) (g.)	Petrol. insoluble from (E) (g.)
1	4	2	7.03	3.20	3.00	0.20
2	16	2	4.47	1.21	0.91	0.30
3	4	2	4.56	0.38	0.202	0.18
4	16	2	3.65	0.20	0.100	0.100
5	4	2½	4.32	0.20	0.100	0.100
A	B	C	D	E	Total 4.39	Total 0.800

The petrol. soluble fraction was not further examined.

The petrol. insoluble material (800mg.) was dissolved in dry methanol (32 c.c.) containing glacial acetic acid (5 c.c.) and to this solution was added Girard's reagent P (2.0g.) and the mixture heated for 1 hour on the water bath. The methanol was removed at 0° by distillation under high vacuum (2mm.) and the residue made alkaline to phenolphthalein with sodium carbonate solution (10%; 40 c.c.). The cooled solution was extracted with cold ethyl acetate (600 c.c.), the ethyl acetate extracts combined, dried (Na_2SO_4) and the solvent removed under reduced pressure to yield a residue (690mg., gum). This fraction was not further examined.

The aqueous residue was made acid to Congo Red with concentrated HCl (15 c.c.) and the acidic solution was extracted with cold ethyl acetate (600 c.c.), the ethyl acetate extracts combined, dried (Na_2SO_4) and the solvent removed under reduced pressure and yielded 70mg. ketonic material as a gum.

The ketones (70mg.) were dissolved in dry benzene (50 c.c.) and the solution was chromatographed on alumina (Grade II, 10g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene	100 c.c.	1	30mg. (gum)
Benzene	300 c.c.	2	11mg. (gum)
Ether	200 c.c.	3	Trace
Ethanol	200 c.c.	4	20mg. (gum)

Fractions 1, 2, 4 were dried in vacuo over a period of 24 hours and examined for absorption in the ultraviolet.

Fraction 1 showed a maxima 225mu (0.47); the others showed no selective absorption.

These fractions were not further examined.

The extraction made on a large scale followed closely the above procedure, 240 gallons were extracted and the chloroform concentrates were worked up in a manner shown below.

A	B	C	D	E	F	G
Batch No.	Volume Bile used (gallons)	Weight of batch after removal of chl.	Weight of ether insoluble residue (from C)	Weight of neutral fraction (from D)	Petrol. soluble fraction (from D)	Petrol insol. Fr. (from D)
1	50	780g.	160g.	215g.	9g.	152g.
2	40	640g.	127g.	171g.	14g.	91g.
3	50	712g.	185g.	201g.	17g.	111g.
4	50	807g.	173g.	131g.	18g.	103g.
5	50	813g.	192g.	183g.	18g.	124g.
			Total	901g.*	76g.	581g.

* Weight is approximate. The material was greasy and could not be freed from solvent.

Petrol Soluble Fraction.

The petrol soluble fraction (581g.) was dissolved in methanol (6 litres) and the solution allowed to stand at room temperature for several hours. The crystalline precipitate was filtered and dried. Yield = 250g., m.p. 146-8°, $[\alpha]_D = -38°$ ($\ell = 1$, c = 0.63 in $CHCl_3$). A specimen of this material was undepressed on admixture with an authentic specimen of cholesterol, m.p. 146-148°.

The methanol mother liquors were evaporated to yield a gum 134g. This material was not further investigated.

CHROMATOGRAPHY OF PETROLEUM INSOLUBLE FRACTION

The petroleum insoluble fraction (76g., m.p. 100-160 , $([\alpha]_D)$ = - 22.2 (chloroform) was dissolved in dry benzene (4,200 c.c.) and the solution was chromatographed on alumina (Grade III ; 1 Kg.). The Column was 6 cm. in diameter and 47 cm. long. The volume of eluent examined in all cases was 500 c.c. When all the solution was added, the column presented the following appearance:-

Band No.	Depth (ins).	Colour
1	1/5"	brown
2	1/5"	orange
3	1/5"	grey
4	7/8"	dark brown
5	1/4" (approx.)	pale yellow
6	1/2"	pale yellow
7	1/2"	pale yellow
8	1/10"	yellow-brown
9	1/10"	yellow-brown
10	1/10"	greenish-yellow

Eluent.	Fraction No.	ELUATE. Yield(mg.)	m.p.	Description.
Benzene.	1,2,3,4.	Nil.	-	-
"	5.	50.	-	Brown Gum.
"	6.	30.	-	Yellow-green gum.
"	7.	28.	-	Green Gum.
"	8.	50.	-	Brown Gum.
"	9.	150.	-	" "
"	10.	4230.	147-150°(S.105-148°).	Brown Solid.
"	11.	5050.	135-137°(S.125°).	White Solid.
"	12.	5000.	148-149°(S.138-147°).	" "
"	13.	2500.	143-145°(S.141°).	" "
"	14.	3000.	143-145°(S.141°).	" "
"	15.	2642.	143-145°(S.141°).	" "
"	16.	2175.	143-145°(S.141°).	" "
"	17.	1850.	143-145°(S.141°).	" "
"	18.	1770.	143-145°(S.141°).	" "
"	19.	1601.	144-147°(S.142°).	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
Benzene.	20.	1216.	143-145°(S.140°).	White Solid.
"	21.	650.	142-144°(S.140°).	" "
"	22.	350.	139-140°(S.136°).	" "
"	23.	130.	150-152°(S.140-50°).	" "
"	24.	100.	161-163°(S.120-158°).	" "
"	25.	60.	157-8°(S.150°).	" "
"	26.	42.	162-165°(S.158°).	" "
"	27.	346.	155-8°(S.150°).	" "
"	28.	30.	154-155°(darks.slight). S.150°	" "
"	29.	29.	187°(S.145-186°).	" "
"	30.	19.	150-151°(S.145°).	" "
"	31.	31.	168°(S.120-165°).	" "
"	32.	33.	144-50°(S.143°)C.210°.	" "
"	33.	29.	168°(S.135-165°).	" "

Eluent.	Fraction No.	E L U A T E.		
		Yield(mg.)	m,p.	Description.
Benzene	34.	22.	150-165°.	White Solid.
"	35.	32.	150-165°(S.135°)C.170°.	" "
"	36.	24.	155-160°(S.135°)C.165°.	" "
"	37.	27.	170-200°(S.120°).	" "
"	38.	36.	146-151°(S.135°)C.160°.	" "
"	39.	29.	150-7°(S.135°)C.158°.	" "
"	40.	48.	155-160°(S.135°)C.162°.	" "
Benzene 99%. Ether 1%.	41.	49.	155-157°(S.145°).	" "
" "	42.	49.	143-146°(S.135°).	" "
" "	43.	50.	150-3°(S.149°).	" "
" "	44.	59.	150-3°(S.149°).	" "
" "	45.	73.	151-3°(S.135°).	" "
" "	46.	52.	146-151°(S.135°).	" "
Benzene 99%. Ether 1%	47.	54.	150-152°(S.135°).	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
Benzene 95%; Ether 5%.	48.	50.	145-148°(S.135°).	White Solid.
" "	49.	83.	150-155°(S.140°).	" "
" "	50.	190.	158-162°(S.130°)C.174°.	" "
" "	51.	170.	155-160°(S.135°)C.165°.	" "
" "	52.	166.	150-172°(S.145°).	" "
" "	53.	120.	155-160°(S.135°)C.167°.	" "
" "	54.	161.	145-165°(S.135°).	" "
" "	55.	119.	155-170°(S.135°).	" "
" "	56.	124.	158-163°(S.130°)C.171°.	" "
" "	57.	100.	159-165°(S.145°)C.174°.	" "
" "	58.	144.	160-165°(S.145°)C.172°.	" "
" "	59.	108.	163-172°(S.135°)C.178°.	" "
" "	60.	100.	165-172°(S.150°)C.175°.	" "
" "	61.	100.	164-174°(S.150°)C.180°.	" "
" "	62.	103.	168-174°(S.150°)C.180°.	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
Benzene 95%; Ether 5%.	63.	100.	165-168°(S.145°)C.175°.	White Solid.
B.95%;E.5%.	64.	100.	164-174°(S.150°)C.180°.	" "
" "	65.	79.	163-168°(S.150°)C.175°.	" "
" "	66.	121.	178-9°(S.158°)C.180°.	" "
" "	67.	75.	170-2°(S.160°)C.172°.	" "
" "	68.	74.	170-175°(S.160°)C.175°.	" "
" "	69.	70.	168-175°(S.160°)C.175°.	" "
" "	70.	422.	175-179°(S.170°)C.179°.	" "
" "	71.	930.	182-185°(S.175°)C.196°.	" "
" "	72.	820.	183-187°(S.175°)C.212°.	" "
" "	73.	755.	184-198°(S.180°)C.215°.	" "
" "	74.	407.	185-210°(S.180°)C.220°.	" "
B.75%;E.25%.	75.	72.	183-4°(S.172°)C.205°.	" "
" "	76.	359.	208-228°(S.185°)C.235°.	" "

Eluent.	Fraction No.	ELUATE		
		Yield(mg.)	m.p.	Description.
B.75%;E.25%.	77.	396.	184-187°(S.180°)C.212°.	White Solid
" "	78.	426.	183-185°(S.178°)C.215°.	" "
" "	79.	277.	180-187°(S.175°)C.200°.	" "
" "	80.	97.	176-182°(S.165°)C.190°.	" "
" "	81.	283.	182-220°(S.170°)C.227°.	" "
" "	82.	381.	227-232°(S.195°)C.238°.	" "
" "	83.	356.	218-230°(S.210°)C.240°.	" "
" "	84.	103.	182-192 (S.175°)C.225°.	" "
" "	85.	169.	179-195°(S.165°)C.215°.	" "
" "	86.	350.	183-205°(S.165°)C.222°.	" "
" "	87.	217.	185-205°(S.165°)C.222°.	" "
B.50%;E.50%.	88.	0,110.	169-175°(S.150°)C.210°.	" "
" "	89.	0,165.	162-165°(S.145°)C.208°.	" "
" "	90.	0,342.	158-175°(S.145°)C.196°.	" "
" "	91.	0,313.	152-160°(S.100°)C.165°.	" "
" "	92.	0,291.	147-170°(S.90°)C.182°.	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
B.50%;E.50%.	93.	0,200.	95-130°(S.85°).	White Solid.
" "	94.	0,151.	95-120°(S.85°).	" "
" "	95.	0,184.	85-90°(S.80°).C.140°.	" "
" "	96.	0,125.	85-90°(S.80°).	" "
" "	97.	0,093.	-	-
" "	98.	0,135.	82-90°(S.65°).	White Solid.
" "	99.	0,152.	-	Brown Gum.
" "	100.	0,112.	-	" "
E.75%B.25%.	101.	0,055.	-	Clear Gum.
" "	102.	0,091.	-	" "
" "	103.	0,052.	-	" "
" "	104.	27.	-	Gum.
" "	105.	21.	-	Brown Gum.
" "	106.	114.	-	" "
" "	107.	30.	-	Clear Gum.

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
E.	108.	40.	-	Clear Gum.
"	109.	60.	-	" "
"	110.	62.	-	" "
"	111.	57.	-	" "
B.49;E.49; M.1;Chl.1%.	112.	69.	88-96°(S.70°)C.100°.	White Solid.
"	113.	28.	98-128°(S.95°)C.128°.	Grey Solid.
"	114.	28.	-	Clear Gum.
"	115.	38.	-	" "
"	116.	234.	-	Green Gum.
"	117.	272.	-	" "
"	118.	50.	-	Clear Gum.
"	119.	19.	-	" "
"	120.	16.	-	" "
"	121.	35.	-	" "
"	122.	71.	-	" "
B.45;E.45% M.5%;Chl.5%.	123.	52.	85-90°(S.78°)C.120°.	White Solid.

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
	124.	120.	70-92°(S.45°)C.92°.	White Solid.
"	125.	221.	65-72°(S.45°)C.72°.	" "
"	126.	92.	-	Gum.
"	127.	43.	-	Gum.
"	128.	114.	75-95°(S.65°)C.105°.	White Solid.
"	129.	293.	85-95°(S.65°)C.95°.	" "
"	130.	2632.	85-130°(S.72°(C.130°.	Yellow Solid
"	131.	3000.	95-105°(S.70°)C.130°.	" "
"	132.	56.	95-100°(S.70°)C.100°.	White Solid.
"	133.	466.	105-120°(S.70°)C.120°.	" "
"	134.	76.	92-100°(S.80°)C.100°.	" "
"	135.	526.	92-100°(S.80°)C.100°.	" "
"	136.	1276.	92-120°(S.70°)C.120°.	" "
"	137.	172.	95-100°(S.88°)C.100°.	" "
"	138.	94.	95-105°(S.60°)C.117°.	" "
"	139.	102.	95-105°(S.60°)C.120°.	" "

Eluent.	Fraction No.	E L U A T E.		
		Yield(mg.)	m.p.	Description.
	140.	470.	107-110°(S.95°)C.145°.	White Solid.
"	141.	200.	107-149°(S.92°)C.149°.	" "
"	142.	76.	130-146°(S.100°)C.146°.	" "
"	143.	150.	131-144°(S.100°)C.144°.	" "
B.45%;E.45%; M.5%;Chl.5%.	144.	670.	154-160°(S.95°)C.160°.	" "
" "	145.	423.	155-159°(S.130°)C.159°.	" "
" "	146.	233.	155-160°(S.130°)C.160°.	" "
" "	147.	259.	155-163°(S.135°)C.163°.	" "
" "	148.	301.	157-169°(S.135°)C.179°.	" "
" "	149.	280.	165-178°(S.155°)C.178°.	" "
" "	150.	195.	160-167°(S.130°)C.167°.	" "
" "	151.	167.	170-176°(S.165°)C.176°.	" "
" "	152.	146.	170-178°(S.165°)C.178°.	" "
" "	153.	149.	183-187°(S.165°)C.187°.	" "
" "	154.	186.	182-184°(S.165°)C.184°.	" "
" "	155.	570.	175-177°(S.165°)C.177°.	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
B.45%;E.45%; M.5%;Chl.5%.	156.	652.	189-192°(S.170°)C.192°.	White Solid.
" "	157.	201.	190-192°(S.172°)C.192°.	" "
" "	158.	114.	181-187°(S.172°)C.187°.	" "
" "	159.	185.	192-196°(S.175°)C.196°.	" "
" "	160.	239.	194-196°(S.175°)C.197°.	" "
" "	161.	122.	195-199°(S.175°)C.199°.	" "
" "	162.	45.	178-187°(S.175°)C.190°	" "
" "	163.	78.	185-187°(S.165°)C.187°.	" "
" "	164.	150.	185-188°(S.170°)C.188°.	" "
" "	165.	95.	200-203°(S.190°)C.203°.	" "
" "	166.	100.	192-197°(S.183°)C.197°.	" "
" "	167.	220.	198-203°(S.190°)C.203°.	" "
" "	168.	207.	201-204°(S.195°)C.204°.	" "
" "	169.	147.	200-205°(S.195°)C.205°.	" "
" "	170.	108.	200-203°(S.195°)C.205°.	" "
" "	171.	69.	190-198°(S.185°).C.198°.	" "
" "	172.	45.	190-198°(S.183°)C.198°.	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
B.45%;E.45%; M.5%;Chl.5%.	173.	27.	190-196°(S.175°)C.200°.	White Solid.
" "	174.	79.	194-196°(S.175°)C.200°.	" "
B.40%;E.40%; M.10%;Chl.10%.	175.	35.	190-195°(S.176°)C.198°.	" "
" "	176.	75.	175-182°(S.170°)C.182°.	" "
" "	177.	206.	175-182°(S.170°)C.182°.	" "
" "	178.	373.	170-178°(S.165°)C.178°.	" "
" "	179.	218.	170-174°(S.160°)C.174°.	" "
" "	180.	104.	150-155°(S.110°)C.155°.	" "
" "	181.	38.	130-135°(S.110°)C.135°.	" "
" "	182.	29.	-	Gum.
" "	183.	52.	130-135°(S.130°)C.142°.	White Solid.
" "	184.	76.	132-135°(S.120°)C.142°.	Yellow Solid.
" "	185.	60.	115-120°(S.90°)C.124°.	" "
" "	186.	52.	112-115°(S.108°)C.124°.	" "
" "	187.	212.	155-160°(S.80°)C.160°.	" "
" "	188.	400.	150-160°(S.110°)C.160°.	" "
" "	189.	188.	120-156°(S.118°)C.200°.	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
B.40%;E.40%; M.10%;Chl.10%.	190.	231.	156-158°(S.140°)C.214°.	Yellow Solid.
" "	191.	222.	182-183°(S.110°)C.186°.	" "
" "	192.	202.	182-185°(S.130°)C.190°.	" "
" "	193.	154.	185-186°(S.182°)C.190°.	" "
" "	194.	60.	186-190°(S.182°)C.192°.	" "
" "	195.	95.	185-190°(S.182°)C.190°.	" "
" "	196.	151.	190-193°(S.182°)C.196°.	" "
" "	197.	150.	192-194°(S.182°)C.194°.	" "
" "	198.	123.	196-202°(S.182°)C.204°.	" "
" "	199.	92.	192-196°(S.190°)C.197°.	" "
" "	200.	43.	184-186°(S.182°)C.188°.	" "
" "	201.	12.	184-186°(S.182°)C.188°.	" "
" "	202.	44.	184-186°(S.182°)C.188°.	" "
Methanol.	203.	31.	196-198°(S.182°)C.198°.	" "
"	204.	264.	196-200°(S.182°)C.200°.	" "
"	205.	720.	175-180°(S.160°)C.180°.	Brown Solid.
"	206.	128.	182-184 (S.160)C.185 .	" "

Eluent.	Fraction No.	ELUATE.		
		Yield(mg.)	m.p.	Description.
Methanol.	207.	74.	186-187°(S.170°)C.187°.	Brown Solid.
"	208.	28.	180-200°(S.170°)C.200°.	" "
"	209.	9.	173-175°(S.160°)C.177°.	" "
"	210.	80.	173-180°(S.140°)C.180°.	" "

Abbreviations in above table: B. = Benzene; E. = Ether; Chl. = Chloroform; M.=Methanol; S. = softens; C. = clears.

The above fractions were bulked into twelve groups based on order of elution and sub-fractional mixed m.p's. The results are tabulated below:

Fraction.	Yield(g.).	Components (Column).	m.p.
I	35.12	10/54	135-157°
II	1.248	55/70	174-8° S.155°
III	2.886	70/74	185-208°

Cont'd...

Fraction.	Yield(g.).	Components (Column).	m.p.
IV	1.25	75/78	185-215°
V	1.19	80/89	178-185° S.152° C.212°
V1	1.74	90/92	158-9° S.154° C.161°
V11	12.63	93/141	150-160°
V111	1.73	142/150	155-176°
IX	2.01	151/158	171-185°
X	2.690	159/179	180-202°
X1	1.670	180/190	150-8°
X11	2.680	191/210	167-205°

Fractions 1-9 (inclusive) on evaporation yielded a dark brown gum (310mg.). This material could not be crystallised and was not further examined.

Fraction I (Nos. 10/54 inc.)

The sub-fractions were combined and the solution was evaporated to dryness and the residue (35.31g., m.p. 135-157°) dissolved in methanol (150 c.c.) and the solution concentrated until crystals appeared. The mixture was filtered and the residue (29.2g., m.p.147-8°) shown to be cholesterol (m.p. 147-8°). The mother liquor on concentration yielded a further 4.3g. cholesterol, m.p. 146-8°.

The final mother liquor yielded no crystalline material on further concentration and the solution was evaporated under reduced pressure and methanol was replaced with petroleum (60/80) by two successive distillations under reduced pressure. The amorphous residue (0.91g., m.p. 150-161°) was dissolved in dry petroleum (60/80 : 100 c.c.) and the solution chomatographed on alumina (Grade II; 50g.).

Eluent.	Volume.	Fraction No.	Eluate
Petrol.	1000 c.c.	1	252mg.,m.p.145-9°. crystalline.
Petrol./ benzene (50%)	500 c.c.	2	30mg.,m.p.137-143°. crystalline.
Benzene	200 c.c.	3	10mg.,m.p.132-138°. crystalline.
Ether	1000 c.c.	4	583mg.,m.p.152-162°. amorphous.

Fraction 1.

A specimen of this fraction (m.p. 145-149°) showed no depression on admixture with an authentic specimen of cholesterol (m.p. 146-8°).

Fraction 2.

After two crystallisations from methanol yielded 12mg. plates, m.p.146-147°, a specimen of which was undepressed on admixture with an authentic specimen of cholesterol (m.p. 147-148°). The mother liquors were not further examined.

Fraction 3.

This material was not further examined.

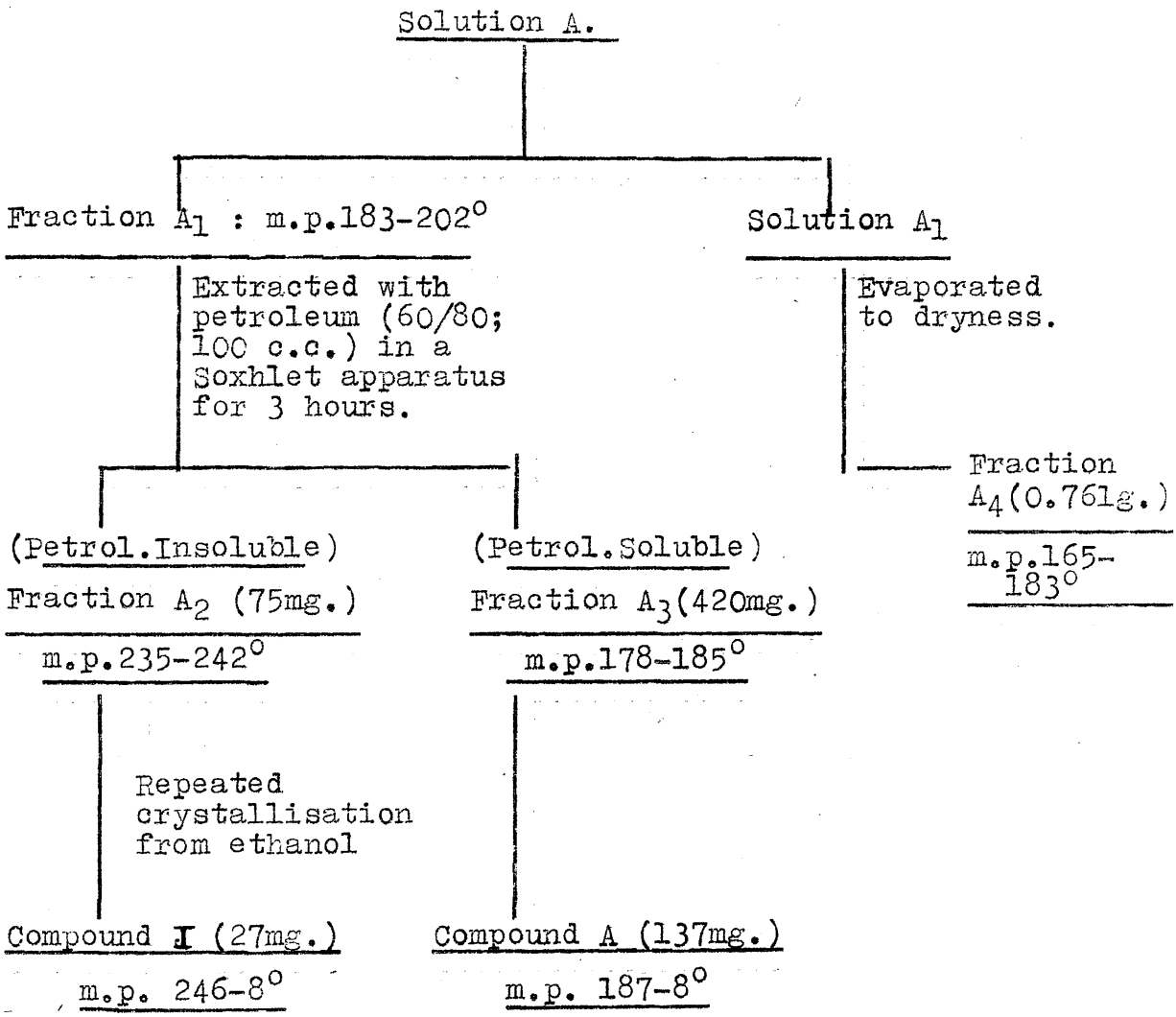
Fraction 4.

A specimen showed no absorption in the ultraviolet (220-400mu). This fraction was not further examined.

Fraction II (Nos.55/70)

The sub-fractions were combined and the solvent removed under reduced pressure, the dried residue (1.24g., m.p.174-8°, softening 158°) dissolved in dry acetone (50 c.c.) and the solution (Solution A) allowed to stand at room temperature for 1 hour.

The fractionation procedure is shown below.



Fraction A_2

The material (75mg., m.p. 235-242°) was crystallised three times from ethanol and afforded Compound I , as needles, m.p. 246-8°. Yield = 27mg.

$[\alpha]_D = -38.8°$ (ℓ =1, c = 0.31 in chloroform).

Found: C = 72.96%; H = 9.86%.

$C_{23}H_{40}O_4$ requires: C = 72.7% ; H = 10.5%.

A specimen of Comound I gave no colouration with tetranitromethane. Compound I showed no absorption in the ultraviolet (220 to 400mu).

The crude product (m.p. 230-238°) obtained by evaporation of the mother liquors was dried (yield = 37mg.) and heated on the boiling water bath for 4 hours with a mixture of pyridine (½ c.c.) and acetic anhydride (2 c.c.). The mixture was cooled and excess solvent removed under reduced pressure and the residue (52mg.) crystallised three times from methanol.

Compound I acetate crystallised as needles, m.p. 218-220° (yield = 10mg.).

$[\alpha]_D$ = - 43.9° (ℓ = 1, c = 0.365 in chloroform).

Found: C = 68.8%; H = 9.5%.

$C_{29}H_{46}O_7$ requires: C = 68.9%; H = 9.1%.

Fraction A_3

The solid (420mg., m.p. 178-185°) after five crystallisations from dry petroleum (b.p. 60/80) afforded Compound A as clusters of fine needles. Yield = 137mg., m.p. 187-8°.

$[\alpha]_D = -24.1^\circ$ ($l = 1$, C = 1.01 in chloroform).

Found: C = 75.5%; H = 10.5%.

C = 75.3%; H = 10.5%.

$C_{22}H_{36}O_3$ requires: C = 75.8%; H = 10.4%.

The substance showed no absorption in the ultraviolet (220 to 400mu). A specimen of the material gave no colouration with tetranitromethane.

The mother liquors were concentrated and the residue, after drying (215mg., m.p. 181-6°) was treated with pyridine (2 c.c.) and acetic anhydride (6 c.c.) and the mixture allowed to stand overnight at room temperature. The solution was decomposed with water, the residue filtered, washed and dried. Yield = 232mg., m.p. 159-168°.

After four crystallisations from methanol Compound A diacetate formed clusters of needles, m.p. 173-4°.
Yield = 92mg.

$[\alpha]_D = -43.1^\circ$ ($l = 1$, C = 0.59 in chloroform).

Found: C = 72.7%; H = 9.5%.

C = 72.5%; H = 9.3%.

$C_{26}H_{40}O_5$ requires: C = 72.2%; H = 9.3%.

% Acetyl found: 20.3%.

Compound A diacetate requires, % Acetyl, 21%.

A specimen of Compound A diacetate showed no absorption in the ultraviolet (220 to 400mu).

Fraction A_4

The dried solid (761mg., m.p. 165-183°) was dissolved in carbon tetrachloride (200 c.c.) and the solution was chromatographed on alumina (Grade II, 30g.).

Eluent	Volume.	Fraction No.	Eluate.
CCl_4	400 c.c.	(i)	Nil.
CCl_4/MeOH(1%)	200 c.c.	(ii)	Nil.
CCl /MeOH(50%)	50 c.c.	(iii)	Nil.
" / "	20 c.c.	(iv)	420mg.m.p.155-165, crystalline.
" / "	100 c.c.	(v)	260mg.,m.p.190-191, crystalline.
" / "	100 c.c.	(vi)	98mg.,m.p.205, crystalline.
Methanol	200 c.c.	(vii)	45mg.,m.p.161-175, amorphous.

Fraction (iv).

Five crystallisations from acetone gave colourless plates of Compound F, m.p. 191-3°, (Yield = 90mg.).

$[\alpha]_D$ = - 32.3° (l = 1, C = 0.124 in chloroform).

Found: C = 74.4%; H = 10.6%.

$C_{20}H_{34}O_3$, requires: C = 74.5%; H = 10.6%.

Compound F showed no absorption in the ultraviolet (220 to 400mu). A specimen of Compound F gave no colouration with tetranitromethane.

To Compound F (53mg., m.p. 191-3°) was added pyridine

(1 c.c.) and acetic anhydride (3 c.c.) and the mixture allowed to stand at room temperature for 24 hours. The mixture was decomposed with water and the residue collected, washed and dried. After three crystallisations from methanol, Compound F acetate, m.p. 171-2°, was obtained as needles (Yield = 16mg.).

$[\alpha]_D$ = - 16° (l = 1, C = 1.83 in chloroform).

Found: C = 71.2%; H = 8.9%.

$C_{24}H_{38}O_5$, requires: C = 71.0%; H = 9.3%.

% Acetyl found = 21.0%.

Compound F diacetate requires, % acetyl = 21.3%.

A specimen of Compound F diacetate showed no absorption in the ultraviolet (220 to 400mu).

Fraction (v).

After several crystallisations from acetone afforded a further quantity of Compound F, m.p. 191-193°, (Yield = 52mg.).

$[\alpha]_D$ = - 33.1° (l = 1, C = 0.157 in chloroform).

A specimen of this material was undepressed on admixture with an authentic specimen of Compound F (m.p. 191-3°).

The mother liquors from fractions (iv) and (v) were combined and the dried residue (510mg.) acetylated with pyridine (3 c.c.) and acetic anhydride (7 c.c.) for 24 hours at room temperature. The reaction mixture was decomposed with water and the crude acetates collected, washed and dried (Yield = 530mg., m.p. 152-163°).

The residue was dissolved in dry benzene (250 c.c.) and the solution was chromatographed on alumina (Grade II, 50g.).

Eluent.	Volume.	Fraction.	Eluate.
Benzene	250 c.c.	a.	200mg., m.p.165-170°
Benzene/MeOH(10%)	350 c.c.	b.	185mg., m.p.155-159°
Methanol	400 c.c.	c.	71mg., m.p.169-171°

Fraction (a).

After four crystallisations from methanol yielded Compound A acetate, m.p. 173-4°, as needles (Yield = 73mg.).

$[\alpha]_D = -41.8^\circ$ ($l = 1$, C = .151 in chloroform).

A specimen of this product was undepressed on admixture with an authentic specimen of Compound A acetate, m.p. 173-4°.

Fraction (b).

After three crystallisations from methanol, Compound F acetate, m.p. 171-2°, was obtained as needles (Yield = 33mg.).

$[\alpha]_D = -17.4^\circ$ ($l = 1$, C = .620 in chloroform).

A specimen of this material was undepressed on admixture with a specimen of Compound F acetate, m.p.171-2°.

Fraction (c).

After one crystallisation from methanol afforded a further quantity of Compound F acetate, m.p. 171-2°(51mg.) a specimen of which was undepressed on admixture with an authentic specimen of Compound F acetate, m.p. 171-2°.

Fraction (vi).

The material (98mg., m.p. 205°) after four crystallisations from methanol afforded Compound I, m.p. 246-8°, as needles. (Yield = 10mg.).

$[\alpha]_D$ = - 38.1° (ℓ = 1, C = .214 in chloroform).

A specimen of this compound showed no depression on admixture with an authentic specimen of Compound I, m.p. 246-8°.

Fraction (vii).

This fraction was not further examined.

Fraction III (No. 70/74).

The sub-fractions were combined and the solvent removed under reduced pressure and the dried residue (2.89g., m.p. 185-208°) extracted with petroleum (b.p.60/80; 350 c.c.) over a period of 72 hours in a Soxhlet apparatus. The petroleum solution was evaporated to dryness under reduced pressure and the residue (Fraction IIIa) was dried. (Yield = 2.2g., m.p. 182-7°). The petroleum insoluble fraction (Fraction IIIb) was dried. (Yield = 0.793g., m.p. 250-259°).

Fraction IIIa.

The material, after four crystallisations from petroleum afforded Compound A, m.p.187-8° as needles. (Yield = 1.21g.)

$[\alpha]_D$ = -21.5° (ℓ = 1, C = 2.41 in chloroform)

A specimen of this material was undepressed on admixture with an authentic specimen of Compound A, m.p. 187-8°.

The mother liquors were evaporated to dryness under reduced pressure and the dried residue (900mg., m.p.181-5°) was acetylated with pyridine/acetic anhydride (4 c.c./10 c.c.) for 24 hours at room temperature. The dried, crude acetate (1.2g., m.p. 155-168°)after four crystallisations from methanol yielded Compound A acetate, m.p. 173-4°, (331mg.) as needles.

$[\alpha]_D = -44.2°$ ($l = 1$, C = .353 in chloroform).

Fraction IIIb.

The solid (792mg., m.p. 250-259) was crystallised six times from toluene and gave Compound B, as needles, m.p. 260-261°. (Yield = 85mg.).

$[\alpha]_D = -58.3$ ($l = 1$, C = 2.681 in chloroform).

Found:	C = 74.4% ;	H = 10.0%
	C = 74.04% ;	H = 10.3%
$C_{19}H_{32}O_3$ requires:	C = 74.03% ;	H = 10.3%

Compound B showed no absorption in the ultraviolet (220 to 400mu).

A specimen of Compound B gave no colouration with tetranitromethane.

The mother liquors were evaporated under reduced pressure and the dried residue (430mg., m.p. 249-257°) was refluxed for 6 hours with a mixture of pyridine (4 c.c.) and

acetic anhydride (12 c.c.). The reaction mixture was cooled, decomposed with water and the residue filtered, washed and dried. (Yield = 300mg., m.p.185-200°). After five crystallisations from methanol Compound B acetate, m.p. 223-5° was obtained as needles (Yield = 61mg.).

$[\alpha]_D$ = - 50.7° (ℓ = 1, C = 0.945 in chloroform).

Found: C = 71.9%; H = 9.8%.

$C_{21}H_{34}O_4$, requires: C = 71.9%; H = 10.0%.

Fraction IV (Nos. 75/78).

The dried material (1.25g., m.p. 185-215°) was extracted with petroleum (b.p. 60/80; 300 c.c.) over a period of 36 hours in a Soxhlet apparatus. The petroleum solution was evaporated to dryness under reduced pressure and the residue (Fraction IVa) was dried (Yield = 1.1g., m.p.182-5°). The petroleum insoluble fraction (Fraction IVb) was dried under reduced pressure. (Yield = 0.2g., m.p. 220-232°).

Fraction IVa.

The solid (1.1g., m.p.182-5°) after three crystallisations from petroleum (b.p. 60/80) gave Compound A, as needles, m.p. 187-8°, a specimen of which was undepressed on admixture with an authentic specimen of Compound A, m.p. 187-8°, previously obtained. (Yield = 0.82g.).

Fraction IVb.

This fraction (0.2g., m.p. 220-232°) was combined

with Fraction Vb (q.v).

Fraction V.

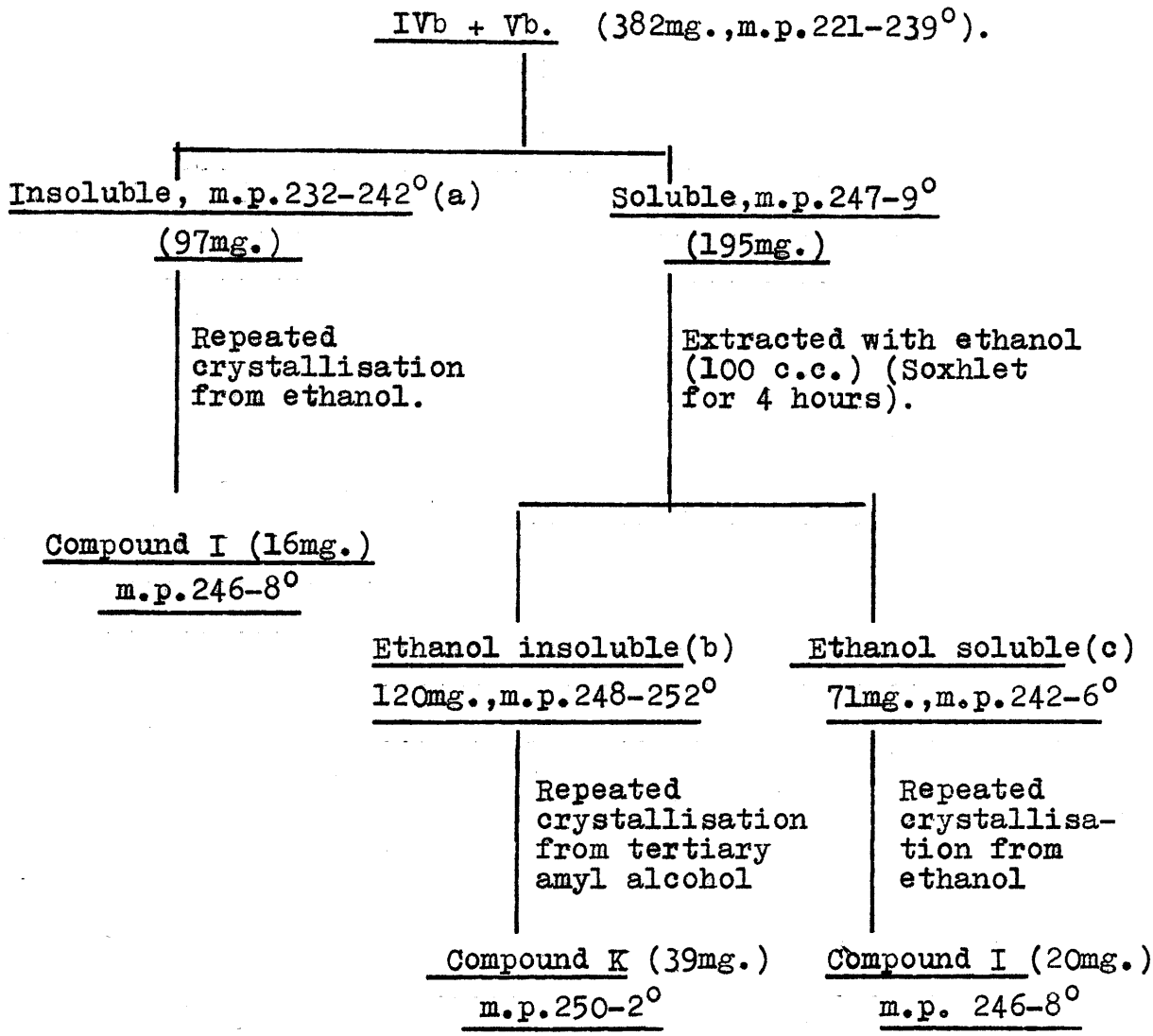
The dried solid (1.19g., m.p. 178-185°) was extracted with dry petroleum (b.p.60/80; 300 c.c.) over a period of 48 hours in a Soxhlet apparatus. The petroleum solution was evaporated to dryness under reduced pressure and the residue (Fraction Va) was dried (Yield = 1.58g.,m.p.181-2°). The petroleum insoluble residue (Fraction Vb) was dried under reduced pressure (Yield = 0.18g., m.p. 223-227°).

Fraction Va.

The dried material (1.58g.) after three crystallisations from petroleum gave Compound A as needles, m.p.187-8°(0.92g.) a specimen of which was undepressed on admixture with an authentic specimen of Compound A, m.p. 187-8°.

Fraction Vb.

The material (0.2g., m.p. 223-237°) was combined with Fraction IVb, (0.2g., m.p.220-232°) and fractionated as described below.



Insoluble (Fraction (a)).

The material (97mg., m.p.232-242°) after four crystallisations from ethanol yielded Compound I, as needles, m.p. 246-8°.* $[\alpha]_D = -38.5°$ (ℓ =1, C = .192 in chloroform).

A specimen of this material was undepressed with admixture with an authentic specimen of Compound I, m.p.246-8°,

*(Yield = 16mg.).

Ethanol Soluble (Fraction (c)).

The solid (71mg., m.p. 242-6°) after three crystallisations from ethanol afforded Compound I, m.p. 246-8°, as needles. (Yield = 20mg.). A specimen of this compound was undepressed on admixture with an authentic specimen of Compound I, m.p. 246-8°.

Ethanol Insoluble (Fraction (b)).

The material (120mg., m.p. 248-252°) was crystallised three times from t-amyl alcohol and gave Compound K, as long, fine, silky needles, m.p. 250-252°. (Yield = 50mg.).

$[\alpha]_D = -71.6°$ ($l = 1$, C = .117 in chloroform).

$[\alpha]_D = -47.3°$ ($l = 1$, C = .343 in pyridine).

Found: C = 75.4%; H = 10.7%.

$C_{21}H_{36}O_3$, requires: C = 75.5%; H = 10.6%.

A specimen of Compound K showed no absorption in the ultraviolet (220 to 400mu) and gave no colouration with tetranitromethane.

The mother liquors were combined and the solvent removed under reduced pressure and the dried residue (34mg., m.p. 245-250°) heated on the boiling water bath for four hours with pyridine (½ c.c.) and acetic anhydride (2 c.c.). After standing 48 hours at room temperature the excess solvent was removed under reduced pressure and the residue crystallised three times from methanol.

Compound K acetate, m.p. 220-4°, was obtained as needles.

Yield = 6mg.

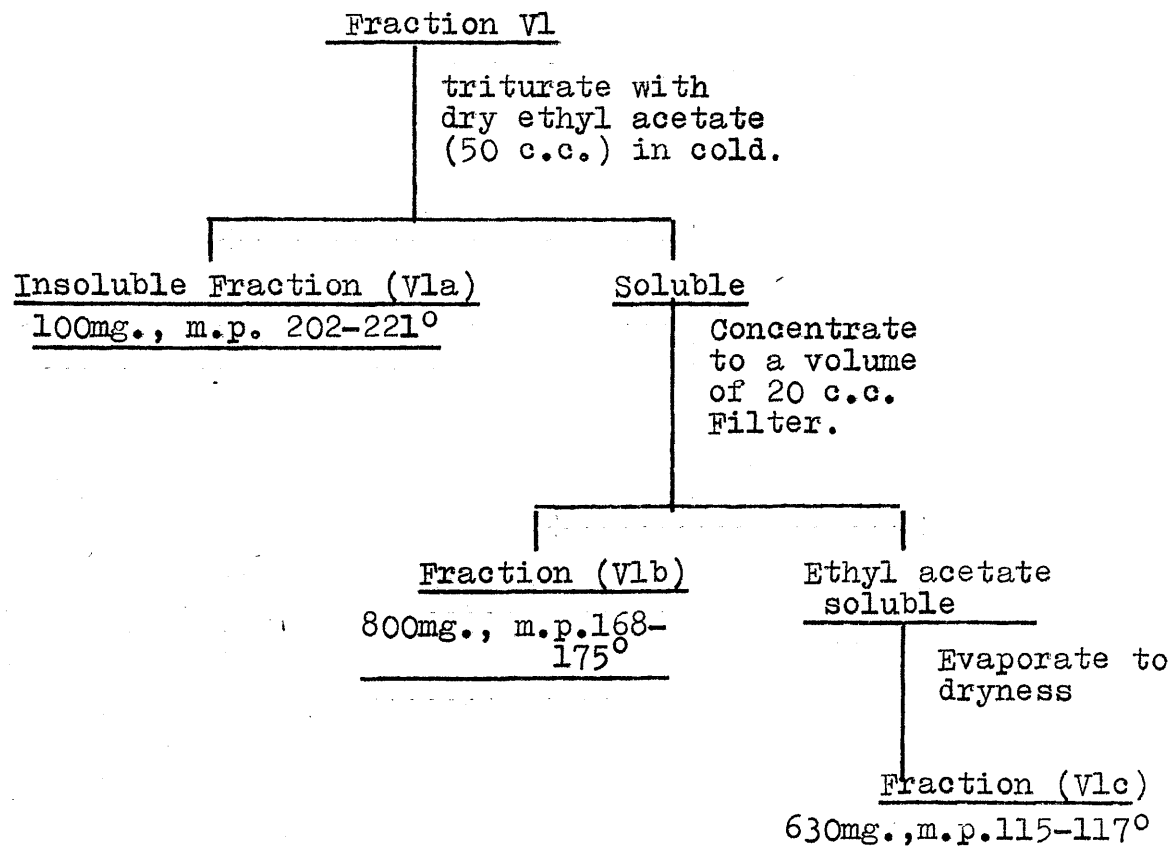
$[\alpha]_D$ = - 50° (l = 1, C = .422 in chloroform).

Found: C = 71.8%; H = 9.7%.

$C_{25}H_{40}O_5$, requires: C = 71.6%; H = 9.5%.

Fraction VL (Nos. 90/92).

The dried solid (1.74g., m.p. 154-161°) was subjected to fractional crystallisation as shown below.



Fraction(V1a).

The solid, (100mg., m.p.202-221°) after six crystallisations from ethanol afforded Compound I, m.p. 246-8°, as needles. Yield = 15mg.

$[\alpha]_D$ = - 39.2° (ℓ = 1, C = .198 in chloroform).

A specimen of this material gave no depression on admixture with a specimen of Compound I, m.p. 246-8°.

Fraction(V1b).

The residue (800mg., m.p.168-175°) after six crystallisations from ethyl acetate afforded Compound A, m.p. 187-9°, as needles. Yield = 120mg.

$[\alpha]_D$ = - 33.9° (ℓ = 1, C = 1.041 in acetone).

$[\alpha]_D$ = - 24.1° (ℓ = 1, C = 0.665 in chloroform).

A specimen of this compound gave no depression on admixture with an authentic specimen of Compound A, m.p. 187-8°.

The mother liquors were combined and the solvent removed under reduced pressure and the dried residue (373mg., m.p. 157-170°) acetylated with pyridine (3 c.c.) and acetic anhydride (10 c.c.) over a period of 24 hours at room temperature. The acetylation mixture was decomposed with water, the crude acetate filtered, washed and dried. Yield = 416mg., m.p. 151-159°. After six crystallisations from methanol, Compound A acetate, m.p. 173-4° (needles), was obtained. (Yield = 82mg.)

$[\alpha]_D$ = - 42.8°(ℓ = 1, C = 0.834 in chloroform).

Fraction(V1c).

The dried amorphous product (630mg., m.p.115-157°) was dissolved in dry benzene (300 c.c.) and the solution was chromatographed on alumina (Grade II, 50g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene.	500 c.c.	1	Nil.
Benzene/MeOH(1%)	500 c.c.	2	Nil.
Benzene/MeOH(10%)	200 c.c.	3	37mg.,m.p. 184-8°, crystalline.
Benzene/MeOH(50%)	450 c.c.	4	230mg., m.p. 131-165°, amorphous.
Methanol.	500 c.c.	5	320mg., m.p. 135-162°, amorphous.

Fraction 3.

After two crystallisations from ethyl acetate afforded a further quantity of Compound A, m.p. 187-9°, as needles. (Yield = 11mg.).

A specimen of this material was undepressed on admixture with an authentic specimen of Compound A, m.p. 187-8°.

Fractions 4/5 were not further examined.

The fraction after drying (12.63g., m.p.150-160°) revealed no crystalline material on microscopic examination.

A quantity of the amorphous product (4.2g.) was dissolved in dry benzene 800 c.c.) and the solution was chromatographed on alumina (Grade II, 100g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene.	3,000 c.c.	1	Nil.
Benzene/ether(1%).	2,000 c.c.	2	Nil.
Benzene/ether(5%).	1,250 c.c.	3	Nil.
Benzene/ether(10%).	1,750 c.c.	4	Nil.
Benzene/ether(20%).	1,500 c.c.	5	Nil.
Benzene/ether(40%).	1,750 c.c.	6	Nil.
Benzene/ether(80%).	4,500 c.c.	7	.356g., gum.
Ether (100%).	3,000 c.c.	8	1.05g.,m.p.123-157°, amorphous.
Ether (100%).	4,000 c.c.	9	.808g.,m.p.127-149°, amorphous.
Ether (100%).	2,000 c.c.	10	.121g., m.p.131-152°, amorphous.
Benzene/MeOH(10%).	500 c.c.	11	2.873g.,m.p.120-161°, amorphous.
Methanol.	1,000 c.c.	12	.208g., m.p.139-153°, amorphous.

Fractions 7, 8, 9, 10, 12 were not further examined.

Fraction 11.

The amorphous solid (2.873g., m.p.131-152°) was dissolved in pyridine (4 c.c.) and acetic anhydride (15 c.c.) added and the mixture warmed 4 hours on the water bath and then allowed to stand overnight at room temperature. The mixture was decomposed with water and the crude acetates taken up in ether (500 c.c.), the ethereal solution washed with water (1 litre), then with dilute hydrochloric acid (10%; 2 litres) and sodium carbonate solution (2%; 1.5 litres) and finally again with water (1 litre). The ethereal solution was dried (Na_2SO_4) and excess solvent removed on the water bath and the ether displaced with benzene by successive distillations under reduced pressure. The benzene-dried residue (3.1g., gum) was redissolved in benzene (600 c.c.) and the solution was chromatographed on

alumina (Grade II, 50g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene.	1,000 c.c.	1	700mg., gum.
Benzene/ether(1%).	1,500 c.c.	2	352mg., gum.
Benzene/ether(20%).	2,000 c.c.	3	571mg., gum.
Benzene/ether(50%).	2,250 c.c.	4	470mg., gum.
Benzene/ether(80%).	3,000 c.c.	5	68mg., gum.
Ether.	2,000 c.c.	6	897mg., gum.

The fractions obtained could not be crystallised from organic or aqueous organic solvents.

These fractions were not further examined.

Fraction(VIII) (Nos.142/150).

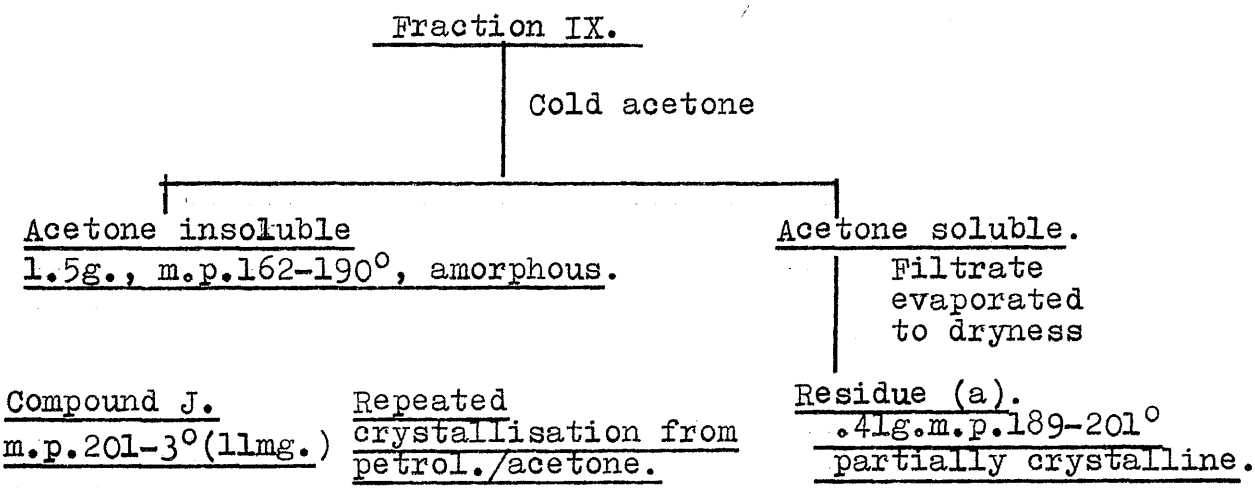
The dried product (1.73g., m.p.155-176°) was dissolved in benzene/chloroform (1%; 500 c.c.) and the solution was chromatographed on alumina (Grade II, 40g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene/$CHCl_3$(1%).	500 c.c.	1	320mg.,m.p.120-130°, amorphous.
" / "	500 c.c.	2	Nil.
Benzene/$CHCl_3$(5%).	1000 c.c.	3	341mg.,m.p.160-172°, amorphous.
" / "	1500 c.c.	4	170mg.,m.p.160-172°, amorphous.
" / "	2000 c.c.	5	16mg.,m.p.160-172°, amorphous.
Benzene/$CHCl_3$(10%).	500 c.c.	6	113mg.,m.p.160-170°, amorphous.
" / "	500 c.c.	7	116mg.,m.p.157-171°, amorphous.
" / "	500 c.c.	8	60mg.,m.p.151-159°, amorphous.
" / "	1000 c.c.	9	9mg.,m.p.143-153°, amorphous.
Benzene/Chl.(50%).	200 c.c.	10.	404mg.,m.p.173-188°, amorphous.
" / "	300 c.c.	11	40mg.,m.p.174-190°, amorphous.
Chloroform.	2000 c.c.	12	112mg.,m.p.180-193°, amorphous.

Fractions 1 to 12 were not further examined.

Fraction (IX) (Nos. 151/158).

The product (amorphous, 2.01g., m.p.171-185°) was triturated with cold acetone (100 c.c.) and the mixture was filtered.



Residue (a).

The product (410mg., m.p. 189-201°) after eight crystallisations from petrol./acetone yielded Compound J, m.p. 201-203°, as prisms. Yield = 11mg.

$[\alpha]_D$ = + 39.9° (l = 1, C = .801 in chloroform).

Found: C = 71.9%; H = 10.4%.

C = 71.7%; H = 10.7%.

$C_{21}H_{37}O_4$, requires: C = 71.4%; H = 10.5%.

A specimen of Compound J showed no absorption in the ultraviolet (220 to 400mu). Compound J gave no colouration

with tetranitromethane.

The amorphous product (312mg., m.p.177-195°) obtained on concentration of the mother liquors was not further examined.

Acetone Insoluble Fraction.

The amorphous solid (1.5g., m.p.162-190°) was dissolved in dry chloroform (500 c.c.) and the solution was chromatographed on alumina (Grade II, 70g.).

Eluent.	Volume.	Fraction No.	Eluate.
Chloroform.	1000 c.c.	1	Nil.
Chloroform/MeOH(1%).	500 c.c.	2	Nil.
Chloroform/MeOH(10%).	1000 c.c.	3	21mg.,m.p.157-189°, amorphous.
Chloroform/MeOH(20%).	1000 c.c.	4	137mg.,m.p.153-178°, amorphous.
Chloroform/MeOH(50%).	2000 c.c.	5	215mg.,m.p.162-189°, amorphous.
" / "	2000 c.c.	6	311mg., m.p.164-190°, amorphous.
Methanol.	300 c.c.	7	476mg.,m.p.162-187°, amorphous.

Fractions 3/7 were not further examined.

Fraction X (Nos.159/179).

The dried material (2.69g., m.p.180-202°) was dissolved in benzene/ether mixture (10% ; 1000 c.c.) and the solution was chromatographed on alumina (Grade II, 50g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene/ether(10%).	2,250 c.c.	1	387mg., gum.
Benzene/ether(20%).	2,100 c.c.	2	20mg., m.p.140-165°, amorphous.
Benzene/ether(40%).	1,750 c.c.	3	28mg.,m.p.150-162°, amorphous.

Eluent.	Volume.	Fraction No.	Eluate.
Benzene/ether(78%).	10,000 c.c.	4	59mg.,m.p.152-175°, amorphous.
Ether.	2,000 c.c.	5	16mg., resin.
Ether/MeOH (1%).	2,250 c.c.	6	325mg.,m.p.198-204°, amorphous.
Ether/MeOH (1%).	8,000 c.c.	7	455mg.,m.p.200-202°, partially crystalline.
Ether/MeOH (5%).	2,000 c.c.	8	1250mg.,m.p.185-190°, partially crystalline.
Ether/methanol (5%).	4,500 c.c.	9	245mg.,m.p.150-161°, amorphous.
Methanol.	1,250 c.c.	10	381mg.,m.p.200-213°, partially crystalline.

Fractions 2, 3, 4, 5, 6 and 9 were not further examined.

Fraction 7.

The product (455mg., m.p.200-2°) after eight crystallisations from petrol./acetone and finally two further crystallisations from acetone afforded Compound D, as cubes, m.p. 202-204°.

Yield = 15mg.

$[\alpha]_D$ = + 23.5° (l = 1, C = 2.497 in chloroform).

Found: C = 72.5%; H = 10.0%.

C = 72.5%; H = 10.5%.

$C_{22}H_{37}O_4$, requires: C = 72.3%; H = 10.2%.

Compound D showed no absorption in the ultraviolet (220 to 400mu). A specimen of Compound D gave no colouration with tetranitromethane.

Liebermann Reaction - blood red colouration (both layers).

The mother liquors were evaporated and the dried, crude amorphous product (250mg., m.p. 191-200°) was acetylated with pyridine (2 c.c.) and acetic anhydride (7 c.c.) for two hours at 100° (water bath) and then allowed to stand at

room temperature for 48 hours. The mixture was decomposed with water, the crude acetates filtered, washed and dried. Yield = 257mg., m.p. 40-72°, amorphous.

The product could not be crystallised from organic solvents. The melting point was not raised by prolonged drying under high vacuum (48 hours at 1 mm. over P_2O_5). This material was not further examined.

Fraction 8.

The product (1.25g., m.p. 185-190°) after 10 crystallisations from petrol./acetone and finally a further two crystallisations from acetone gave colourless prisms of Compound C, m.p. 191-4°. Yield = 27mg.

$[\alpha]_D$ = + 24.3° (l = 1, C = 1.27 in chloroform).

Found: C = 72.9%; H = 10.6%.
C = 72.8%; H = 10.7%.

$C_{24}H_{42}O_4$, requires: C = 73.1%; H = 10.2%.

A specimen of Compound C gave no colouration with tetranitromethane. The compound showed no absorption in the ultraviolet (220 to 400mu).

Liebermann Reaction - Red to Green (both layers).

The mother liquors were evaporated to dryness and the crude, amorphous product (200mg., m.p. 182-190°) was acetylated with pyridine (2 c.c.) and acetic anhydride (7 c.c.) over a period of 48 hours at room temperature. The reaction mixture was decomposed with water, the crude acetates filtered, washed and dried. Yield = 210mg., m.p. 60-72°.

The material could not be crystallised from organic solvents. Prolonged drying (1 mm. pressure, over P_2O_5 at 35°) did not change the melting point. This product was not further examined.

Fraction 10.

The material (381mg., m.p. 200-213°) after five crystallisations from petrol./acetone and a further two crystallisations from acetone afforded Compound E, m.p. 214-216°, as plates (micro). Yield = 17mg.

$[\alpha]_D$ = + 32.9° (l = ½, C = .338 in ethanol).

Found: C = 68.4%; H = 10.0%.
C = 67.8%; H = 10.6%.

$C_{21}H_{38}O_5$, requires: C = 68.1%; H = 10.3%.

A specimen of Compound E showed no absorption in the ultraviolet (220 to 400mu). The compound gave no colouration with tetranitromethane.

The crude product obtained by evaporation of the mother liquors (140mg., m.p. 201-211°) was refluxed with acetic anhydride (3 c.c.) and anhydrous sodium acetate (0.1g.) over a period of 2 hours. The reaction mixture was cooled and decomposed with water. The crude acetate was filtered, washed and dried. Yield = 117mg., m.p. 71-93°. This material could not be resolved by crystallisation. Prolonged drying under vacuum (1 mm.) did not affect the melting point. This material was not further examined.

Fraction X1 (Nos.180/190).

The solid (1.67g., m.p. 150-158°) was dissolved in pure, dry chloroform, (450 c.c.) and the solution was chromatographed on alumina (Grade II, 60g.).

Eluent.	Volume.	Fraction No.	Eluate.
Chloroform.	500 c.c.	1	467mg.,m.p.102-146°, crystalline.
"	500 c.c.	2	250mg., m.p.160-170°, crystalline.
"	1000 c.c.	3	80mg., m.p.168-184° amorphous.
Chloroform/MeOH(10%)	500 c.c.	4	591mg., resin.
Methanol.	1000 c.c.	5	205mg., resin.

Fraction 1.

The product (467mg., m.p.102-146°) after seven crystallisations from petrol./acetone followed by two crystallisations from acetone afforded Compound C, m.p. 191-3°, as prisms. Yield = 10mg.

A specimen of this material was undepressed on admixture with an authentic specimen of Compound C.

The mother liquors were combined and the solvent was removed under reduced pressure and the dried residue, (332mg., m.p. 134-147°), after five crystallisations from benzene/petrol. (b.p.60/80) and finally two crystallisations from petrol./ethanol afforded Compound G, as prisms, m.p. 148-150°. (Yield = 30mg.)

$[\alpha]_D$ = + 28.7° (ℓ = 1, C = .168 in chloroform).

Found: C = 69.1%; H = 10.2%.
C = 69.4%; H = 10.5%.

$C_{22}H_{40}O_5$, requires: C = 68.8%; H = 10.4%.

A specimen of Compound G showed no absorption in the ultraviolet (220 to 400mu). A sample of Compound G gave a yellow colouration with tetranitromethane.

The mother liquors were not further examined.

Fraction 2.

The solid (250mg., m.p. 160-170°) after eight crystallisations from petrol./acetone gave colourless prisms of Compound H, m.p. 181-3°. Yield = 27mg.

$[\alpha]_D$ = + 32.8° (ℓ = 1, C = .730 in chloroform).

Found: C = 69.5%; H = 10.6%.
C = 69.5%; H = 10.6%.

$C_{24}H_{44}O_5$, requires: C = 69.9%; H = 10.4%.

A specimen of Compound H showed no absorption in the ultraviolet (220 to 400mu).

Compound H gave no colouration with tetranitromethane.

The mother liquors were not further examined.

Fraction 3.

The material (80mg., m.p. 168-184°) could not be resolved by crystallisation from petrol., petrol./acetone or petrol./benzene.

This fraction, and fractions 4 and 5 were not further examined.

Fraction XII (Nos. 191/210).

The brown solid (2.68g., m.p. 167-205°) was extracted with dry chloroform (350 c.c.) over a period of 100 hours in a Soxhlet apparatus. The chloroform insoluble fraction (1.6g., m.p. 180-220°) (amorphous), was not further examined.

The chloroform solution was evaporated to dryness under reduced pressure and the residue (930mg., m.p.178-190°, amorphous) was dissolved in dry chloroform (500 c.c.) and the solution was chromatographed on alumina (Grade II, 50g.).

Eluent.	Volume.	Fraction No.	Eluate.
Chloroform.	1,250 c.c.	1	16mg., gum.
Chloroform/MeOH(1%).	1,000 c.c.	2	16mg., gum.
Chloroform/MeOH(2%).	1,000 c.c.	3	25mg., gum.
Chloroform/MeOH(5%).	500 c.c.	4	207mg., m.p.145-180°, amorphous.
" / "	250 c.c.	5	413mg., m.p.170-198°, amorphous.
" / "	500 c.c.	6	30mg., m.p.183-200°, amorphous.
Chloroform/MeOH(10%).	250 c.c.	7	223mg., m.p. 187-212°, amorphous.
Methanol.	1,000 c.c.	8	100mg., m.p.191-215°, amorphous.

These fractions could not be resolved by crystallisation from petrol./acetone or petrol./ethanol.

The fractions were not further examined.

SECTION B.

Compound A Diacetate (X).

To compound A (2.60g., crude, m.p. 180-2°) in dry pyridine (20 c.c.) was added acetic anhydride (dry, distilled, 21 c.c.) and the mixture warmed gently until solution was attained. After standing 24 hours at room temperature, the mixture was decomposed with water (1 litre), the residue filtered, washed with water (1½ litres) and dried. Yield = 2.95g., m.p. 157-160°. After three crystallisations from methanol, Compound A diacetate, m.p. 173-4°, was obtained as needles.

$[\alpha]_D$ = - 43.1°(l = 1, C = 0.59 in chloroform).

Found: C = 72.7% ; H = 9.5%.
C = 72.5% ; H = 9.3%.

$C_{26}H_{40}O_5$, Requires: C = 72.2% ; H = 9.3%.

% Acetyl = 21% (Calcd.).
= 20.3% (Found).

Molecular Weight (Rast), Found, 450, 460, 470.
Calculated for $C_{26}H_{40}O_5$, 432.

A specimen of the diacetate was found to contain no active hydrogen.

To Compound ~~A diacetate~~ (300mg., m.p. 173-4°) in dry pyridine (5 c.c.) was added acetic anhydride (6 c.c.) and the mixture was heated on the boiling water bath for 6 hours. The mixture was cooled, decomposed with water, the residue filtered and dried and crystallised several times from methanol. Compound A diacetate crystallised as needles, m.p.173-4°, a specimen of which was undepressed

on admixture with starting material, m.p. 173-4°.
Yield = 207mg.

Compound A (1X) (X ⟶ IX).

To Compound A diacetate (552mg., m.p. 173-4°) was added methanolic potassium hydroxide (5%; 60 c.c.) and the mixture was refluxed for 3 hours on the water bath. About one third of the volume of the mixture was removed by distillation under reduced pressure, the residue cooled and decomposed with water. The precipitate was filtered, washed and dried. Yield = 420mg., m.p. 182-4°. After two crystallisations from petroleum (b.p. 60/80), Compound A, m.p. 187-8° was obtained as clusters of fine needles. Yield = 320mg.

$[\alpha]_D = -23.2°$ ($l = 1$, C = 2.003 in chloroform).

A specimen of this material was undepressed on admixture with a specimen of Compound A, m.p. 187-8°.

Oxidation of Compound A diacetate.

To the diacetate (500mg., m.p. 173-4°) in stabilised acetic acid (10 c.c.) was added a solution of chromic acid (90mg.) in water (½ c.c.) and stabilised acetic acid (5 c.c.), slowly and with stirring. After standing at room temperature for 24 hours the mixture was warmed at 40° (water bath) over a period of 4 hours, cooled, methanol (2 c.c.) added and the mixture decomposed with water (1 litre).

The precipitate was filtered, washed and dried. Yield = 430mg., m.p. 157-170°.

The solid (430mg., m.p.157-170°) was dissolved in dry benzene (500 c.c.) and the solution was chromatographed on alumina (Grade II; 30g.).

Eluent.	Volume.	Fraction No.	Eluate.
Benzene.	100 c.c.	1	Nil.
"	300 c.c.	2	265mg.,m.p.166-168°.
"	400 c.c.	3	131mg.,m.p.164-167°.
Ether.	200 c.c.	4	35mg., gum.

Fractions 2/3 were combined and crystallised twice from methanol and yielded a solid, m.p. 173-4°, (Yield = 200mg.) undepressed on admixture with an authentic specimen of Compound A diacetate, m.p. 173-4°. Fraction 4 was not further examined.

Treatment of Compound A with Hydrazine Hydrate.

To Compound A (100mg., m.p. 187-188°) in ethanol (95%; 6 c.c.) was added hydrazine hydrate (90%; 1 c.c.) and the mixture was heated in an autoclave over a period of 24 hours at 200°. The cooled mixture was transferred to a flask, the solvent removed under reduced pressure and the residue (131mg.) dissolved in ether (50 c.c.) and the ethereal solution extracted with water (8 x 50 c.c.) and finally dried ($MgSO_4$). The ether was removed by distillation under reduced pressure and the residue (94mg.)

after two crystallisations from petroleum afforded Compound A as needles. Yield = 58mg., m.p. 187-8°. A specimen of this material was undepressed on admixture with an authentic specimen Compound A, m.p. 187-188°.

Treatment of Compound A diacetate with Hydroxylamine Hydrochloride.

To Compound A diacetate (120mg., m.p.173-4°) in ethanol (45 c.c.) was added hydroxylamine hydrochloride (120mg.) and sodium acetate (135mg.) in water (15 c.c.) and the mixture was refluxed for 4 hours. The mixture was cooled and decomposed with water (400 c.c.), the precipitated solid filtered, washed with water and dried. Yield = 112mg., m.p. 162-8°. After two crystallisations from methanol, Compound A diacetate, m.p. 173-4° was obtained as needles. Yield = 81mg.

A specimen of this compound was undepressed on admixture with a specimen of Compound A diacetate, m.p. 173-4°.

Hydrogenation of Compound A diacetate in neutral solution.

To a suspension of platinum catalyst (previously obtained by reduction of platinum oxide (50mg.)) in absolute ethanol (6 c.c.) was added a solution of Compound A diacetate (53mg., m.p.173-4°) in absolute ethanol (5 c.c.) and the mixture was hydrogenated over a period of 5 hours at room temperature. No hydrogen was

absorbed. The catalyst was removed by filtration and the filtrate concentrated to a volume of 3 c.c. and allowed to stand at room temperature for several hours to yield Compound A diacetate, needles, m.p.173-4°. Yield = 42mg.

$[\alpha]_D = -44.7°$ ($l = 1$, C = 1.027 in chloroform).

A specimen of this solid was undepressed on admixture with an authentic specimen of starting material, m.p.173-4°.

Partial Hydrolysis Compound A diacetate (X— X1)

To compound A diacetate (492mg., m.p. 173-4°) in dry methanol (20 c.c.) was added a solution of potassium bicarbonate (4 c.c. satd.soln. $KHCO_3$ 4 c.c. water) and the mixture warmed on the water bath until complete solution was attained (a further 2 c.c. methanol was added). The mixture was allowed to stand at room temperature for 48 hours and water (20 c.c.) was added and the solution was evaporated to dryness under reduced pressure (2mm., water bath temperature). The residue (510mg.) was triturated with water, the suspension filtered and the residue washed with water, then with dilute hydrochloric acid (2%) and finally with water. The dried residue (385mg., m.p. 133-144°) after four crystallisations from methyl alcohol gave Compound A monoacetate, m.p. 90-91°, (needles from aqueous methanol). Yield = 203mg.

$[\alpha]_D = -19.3^\circ$ ($l = 1$, C = .337 in chloroform).

Found: C = 71.9% ; H = 9.7%.

$C_{24}H_{40}O_4 \cdot \frac{1}{2}H_2O$, requires: C = 71.3% ; H = 10.4%.

Oxidation of Compound A Monoacetate.

To Compound A monoacetate (186mg., m.p. 90-1°) in glacial acetic acid (5 c.c.) was added a solution of chromic acid (60mg.) in water (1 c.c.) and glacial acetic acid (2 c.c.) and the mixture was allowed to stand at room temperature for 24 hours and then warmed for 3 hours at 40° (water bath). The mixture was cooled, poured into water (200 c.c.) and the residue collected, washed with water and dried. Yield = 80mg., m.p. 140-6°. After four crystallisations from aqueous methanol, Compound IV was obtained as needles, m.p. 152-3°. Yield = 15mg.

$[\alpha]_D = -34.3^\circ$ ($l = 1$, c = 0.291 in chloroform).

Found: C = 70.9% ; H = 9.4%.

$C_{24}H_{36}O_4 \cdot H_2O$, requires: C = 70.5% ; H = 9.4%.

Hydrogenation of Compound A diacetate in acid solution (X ⟶ XV).

The catalyst (54mg., PtO_2) was suspended in glacial acetic acid and was reduced in an atmosphere of hydrogen over a period of four hours, (absorption = 18.6 c.c.). To the suspension was added a solution of Compound A diacetate (100mg., m.p.173-4°) in glacial acetic acid (5 c.c.) and

the mixture was hydrogenated at room temperature until hydrogen absorption had ceased (absorption = 9 c.c.). The acetic acid solution was filtered and the solvent was removed under reduced pressure (2mm., water bath) and the residue was extracted with water (3 x 20 c.c.) then with sodium bicarbonate solution (10%; 6 x 25 c.c.) and finally again with water (5 x 15 c.c.) and the ethereal solution dried ($MgSO_4$). Removal of solvent yielded a syrupy residue, m.p. 52-120° (92mg.), which after four crystallisations from petroleum (b.p. 60/80) gave Compound VI as needles, m.p. 113-114°.

Yield = 22mg.

$[\alpha]_D$ = + 19.8° (ℓ = 1, C = 0.456 in chloroform).

Found: C = 71.2% ; H = 10.4%.

$C_{26}H_{42}O_5$, requires: C = 71.6% ; H = 10.1%.

Oxidation of Compound A (IX ⟶ XIII).

To Compound A (730mg., m.p. 187-8°) in stabilised acetic acid (15 c.c.) was added a solution of chromic acid (425mg., 1.1 mols O_2) in water (2 c.c.) and acetic acid (8 c.c.). The mixture was allowed to stand at room temperature for 24 hours and then warmed for 3 hours at 50° (water bath), cooled to room temperature and decomposed with water. The aqueous solution was extracted with ether (5 x 100 c.c.) and the ethereal

solution was washed with water (10 x 50 c.c.), sodium carbonate solution (5%; 10 x 40 c.c.) and finally with water. The ethereal layer was dried ($MgSO_4$). The ethereal solution on evaporation yielded 127 mg., gum from which no further crystalline material could be obtained.

The combined aqueous and alkaline extracts were acidified with concentrated hydrochloric acid and the solution allowed to stand overnight at room temperature. The precipitate was filtered, washed with water and dried. Yield = 130mg., m.p. 182-7°. The material, after five crystallisations from aqueous acetone, afforded Compound VI as prisms, m.p. 193-4°. Yield = 20mg.

$[\alpha]_D = -34.7°$ ($\ell = 1$, C = 1.24 in ethanol).

Found: C = 72.6% ; H = 8.6%.

C = 72.5% ; H = 9.6%.

$C_{22}H_{32}O_4$, requires: C = 73.1% ; H = 8.8%.

Clemensen Reduction of Acid Product (XIII ⟶ XIV).

To granulated zinc (4g.) was added a solution of mercuric chloride (0.5g.) in water (10 c.c.) and the mixture was agitated vigorously for 10 minutes. When the zinc surface had assumed a dark grey colour, the mercuric chloride solution was decanted off and a solution of Compound VI (crude, 70mg., m.p. 187-194°) in glacial acetic acid (5 c.c.) and concentrated hydrochloric acid was added and the mixture refluxed on the oil bath over

a period of 3 hours. On cooling, the solution was filtered, the filtrate evaporated to dryness (oil bath, vac. 10mm.) and the residue dissolved in ether (100 c.c.). The ethereal layer was extracted with sodium carbonate solution (10%; 5 x 20 c.c.), the alkaline extracts bulked and acidified with concentrated hydrochloric acid. The precipitate was filtered, washed and dried.
Yield = 30 mg., indefinite m.p. - softens 90°, begins to froth 150° - not completely melted at 360°. The material could not be purified.

B I B L I O G R A P H Y

BIBLIOGRAPHY

1. Fieser and Fieser, "Natural Products Related to Phenanthrene" Reinhold, N.Y. 1949.
2. Gilman, Organic Chemistry, Vol.II (John Wieley & Sons, N.Y., 1943)
3. Shoppee, Ann.Reports, 1946, 43, 200.
4. Shoppee, Ann.Reports, 1947, 44, 170.
5. Wieland and Weil, Z.physiol. Chem., 1912, 80, 287.
6. White, Biochem.J., 1929, 23, 165.
7. Wieland, Kapitel, Z.physiol. Chem., 1932, 212, 269.
8. Letsche, Z.physiol. Chem., 1909, 60, 462.
9. Cortese and Baumann, J.Amer.Chem.Soc., 1935, 57, 1393.
10. Kazuno and Jamasaki, Z.physiol. Chem., 1934, 224, 160.
11. Pregl and Buchtala, Z.physiol. Chem., 1911, 74, 198.
12. Schryver, J.Physiol., 1912, 44, 265.
13. Main, Biochem. J., 1917, 11, 11.
14. Wieland, Z.physiol.Chem., 1919, 106, 181.
15. Wieland and Reverey, ibid., 1924, 140, 186.
16. Wieland and Jacobi, ibid., 1925, 148, 232.
17. Wieland and Kishi, ibid., 1933, 214, 47.
18. Windaus, Bohne and Schwarzkopf, ibid., 1924, 140, 177.
19. Fischer, ibid., 1911, 73, 204.
20. Wieland and Weyland, ibid., 1920, 110, 123.
21. de Founcroy, Ann.Chim., 1779, 3, 242.

22. Chevreul, ibid., 1815, 95, 1.

23. Planer, Ann., 1861, 118, 25.

24. Wislicenus and Moldenhauer, ibid., 1868, 146, 175.

25. Haslewood, Biochem.J., 1946, 40, 52.

26. Schoenheimer, et.al., Z.physiol. Chem., 1930, 192, 73.

27. Schoenheimer, et.al., ibid., 1930, 192, 93.

28. Gardner and Gainsborough, Biochem. J., 1934, 28, 1635.

29. Pearlmann, J.Amer.Chem.Soc., 1944, 1, 806.

30. Pearlmann, J.Biol.Chem., 1946, 166, 473.

31. Blix and Löwenhielm, Biochem.J., 1928, 22, 1313.

32. Bergström and Wintersteiner, J.Biol.Chem., 1941, 141, 597.

33. Bergström and Wintersteiner, ibid., 1942, 143, 503.

34. Bergström and Wintersteiner, ibid., 1942, 145, 309, 327.

35. Städler, Ann., 1864, 132, 325.

36. Fischer, Z.physiol.Chem., 1911, 78, 227.

37. Morton, A.A., "The Chemistry of the Heterocyclic Compounds", McGraw Hill, London & N.Y., 1946.

38. Watson, C.J., Blood, 1946, 1, 99-120.

39. Farnsteiner, Z.Untersuch. Nahru.Genussm., 1889, 2, 1-27.
ibid., 1901, 4, 63-65
ibid., 1903, 6, 161-166.

40. Bosworth; A.W., and Helz, G.E., J.Biol.Chem., 1936, 112, 489-492.

41. Lapworth, Pearson & Mottram, Biochem.J., 1925, 19, 7-18.

42. Skellon, J.Soc.Chem.Ind., 1931, 50, 131-134T.

43. Edmann and Bedford, Ber., 1909, 42, 1324-1333.

44. Smith & Chibnall, Biochem J., 1932, 26, 218-234.

45. Breusch, Z.physiol.Chem., 1934, 227/228, 242.

46. Hatcher, Proc.Soc.Exptl.Biol.Med., 1924, 22, 141-2.

47. Sauermann, Z.physiol.Chem., 1935, 231, 92.

48. Nagakawa, and Fujikawa, J.Biochem., 1930, 12, 399.

49. Gsell-Busse, Archir.für Exptl.Path., 1929, 139, 329.

50. Bloch, et.al., J.Biol.Chem., 1943, 149, 511.

51. Channon, Biochem.J., 1925, 19, 425.

52. Randles and Knudson, J.Biol.Chem., 1925, 66, 459.

53. Eckstein and Treadwell, ibid., 1935, 112, 373.

54. Schoenheimer and Breusch, ibid., 19

55. Rittenberg and Schoenheimer, ibid., 1942, 143, 297.

56. Bloch and Rittenberg, ibid., 1942, 145, 625.

57. Bloch and Rittenberg, ibid., 1945, 159, 45.

58. Bloch and Rittenberg, ibid., 1945, 160, 417.

59. Bloch, Borek and Rittenberg, ibid., 1946, 162, 441.

60. Hench, Kendall, et.al., Proc.Staff.Meet.Mayo Clinic, April 1949, 24, 181.

61. Hench, Kendall, et.al., ibid., May 1949, 24, 277.

62. Freudweiler, Pharm.Acta.Helv., 1938, 13, 176.

63. Girard and Sandalescu, Helv.Chim.Acta., 1936, 19, 1905.

64. Fieser and Fieser, "Chem.Nat.Prod.Rel.to Phenanthrene", 1949, p.283.

65. Jacobs and Steidelberger, J.Biol.Chem., 1929, 81, 765.

66. Smith, J., 1935, p.1305.

67. Kendall, et.al., J.Biol.Chem., 1937, 120, 719.

68. Reichstein, Helv.Chim.Acta., 1937,19,29.

69. Reichstein, ibid., 1936, 19, 407.

70. Steiger and Reichstein, ibid., 1937, 20, 817.

71. Wieland, et.al., Z.physiol.Chem., 1932, 211, 261.

72. Yamasaki and Kyogoku, ibid., 1935, 233, 29.

73. Borsche and Hallwass, Ber., 1922, 55, 3324.

74. Borsche and Hallwass, Ber., 1922, 55, 3318.

75. Djerassi, et.al., J.Amer.Chem.Soc., 1951, 73, 4496.

76. Djerassi, et.al., ibid., 1951, 73, 1951.

77. Fieser, et.al., ibid., 1951, 73, 2397.

78. Fieser and Fieser, "Chem.Nat.Prod.Rel.to Phenanthrene", 1949, p.219 et seq.

79. Von Euw, Lardon and Reichstein, Helv.Chim.Acta, 1944, 27, 821.

80. Lardon and Reichstein, ibid., 1944, 27, 713.

81. Djerassi, et.al., J.Amer.Chem.Soc., 1951, 73, 3546.

82. Salamon and Reichstein, Helv.Chim.Acta, 1947, 30, 1929.

83. Reichstein, et.al., ibid., 1942, 25, 805.

84. Barton, J.Soc.Chem.Ind., 1948, p.755.

85. Marker, et.al., J.Amer.Chem.Soc., 1938, 60, 210.

86. Marker, et.al., ibid., 1938, 60, 1061.

87. Marker, et.al., ibid., 1938, 60, 1561.

88. Marker, et.al., ibid., 1938, 60, 2719.

89. Klyne, Nature, 1950, 166. 559.

90. Goldberg and Mannier, Helv.Chim.Acta, 1940, 23, 376.
" " " , ibid., 1940, 23, 840.

91. Fieser and Fieser, ChemNat.Prod.Rel.to Phenanthrene" 1949, p. 377 et. seq.

92. Sarett, J.Biol.Chem., 1946, 162, 601