I. ANALYSIS OF ANIMAL FATS.

II. CYCLISATION IN THE FRIEDEL-CRAFTS KETONE SYNTHESIS.

With an addendum on

The Structure of Byssochlamic Acid.

T H E S I S

for the Degree of

Doctor of Philosophy

by

Robert P. Paton, B.Sc.

The University of Glasgow.

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C O N T E N T S.

	Page
PART I. ANALYSIS OF ANIMAL FATS.	
Introduction and Summary	1
PART IA. PREPARATORY WORK.	
Semi-micro saponification equivalent.	
Introduction	9
Discussion	9
Experimental and results	13
Low temperature crystallisation	
Introduction	15
Discussion and conclusions	16
Experimental and results	18
Trial distillation of methyl esters	
Introduction	21
Experimental	22
Results	24
Discussion	28
Extraction of fats	30
PART IB. DETERMINATION OF FATTY ACID COMPOSITION OF DEER, CAMEL AND PYTHON FATS.	
Introduction	33
Method of analysis	35
Method of calculation	41
Deer fat	
Introduction	49
Results	50
Camel fat	
Introduction	56
Results	57
Discussion on deer and camel fats	63

C O N T E N T S (Contd.)

	Page
Python fat	
Introduction	66
Results	67
Discussion	76
References	78.
PART II. CYCLISATION IN THE FRIEDEL-CRAFTS KETONE SYNTHESIS.	
Summary	80
Introduction	82
Discussion	87
Experimental	108
References	131.
Addendum. The Structure of Byssochlamic Acid.	
Summary	133
Introduction	134
Discussion	138
Experimental	142
References	148.

PART I.

Analysis of Animal Fats.

Introduction and Summary.

In discussing land animal fats Hilditch[1] (1947, p.12) draws attention to the scarcity of detailed analyses in this group except for a few common animals. Later he writes[1] (p.65), "Discussion of the ultimate composition of these various groups of animal fats is subject to the usual limitations, namely, paucity of detailed fatty acid analyses in spite of abundant data on saponification values, etc., of individual fats or of their corresponding "mixed fatty acids"."

In order to augment the limited number of detailed analyses and widen the field of discussion, he has been forced to attempt an approximation of the fatty acid composition of some of the larger land animal depot fats from the above type of data, the fats of this class being on the whole less complex than those of the reptiles and amphibians.

Barker and Hilditch[2] (1950) further emphasise this lack of detailed information and they list land animal fats satisfactorily analysed. These are lion, Ceylon bear, giant panda, baboon, emu, kangaroo, tiger, puma, black bear, some species of deer and a relatively large number of analyses of the depot fats of oxen, sheep and pigs.

Analyses have since been reported for hyppopotamus fat[2] (Barker and Hilditch, 1950), badger[3] (Gupta, Hilditch and Meara, 1950), horse[4] (Shorland et al., 1950; 1952; Gupta and Hilditch, 1951), rabbit[5] (Clément and Meara, 1951), tiger[6] (Pathak and Agarwal, 1952). In the amphibian and reptile classes Barker and Hilditch name frog, tortoise, lizard and turtle. Toad[7] (Cattaneo et al., 1951), moccasin[8a] (Pollard and McLaughlin, 1952), and crocodile fat[8b] (Gunstone and Russell, 1953) have since been analysed.

The extensive gap in our knowledge of animal fats in comparison to seed fats especially, prompted the initiation in this department of a series of researches of which the present work is the beginning.

The outstanding importance of the large range of vegetable oils to commerce has undoubtedly actuated the investigation of so many seed fats and the search for methods of increasing the supply and quality of fats and oils for food, soap and the paint industry has been intensified during the last twenty years. To a less extent this applies also to fats of aquatic origin which have been more widely studied than those of the land animals. The fats of the less common animals, especially the carnivora, have obviously little commercial importance and this fact, combined with the difficulty of obtaining samples from natural sources, probably explains

their neglect in the past. Seeds are a very convenient form to collect, dry, transport and store if not required for immediate use, but the removal of fat from an animal and its transport, especially in a warm climate, may well prove futile in the end for analytical purposes. As Hilditch suggests, however, this obstacle can be and was overcome by obtaining samples from zoological authorities, although the diet and environment of the animal are almost certainly not those of its natural habitat, to which may be added the complication that the animal has probably died of disease, senility or other causes.

The work done on the fats of oxen, pigs, the white rat, etc., to determine the effect of diet, age, sex, etc., on their depot fatty acids is of the greatest importance. Herein lies the main difference between vegetable and animal fats. Although temperature of climate, etc., does affect the nature of seed fats, they are, on the whole, more uniform in composition and in the particular fatty acids present than many animal fats. For example, Hilditch[1] (p.152) has been able to classify the seed fats according to their fatty acid analysis and this corresponds very closely to their botanical groups. With the possible exception of oxen[9] the animal fats can be considerably affected by ingestion of dietary fat.

On the other hand, the composition of these fats tends to be more uniform when the animal is fed on its normal, low fat content diet[1] (p.102).

All the more usual fatty acids of the vegetable oils have been isolated, characterised and their ultra violet absorptions determined after isomerisation in the case of the polyunsaturated acids. It is thus possible to carry out a complete analysis, naming with certainty the individual acids except those occurring in traces. A number of the fatty acids found in animals, however, especially those of the sea animals, amphibians and reptiles, have not been fully characterised. Many acids have been isolated, but their constitutions are still in doubt in many cases.

The land animal fats are on the whole less complex than those of marine origin and their main polyethenoid acid, Arachidonic acid $C_{20}H_{32}O_2$, which has four double bonds, has been characterised and its absorption after alkaline isomerisation studied[10]. Small amounts of polyethenoid acids of the C_{22} series are also believed to occur in land animal fats[1] (p.433). A "linoleic" acid not giving the usual derivatives is referred to in sheep and ox fat analyses[11].

The newer methods of analysis recently developed for the seed oils have been applied to some animal fats, but the isomerisation technique, invaluable in seed oil work, can be

complicated by the presence of even small amounts of polyethenoid acids of the C_{20} and C_{22} series from dietary fish oils. In many cases, however, the same linoleic and linolenic acids as occur in the seed oils are present.

Another difficulty, in comparing vegetable and animal fats, is the smaller quantities available to the worker of any one sample, especially from the smaller animals. Larger samples have been received than was at first anticipated, but in many cases the amount is much less than that considered necessary for reliable results for minor component acids. Again, to obtain enough material it is often necessary to take accumulated samples from different parts of the animal and the result obtained is an average analysis only, since composition may vary considerably with location in the body. The ultimate aim, of course, would be the fat composition in the various sites as has been done with oxen, pigs, etc., but by present procedure this would be impossible in the case of small animals unless by accumulated samples from a number of individuals.

The initial stage, that of obtaining samples of the less common animals, has been overcome, as stated above, by the cooperation of zoological authorities and other sources. About twenty samples of a wide variety have been sent to us during a year and these have been extracted and stored in a

refrigerator for future work. Treatment prior to extraction of these samples required investigation since the presence of disease in some of the tissues made sterilisation desirable. Apparatus has been designed for extraction of animal fats varying from hard tallows to liquid fish oils. This procedure was quite different from the seed oil method since tissue is often tough and resistant to breakdown.

As some of the samples were too small for analysis by standard procedure, considerable time was spent in testing the reliability of the usual methods on the small scale, and the use of approximately 0.6 g. for the saponification equivalent measurement has given satisfactory results.

Mixtures of fatty acids similar to those in animal fats have been subjected to low temperature crystallisation and the optimum conditions found. Crystallisation from methyl alcohol at -20° has proved as effective and less troublesome than the usual lead salt separation of saturated from unsaturated acids. The technique has been improved and a low temperature cabinet for better control of conditions is being made.

The efficiency of two fractionating columns (Towers and Dixon 6") in separating various quantities of mixed esters has been investigated. It has been shown that fractional distillation of small quantities (12 g. and 6 g.) with subsequent use of the semi-micro technique for the saponification equivalent

gave results almost identical with those obtained by distilling larger quantities (40 g. and 25 g.) of the same ester mixture.

The analysis of three fats has been carried out using the Hilditch procedure modified by the above preparatory work.

The method of analysis is to hydrolyse the fat, extract the mixed acids and subject these to low temperature fractional crystallisation to give three or more fractions. These are then esterified and fractionally distilled to twelve or more ester fractions each. The saponification equivalent, iodine value and weights of the ester fractions combined with alkali isomerisation data provide the information for calculation of the acid composition. This is followed by identification of each acid present in appreciable amounts and concentrated by the fractionation during analysis.

Except in one case the fatty acids identified in these fats were all the usual acids occurring in seed oils. This was a hexadecenoic acid found in deer fat which gave a dihydroxy derivative having a different melting point from that of the usual one. A hexadecenoic acid giving a dihydroxy derivative of the same melting point as the deer fat derivative has been isolated from sperm oil[12], but the position of the double bond was not determined.

PART IA.

Preparatory Work.

SEMI-MICRO SAPONIFICATION EQUIVALENT.

Introduction.

When analysing fat samples of 30 - 40 g.or even more it is often necessary to utilise ester fractions of about 1 g. requiring estimation of the saponification equivalent on about 0.6 g. of esters; standard procedure requires 3 g. This part of the preparatory work set out to test available methods for suitability in fat analysis and to find the degree of accuracy attainable compared with that of the 3 g. scale.

The double indicator method of Marcali and Rieman[13] was found to be unsatisfactory and the standard macro procedure has been adapted to give satisfactory results on the semi-micro scale.

Discussion.

Accuracy is claimed for the double indicator method since it avoids the necessity of using a constant volume of alcoholic potassium hydroxide and making separate blank determinations. In practice the method was found very tedious, due to the time taken for the benzene layer to settle, and did not give the desired accuracy. It was shown experimentally:-

(I) Carbon dioxide guards are unnecessary (Expt.4).

(II) A blank titration of 10 ml. of alcoholic potassium

hydroxide gave a difference of about 0.1 ml. between the two indicators and this definitely affected results; this is not mentioned in the account of the method (Expt.4).
(III) Thymolphthalein was found to be more useful than phenolphthalein in cases where the solutions are coloured.
(IV) The time of reflux in American references is usually ½ an hour in undiluted half normal alcoholic potassium hydroxide solution. This was found to give the same results as 1½ hours reflux with neutral alcohol added, the usual method (Expt.9.).

Although some of these results were good (Expts.6 and 7), they were on the whole not satisfactory and this fact combined with the tediousness of the method caused attention to be turned to testing the standard macro method on the smaller scale. This work showed:- (i) By allowing 15 seconds drainage time for the pipette, the maximum difference in delivery was equal to 0.3 in a saponification equivalent of 300 (Expt. 11). (ii) Twelve blanks under varying conditions showed that it should be possible to keep the maximum difference to 0.7 in an equivalent of 300; superfluity of carbon dioxide guards was confirmed (Expt.14). (iii) ½ hour reflux for saponification was shown to be sufficient (Expt.16).

An average maximum difference of about 1.0 (Expt.17b-19) was found for 4 results and it was decided to accept this as a reasonable practical figure for the semi-micro scale.

These values were finally compared with those obtained on the macro scale (Expt.20) and, to have the same conditions as in actual analysis, freshly distilled methyl oleate and myristate were tried on both scales.

The collective results are shown in Table I. The maximum deviation and the mean deviation from the average saponification equivalent on the semi-micro scale compare favourably with those on the macro scale. The figures for mean deviation were accepted as being reasonable on the grounds that during a macro titration the end point, under ideal conditions, could be obtained within $\frac{1}{2}$ drop, that is, within 0.02 ml. in a $(V_B - V_S)$ of 20.00 ml., or a deviation of 1 in 1000 or 0.3 in a saponification equivalent of 300. This in fact is the figure for the average maximum deviation for all the materials in the table, neglecting the last two very dark solutions. The corresponding figure for the semi-micro scale is 0.4, a very slight increase as might be expected.

	Macro	Semi-micro
Average max. deviation	0.3	0.4
Average mean deviation	0.2	0.25

The value Δ S.E. in Table I was not consistently positive or negative and no constant correction could be applied. In some cases Δ S.E. was rather larger than was hoped, but on the whole results obtained with the semi-micro

Table I.

Table of results comparing semi-micro and macro methods.

Substance	Semi-micro Av.S.E.	Semi-micro Max. Devn.	Semi-micro Mean Devn.	Macro Av.S.E.	Macro Max. Devn.	Macro Mean Devn.	Δ S.E.
Methyl myristate *	243.3	0.1	0.1	242.6	0.1	0.0	+0.7
Methyl oleate *	291.7	0.4	0.3	292.1	0.3	0.1	-0.4
Myristic acid	230.3	0.1	0.1	229.5	0.2	0.1	+0.8
Oleic acid	280.3	0.6	0.3	280.4	0.4	0.3	-0.1
Olive oil	292.2	0.6	0.4	291.5	0.5	0.3	+0.7
Groundnut oil	296.2	0.6	0.3	295.0	0.3	0.2	+1.2
Rape oil	308.6	0.4	0.3	309.5	1.2	0.8	-0.9
Tallow	263.9	0.9	0.6	Too dark for end point			-

These were the average of four determinations in all cases.

* These were distilled and colourless, similar to the material obtained when doing a fatty acid analysis of a fat or oil.

procedure gave only slightly less accurate results than the macro method, as the above mean deviations show.

Experimental and Results.

Double indicator method[13]. About 0.5 g. of material is weighed into a 50 ml. conical flask and about 5 ml. of approx. ½ normal alcoholic potassium hydroxide solution added. The solution is refluxed gently, using a carbon dioxide guard, for ½ hour. The flask is removed, 2 drops of 1% phenolphthalein solution added and the solution titrated with standard half normal hydrochloric acid till the pink colour just disappears. 3 drops (0.18 ml.) of aqueous 0.001 M bromophenol blue and 1 ml. of benzene are added. The titration is continued till the colour of the aqueous layer is green. The difference between the two readings at the end points is $(V_B - V_S)$.

$$\text{S.E.(i.e. saponification equivalent)} = \frac{\text{weight used x 1000}}{(V_B - V_S) \text{ x normality of HCl}}$$

Expt.	Purpose	Conditions
1	Test double indicator method	0.3 g. oleic acid used.
2	Std. $\frac{1}{25}$N HCl replaced H_2SO_4.	As in expt.1.
3	0.6 g. oleic acid tried.	As in expt.2.
4	Test effect of CO_2 & attack by pot.hydroxide on flask	Direct titration of 5 ml. KOH soln. with and without CO_2 guard. Average of 3 titrations.
5	3 ml. benzene added before 1 st. e.p. to remove brown colour from aqueous layer.	As in expt.3.
6	Groundnut oil used.	As in expt.3.
7	T.p. used for 1 st. e.p. with oleic acid & groundnut oil	As in expt.6. Blanks subtracted from (V_B-V_S)
8	Microburette calibrated and corrections applied to expt.9.	
9	Check semi-micro against macro for groundnut oil using corrections	As in expt.7.
10	Test one indic.method (t.p.)	As in standard macro method
11	Check delivery of automatic pipette	a. No drainage time. b. 15 secs. drainage.
12	Repeat of expt.10 allowing drainage time.	As in expt.10.
13	Weights checked against standard weights.	
14	Check all stages by doing blanks, varying conditions	Direct titration of 10 ml. alc. KOH soln. CO_2 guard rechecked.
15	Repeat of expt.12, new wts.	As in expt.12, Gr. oil used.
16	Check time of reflux.	As in expt.15 varying time of reflux. 3 results each.

Results	Remarks
Ep. indefinite K_2SO_4 pptd.	Precipitate masked end point.
3 results, m.d. 3.4	Poor results. Try larger scale.
4 results, m.d. 1.7	Improvement. Soln. brown .·. e.p. masked.
a. No reflux .028 ml. b. ½ hr. " .047 ml. c. ½ hr. " (guard) .047 ml. d. 1 hr. " " .060 ml.	1. CO_2 guard unnecessary. 2. Blank required for each expt; this to be subtracted from (V_B-V_S). 3. Increase on reflux is attack on glass?
Colour remained in aqueous layer. $CHCl_3$ & CCl_4 only slightly better.	E.p. not sharp and layers slow to separate. Try groundnut oil since no colour develops on reflux.
4 results m.d. 0.8	Good result. Try thymolphthalein (t.p.).
Oleic: 2 results, md. 0.5 Gr oil: 2 " " 0.7	.·. t.p. gives better end point.
Macro 1½ hr. reflux ½ hr. reflux 3 res. md. 0.3 3 res. md. 0.2 Semi-micro Double indic. t.p. only 4 res. md. 1.8 4 res. md 0.7	1. ½ hr. reflux enough on macro scale. 2. Calculation using t.p. end point only gave more accurate results. 3. Macro and s-micro results compare favourably.
4 results, m.d. 0.8	Good results but may be improved.
a. Error 4 in 1000 b. " 5 in 10,000	Error in b. for 4 ml. titration is 0.3 in a S.E. of 300.
8 results, m.d. 2.2 but one low result.	M.d. 1.1 only for 7 results if the low result is neglected.
Error in 1 g.wts. 1 in 300	Wts. with negl. error used subsequently.
12 blanks, m.d. 0.010 ml. or 0.7 in S.E. of 300	1. CO_2 guard unnecessary (as in expt. 4) 2. Md. of 0.7 in SE of 300 seems possible.
4 results, m.d. 1.8	m.d. still too large.
Time (mins.) 20 40 60 Av. S.E. 295.1 294.3 293.9 M.d. 1.2 2.2 1.7	Av. SE falls with time but not individually. 30 mins. reflux considered enough. Gr. oil may contain interfering substance.

Expt.	Purpose	Conditions	Results and remarks.
17a	Olive oil & myristic acid tried	As in expt.15	Olive oil: 4 res., m.d. 1.3 Myr. acid: 5 " " 1.1
17b	All glass tip fitted to burette	"	Olive oil: 4 res., m.d. 0.8 Myr.acid: 4 " " 0.2 These are good results
17c	Repeat of 17 b to check constancy	As in expt.17b	Olive oil: 4 res., m.d. 1.1 Myr. acid: 4 " " 1.0
18	Back to groundnut oil & oleic acid	"	Oleic acid: 4 res.,m.d. 1.2 Gr. oil : 4 " " 1.1
19	Try method on fats which darken	"	Rape oil: 4 res., m.d. 0.8 Tallow : 4 " " 1.6 Tallow e.p. masked by colour
20	Repeat of above 6 materials on macro scale	"	Olive oil: 4 res.,m.d. 0.9 Myr. acid: " " 0.3 Gr. oil : " " 0.7 Oleic acid: " " 0.8 Rape oil : " " 2.3 Tallow : " " 6.8 The last 2 are poorer than on small scale. Larger vol. makes end point even more obscure.
21	50 ml. burette calibrated.		Correction (up to 0.15 ml.) applied to expt.20 av. values.
22	Test method on newly distilled methyl esters	As in expt.17b 4 res.each See recommended method p.14	Macro scale av.S.E. m.d. Methyl oleate : 292.1 0.4 " myristate : 242.6 0.1 Semi-micro scale " oleate : 291.7 0.8 " myristate : 243.3 0.2

Abbreviations

e.p. = end point
av. = average
res. = results
myr. = myristic
indic.= indicator
m.d. = maximum difference
t.p. = thymolphthalein
gr. = groundnut
S.E. = saponification equivalent

Recommended semi-micro method for saponification equivalent.

This is essentially the standard 3 g. method with the necessary precautions to ensure accuracy on the smaller scale.

Method. Weigh 0.6 - 0.65 g. of the ester in a glass capsule, transfer this to a 100 ml. conical flask and run in a constant volume (10 ml.) of ½ normal alcoholic potassium hydroxide from an automatic pipette, allowing 15 seconds drainage time. Reflux gently for ½ an hour using a long air condenser, but no carbon dioxide guard is necessary. Blanks are done in the usual way. Remove the condenser and wash the tip into the flask with a little alcohol neutral to the indicator. Add 2 drops of thymolphthalein solution (1% in alcohol) and titrate with standard ½ normal hydrochloric acid solution till colourless.

A calibrated 5 ml. burette graduated to 0.01 ml. was used and read to 0.001 ml. by means of an optical unit supported at right angles to the burette to prevent parallax error. The all glass burette tip was drawn to a fine point and greased ¼" up to prevent drops running up. Aldehyde free alcohol (rectified spirits) should be used, otherwise the solution darkens on reflux or standing.

LOW TEMPERATURE CRYSTALLISATION.

Introduction.

Low temperature crystallisation has recently become of great importance in the study of fats. Its use in place of lead salt separation of saturated from unsaturated acids is of advantage especially for highly unsaturated vegetable and marine oils[1] (p.471). It has been applied to estimation of saturated acids[14], study of glycerides[1] (p.412) and to component fatty acid analysis[15]. Recent work on animal fats[4,5] reports the use of this technique instead of lead salt treatment, crystallising the mixed acids from ether or acetone at -30° to -35° to separate the saturated acids.

The method is used as the first stage in the analysis, to divide the mixed acids from hydrolysis of the fat into 3 or more fractions containing mainly saturated, monoethenoid and polyethenoid acids. Where a wide range of acids are present, as in some animal fats, this simple division is not achieved; for example, the lower saturated acids will crystallise with the higher monoethenoid acids. Nevertheless, low temperature crystallisation is the first step in the simplification of the fatty acid mixture.

It was decided to compare the efficiency of low temperature crystallisation with that of the lead salt separation of saturated acids when applied to animal fats of the type to

be analysed later. Such factors as the best solvent, concentration, time allowed for crystallisation and temperature, taking into account the facilities available in the department, were varied to find the optimum conditions. A deep freeze cabinet maintained at -20° offered the possibility of overnight crystallisation, a time-saving arrangement.

Discussion and Conclusions.

Overnight crystallisation of tallow acids was shown to allow ample time to reach equilibrium (Expt.1, etc.). Ethyl acetate, petroleum-ether (40-60°) and methyl alcohol were the best solvents at -20° and the latter, being cheaper than the first and less volatile than the second, was chosen; pet.-ether (40-60°) tends to evaporate under suction and deposits acids in the filter. Methyl alcohol gave an easily filterable material and could be improved by addition of water. The crystallisations at 0° were unsatisfactory.

Addition of water to methyl alcohol (Expt.3) produced an improvement with a maximum at 5% water, where it equalled the lead salt method with even better iodine values. Above 5% separation was poorer and filtrations slow.

Variation of solvent ratio (Expt.4) showed that 10 ml. per g. of acids gave the best figures.

The use of water in the alcohol became troublesome

since it became concentrated in the acids when removing the solvent and the intentional addition of water was thus abandoned, but it was shown that the alcohol need not be specially dried.

The treatment of the second sample of acids justified the above decision (Expt.5) since 5% water pushed up the iodine value of the saturated fraction. Ethyl acetate equalled methyl alcohol here, but the latter was again preferred for reasons given above.

Conclusions. For the separation of the main bulk of saturated acids in animal fats, overnight crystallisation from methyl alcohol (10 ml. per g. of acids) at -20° gave the most satisfactory results. This has given a slightly lower yield of saturated acids than the lead salt separation, but with an improved iodine value.

The construction of a low temperature cabinet for overnight crystallisation down to -70° has been commenced, but in the meantime this has been achieved in the actual analyses by the use of a 15 litre Dewar flask. Better temperature control is possible using the alcohol cooled filter than with the older type using solid carbon dioxide in a wooden jacket.

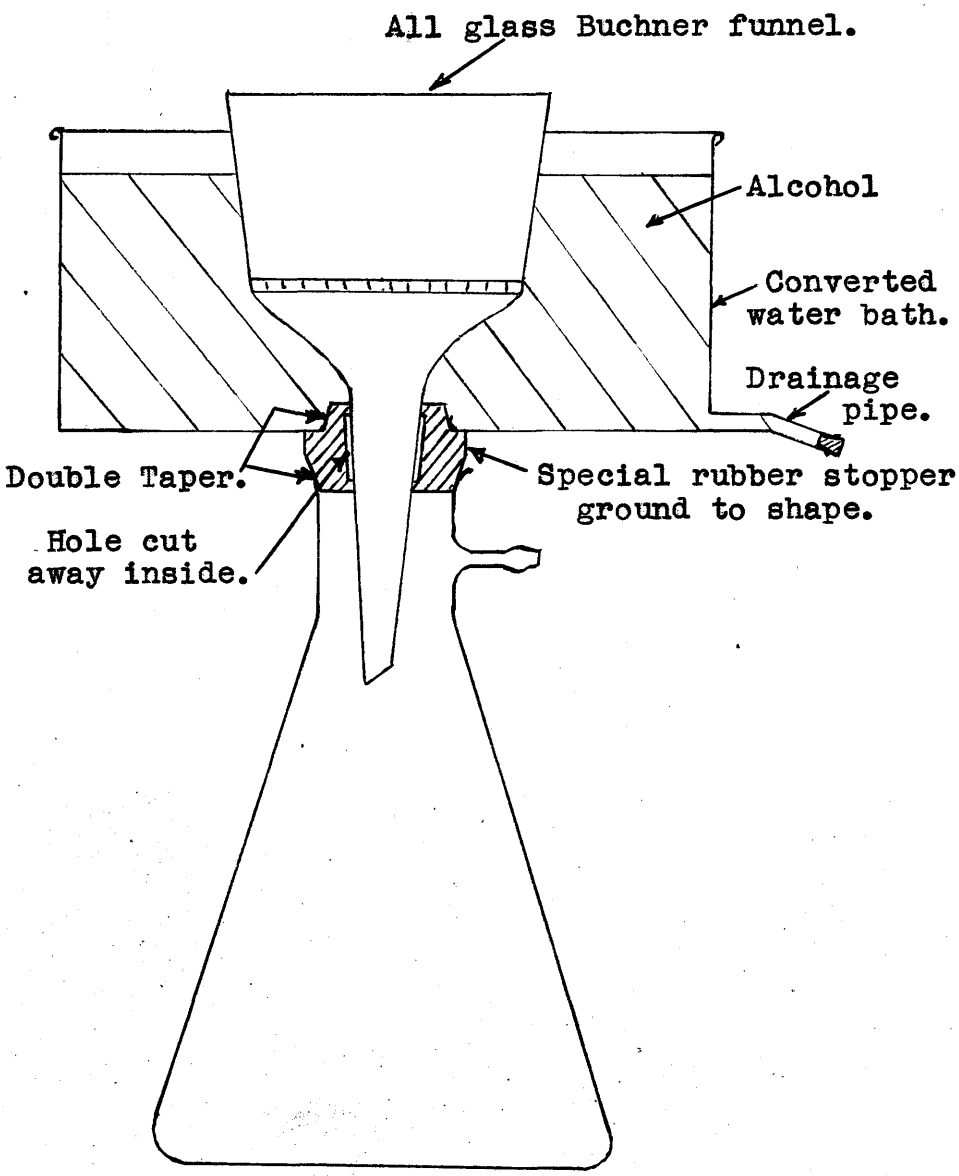

All glass Buchner funnel.
Alcohol
Converted water bath.
Drainage pipe.
Double Taper.
Hole cut away inside.
Special rubber stopper ground to shape.

Figure I.

Experimental and Results.

Method. In all these experiments the mixed acids were dissolved in the required volume of solvent and cooled slowly to crystallisation temperature then left overnight to complete the crystallisation. The following morning the insoluble acids were filtered quickly in the cooled filter (Fig.I), pressing well and washing twice with solvent cooled to the crystallisation temperature. The solids were washed into a flask with warm solvent. After removal of the solvent the weights and iodine value of both fractions were determined.

The Buchner funnel was of Jena glass with a ground surface for the filter paper; visual inspection for thorough cleaning was possible. The special rubber stopper was shaped as shown to support the whole arrangement on the filter flask.

Samples. Two samples were used. A sample of tallow representing the more saturated types of animal fats was hydrolysed and the mixed acids obtained in the usual way. A second more unsaturated sample was prepared by mixing technical palmitic (12%), stearic (13%) and oleic acid (75%).

Results.

Expt.1. 3 crystallisations of tallow mixed acids (I.V. 42.8) at 0° in 10 volumes of dried acetone (calcium chloride) gave the following results:

Cryst$^{n.}$ time (hrs.)	Weights (g)		I.V.	
	soluble	insoluble	soluble	insoluble
17	7.61	2.93	58.2	2.3
41	7.66	2.77	57.7	2.3
84	7.29	2.94	58.6	2.3

Expt.2. A series of crystallisations of the tallow acids using first lead salt separation then crystallisation at 0° and -20° with eight different solvents, all in 10 volume ratio, resulted in the following data:

	0°				-20°			
	insoluble		soluble		insoluble		soluble	
Solvent	% wt.	I.V	% wt.	I.V.	% wt.	I.V.	% wt.	I.V.
Acetone	27.8	2.3	72.2	58.2	46.1	2.7	53.9	75.5
Ether	-		-		35.1	2.8	64.9	62.3
Ethyl acetate	37.9	2.0	62.1	63.3	48.9	2.6	51.1	82.0
Pet.-ether (40-60°)	-		-		51.7	6.0	48.3	80.9
" sol.recrystd.	-		-		8.1	12.5	91.9	85.0
Methyl alcohol	33.7	2.6	66.3	62.0	49.7	3.5	50.3	79.8
" sol.recrystd.	-		-		6.6	20.7	93.4	82.8
Methylene chloride	-		-		47.8	2.0	52.2	79.3
Chloroform	-		-		Small yields and			
Carbon tetra-chloride	-		-		poor filtration			
Lead salt sep$^{n.}$ for comparison of results					52.8	5.6	47.2	83.6

Expt.3. Addition of various percentages of water to methyl alcohol showed an improvement on dry alcohol. 10 ml. per g. was used in all cases.

% water (vol.)	Insoluble % wt.	Insoluble I.V.	Soluble % wt.	Soluble I.V.	Remarks
1	49.2	3.4	50.8	74.3	-
2	50.2	3.5	49.8	82.4	-
5	52.3	3.9	47.7	85.8	As good as Pb salt sep. and better I.V.
10	57.5	10.1	42.5	87.8	Higher yield of insol., but I.V. too high
15	55.1	6.9	44.9	86.4	Poor filtration.

Expt.4. The effect of varying the solvent to solute ratio was studied.

Solvent ratio ml.per g.	5% aqueous methyl alcohol Insoluble % wt.	5% aqueous methyl alcohol Insoluble I.V.	5% aqueous methyl alcohol Soluble % wt.	5% aqueous methyl alcohol Soluble I.V.	Acetone Insoluble % wt.	Acetone Insoluble I.V.	Acetone Soluble % wt.	Acetone Soluble I.V.
5	51.9	4.7	48.1	84.7	-	-	-	-
10	52.3	3.9	47.7	85.8	46.1	2.7	53.9	75.5
20	51.9	4.8	48.1	84.3	47.1	2.4	52.9	75.7
40	50.4	4.7	49.6	81.3	-	-	-	-

Expt.5. The more unsaturated sample of mixed acids (12% palmitic, 13% stearic and 75% oleic) was subjected to crystallisation at -20° overnight using the three best solvents in 10 ml. per g. concentration.

Solvent	Insoluble % wt.	Insoluble I.V.	Soluble % wt.	Soluble I.V.
Pet-ether (40-60°)	24.9	12.3	75.1	93.8
Ethyl acetate	24.0	7.7	76.0	93.5
Methyl alcohol	25.5	8.8	74.5	95.2
5% aqu.Methyl alcohol	29.1	15.8	70.9	96.6

TRIAL DISTILLATION OF METHYL ESTERS.

Introduction.

The second stage in the simplification of the fatty acid mixture in fat analysis is the fractional distillation of the methyl esters of the acid fractions obtained by low temperature crystallisation of the mixed acids. The estimation and identification of minor component acids require an efficient fractionating column and a variety of good columns are now available, among which may be mentioned the spinning band and concentric tube types, both of which have a very small pressure drop over the length of the column.

Two columns were available in the department. A Towers (T.117) electrically heated column, packed with glass helices and capable of dealing with 20 g. and upwards of material and a Dixon[16] column packed with gauze cylinders, having a liquid paraffin jacket round the column and suitable for 5 to 20 g. of esters. It was by using this column for small fractions in conjunction with the semi-micro saponification equivalent procedure that we hoped to analyse the small samples of animal fats.

These columns were tried out under similar conditions to those used in analysis and the results were compared for the analysis of a sample of acids on the macro, using the large column, and the semi-micro scale, using the small column.

Experimental.

A mixture of technical myristic (10%) palmitic (40%) and stearic acid (50% by wt.) was converted to the methyl esters and used to test the Towers column on 40 g. and 25 g. of mixed esters while the Dixon column was tested on 12 g. and 6 g. fractionations.

The procedure in all these fractionations was the same. The weighed sample was added to the flask, the pressure was reduced to 1.0 to 0.1 mms., the column was flooded to wet the helices thoroughly, by forcing the distillation at first, then adjusted to a steady, slow total reflux for one hour to allow the distillation to reach equilibrium; porous chips were used to reduce bumping. The reflux ratio was then set at between 10 and 15 to 1. Fractions of about 3 g. were collected in the large column and 1 g. in the small one taking larger fractions when conditions showed the distillate composition to be constant.

For solid ester fractions, the take off tube and receiver were kept warm to prevent crystallisation. The weight of the residue was kept as small as possible since charring and polymerisation make its iodine value and saponification equivalent unreliable; the residue was washed from the column with acetone.

The fractions were weighed and their iodine value and saponification equivalents determined, the small fractions by the semi-micro method. From this data the percentage composition of the mixed acids was calculated using the method described by Hilditch[1] (p.484).

In all but the 40 g. distillation the fractions were collected singly. A multi-fraction receiver whereby the distillation is not disturbed during changing of receiver tubes, increases the efficiency of the fractionation and for this reason a twelve tube receiver was designed and used for the 40 g. trial which was done last. This receiver was fitted with a nichrome wire heater to keep the solid esters from crystallising. A six tube all Quickfit receiver was made for the Dixon still and was used for the 3 fats reported below, but it was not available for the present experiment.

For smooth working, the Dixon flask heater had to be slowly reduced in temperature as the jacket heater was raised, due to their concentricity, till at the highest boiling fractions the flask heater was turned off. A tap controlled take off similar to the Towers design was fitted to this column to replace the wire control which was troublesome under reduced pressure.

A. 40 g. Distillation in Towers column.

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{12}	C_{14}	C_{16}	C_{18}	Unsat.(g.) C'_{14}	C'_{18} *
A1	2.40	2.5	239.3	0.23	2.11	-	-	0.06	-
A2	2.48	1.6	251.4		1.58	0.86	-	0.04	-
A3	3.33	1.0	268.0		0.24	3.06	-	0.03	-
A4	3.46	0.8	269.7		0.05	3.38	-	0.03	-
A5	3.31	0.6	269.6		0.05	3.24	-	0.02	-
A6	3.33	1.7	270.5		0.03	3.23	-	-	0.07
A7	3.13	15.1	286.1			1.26	1.32	-	0.55
A8	2.84	21.6	295.4			0.24	1.88	-	0.72
A9	3.55	19.5	296.1			0.22	2.52	-	0.81
A10	3.10	15.5	296.4			0.17	2.37	-	0.56
A11	4.40	12.3	297.0			0.16	3.61	-	0.63
A12	4.37	12.0	297.2			0.14	3.62	-	0.61
Total	39.70			0.23	4.06	15.96	15.32	0.18	3.95
		% Esters		0.58	10.23	40.21	38.59	0.45	9.94
		% Acids		0.57	10.15	40.14	38.73	0.44	9.97

A12 Acids Ex. N.S. S.E. = 283.6

* In the unsaturated esters the number of double bonds is indicated by numbers of dashes. Thus oleic ester is C'_{18} and linoleic is C''_{18} etc.

B. 25 g. Distillation in Towers column.

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{14}	C'_{18}
B1	3.05	1.9	245.1	2.65	0.35	-	-	0.05	-
B2	3.15	1.0	267.5	0.28	2.84	-	-	0.03	-
B3	2.89	0.8	268.8	0.13	2.74	-	-	0.02	-
B4	2.84	3.7	271.9		2.69	0.03	-		0.12
B5	2.66	18.7	290.7		0.65	1.43	-		0.58
B6	2.67	19.4	296.3		0.15	1.91	-		0.61
B7	3.92	14.8	296.9		0.16	3.08	-		0.68
B8	3.55	11.8	298.5			3.03	0.03		0.49
Total	24.73			3.06	9.58	9.48	0.03	0.10	2.48
			% Esters	12.38	38.74	38.33	0.12	0.40	10.03
			% Acids	12.28	38.68	38.47	0.12	0.39	10.06

B8 Acids Ex. N.S. S.E. = 284.9.

C. 12 g. Distillation in Dixon 6" still.

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{14}	C'_{18}
C1	0.822	2.3	240.9	0.036	0.768	-	-	-	0.018	-
C2	1.229	1.4	260.8		0.373	0.840	-	-	0.016	-
C3	1.152	0.8	269.2		0.040	1.103	-	-	0.009	-
C4	1.122	1.0	269.9		0.028	1.081	-	-		0.013
C5	1.022	2.1	271.7			0.971	0.026	-		0.025
C6	0.970	11.1	286.2			0.395	0.449	-		0.126
C7	1.306	18.3	295.4			0.111	0.916	-		0.279
C8	1.223	18.1	296.8			0.047	0.917	-		0.259
C9	1.078	16.3	296.8			0.046	0.827	-		0.205
C10	1.407	11.4	296.5			0.072	1.148	-		0.187
C11	0.600	16.4	300.9				0.419	0.066		0.115
Total	11.931			0.036	1.209	4.666	4.702	0.066	0.043	1.209
			% Esters	0.30	10.13	39.12	39.41	0.55	0.36	10.13
			% Acids	0.29	10.05	39.05	39.55	0.55	0.36	10.15

C 11 Acids Ex. N.S. S.E. = 290.2.

D. 6 g. Distillation in Dixon 6" still.

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	Sat.(g.) C_{16}	Sat.(g.) C_{18}	Sat.(g.) C_{20}	Unsat.(g.) C'_{14}	Unsat.(g.) C'_{18}
D1	0.707	2.0	247.7	0.547	0.147	-	-	0.013	-
D2	0.826	1.1	267.8	0.060	0.757	-	-	0.009	-
D3	0.925	0.9	270.4		0.907	0.010	-	0.008	-
D4	0.740	11.1	281.9		0.412	0.232	-		0.096
D5	0.818	19.7	295.7		0.064	0.566	-		0.188
D6	1.557	13.4	297.0		0.060	1.253	-		0.244
D7	0.422	15.3	312.1			0.128	0.219		0.075
Total	5.995			0.607	2.347	2.189	0.219	0.030	0.603
			% Esters	10.13	39.14	36.52	3.65	0.50	10.06
			% Acids	10.05	39.05	36.64	3.67	0.50	10.09

D7 Acids Ex. N.S. S.E. = 296.7.

Table of analysis results for the 4 distillations of the same ester mixture.

Experiment	A	B	C	D
Amount distilled (approx.)	40 g.	25 g.	12 g.	6 g.
No. of fractions	12	8	11	7
Calculated composition (% acids by weight)				
Lauric	0.6	-	0.3	-
Myristic	10.2	12.3	10.0	10.1
Palmitic	40.1	38.7	39.0	39.0
Stearic	38.7	38.4	39.5	36.6
Arachidic	-	0.1	0.6	3.7
Tetradecenoic	0.4	0.4	0.4	0.5
Octadecenoic	10.0	10.1	10.2	10.1

Discussion.

The results from this series of fractionations show that a fairly good agreement has been achieved between large and small scale analysis, especially between A and C. The smaller number of fractions taken in B and D has almost certainly produced a poorer separation and the minor components are consequently partly hidden. For example, the small amount of lauric acid shown in A and C does not appear in B or D. The method of calculation assumes a

binary mixture of saturated acids and this probably is not so in all fractions in the case of a mixture of 6 or 7 components when the number of fractions is small as in B and D. The residue, for reasons mentioned above, is never reliable and in B and D the proportion in the residue is necessarily larger, but the amount in D (0.422 g.) was rather small and, together with the dark colour of the solution, this resulted in a high saponification equivalent producing too high a percentage of C_{20} acid (arachidic) in the final analysis.

We feel certain, however, that the agreement could be improved by experience and by using multi-tube receivers and conclude that, for a mixture of 4 or more acids, it is better, for greater accuracy, to aim at a minimum of 12 ester fractions.

EXTRACTION OF FATS.

A suitable press for expression of oils and soft fats was not available in the department, but mechanical rupture of the fat cells at a temperature at which the fat is liquid can be readily achieved by a steam jacketed homogeniser. The heat required to liquify hard fats is not detrimental in this case. Either oils or very hard fats can therefore be treated in this way followed by solvent extraction. Sterilisation was considered desirable and was found to have little effect on the free fatty acid.

Method. The samples were sterilised on arrival at 120° for 30-40 minutes (depending on size) in a standard domestic pressure cooker. The fat was cut into small pieces and homogenised using steam if necessary; the homogeniser was of standard type, made of copper and nickel plated inside, but designed with a steam jacket. Soft fats or oils were run into a flask, refluxed and filtered with pet.-ether (40-60°) till the residue was free of fat. Hard fats were allowed to harden then broken up and extracted by Soxhlet apparatus to ensure extraction of sparingly soluble glycerides which otherwise crystallised out in the filter. The solvent was removed, the iodine value and free fatty acid determined, except for very small samples, and the fat stored in sealed flasks in the refrigerator.

The following samples were treated in this way and three have been analysed.

Animal	I.V.	F.F.A.	Fat extracted (g.)
Seal 1	178.3	1.5	455
Seal 2	178.6	1.8	1245
Skink	not done	not done	23.8
Camel	35.0	2.1	72
Python	73.1	0.5	580
Mouse	88.0	not done	61
Deer	37.6	1.9	151
Crocodile	84.5	2.1	585
Ostrich	82.2	2.1	705
Chimpanzee	61.6	18.9	465
Rabbit	89.2	1.5	152
Monitor	108.1	not done	67
Hames antelope	85.0	10.1	224
Porcupine	54.4	2.8	366
Deer - Formosan doe	39.2	9.5	235
" - Manchurian doe	35.1	6.1	352
" - Fallow doe	26.2	2.5	195
" - Père David stag	28.7	3.2	339
" - Père David hind	29.8	3.4	217
Puma	71.3	1.9	167

PART IB.

Determination of the Fatty Acid Composition of Deer, Camel and Python Fats.

Introduction.

The aim of the preliminary experiments described above, the fatty acid analysis of some animal fats, has been carried out on deer, camel and python fats, using the standard procedure with modifications resulting from the preparatory work.

The principle of the method is to break down the complex mixture of acids into a large number of fractions each containing not more than two saturated acids, usually accompanied by unsaturated acids of the same number of carbon atoms. This is done in two stages. Firstly, the acids are separated into 3 (or more) acid fractions by low temperature crystallisation comprising mainly saturated, monoethenoid and polyethenoid acids, then the methyl esters of each fraction are distilled under vacuum in a packed, heated column to give ester fractions of simple composition.

The lead salt crystallisation for the separation of the saturated acids was replaced in these analyses by the crystallisation of the mixed acids from methyl alcohol at -20°.

The weight, iodine value and saponification equivalent of each ester fraction determine its composition, but if the fat contains oleic, linoleic and linolenic acids, additional information is required. Older methods include thiocyanogen value, elaidinisation and bromo-addition compounds, but these

are not very accurate. The third is now used mainly for the identification of linoleic and linolenic acids.

The most used method of estimating linoleic and linolenic acids is that described by Mitchell et al.[17] and modified by Hilditch et al.[18]. Both acids are isomerised to conjugated forms under standard alkaline conditions and the resulting equilibrium mixture is estimated spectroscopically. These acids can be estimated in presence of each other or alone; oleic acid does not interfere. This was the method used in these analyses, but in one case the bromo-addition compounds were estimated as a check.

In the case of the deer and camel fats alkaline isomerisation was confined to the mixed acids and the B and C acid fractions, the percentage of linoleic and linolenic acids being small. The python fat was treated in more detail since linoleic acid was a major component; selected ester fractions were also spectroscopically analysed.

Since deer and camel fats are hard and the amounts used were rather smaller than usual for this procedure, each gave a small C acid (most unsaturated) fraction, which in turn resulted in approximately 1 g. ester fractions; the semi-micro saponification equivalent method was therefore employed for these. Other acid fractions were collected in 1.5 to 2 g. ester fractions necessitating half scale determinations of the

saponification equivalent on approximately 1.5 g.

After calculation of the composition of the fat each acid, except those in traces, is identified by isolation of a suitable derivative.

Method of Analysis.

Hydrolysis. If enough fat is available 250 g. are hydrolysed, but the present samples were less; deer 110 g., camel 67 g., and python 200 g. The fat is refluxed for 2 hours in ethyl alcohol (5 ml. per g. of fat) with potassium hydroxide ($^1/_3$ weight of fat). About half of the alcohol is then distilled off, water is added and the solution made acid to Congo Red with 25% sulphuric acid to precipitate the mixed acids. When cool these are extracted with ether and the extract is washed with water till free of sulphuric acid. For highly unsaturated fats a smaller excess of hydroxide is used, refluxing for 1 hour only then, since no solvent is removed, a larger quantity of water is added. The ether extract is dried, the solvent removed and the acids weighed; its characteristics and in some cases the spectrographic data are determined.

Low temperature crystallisation. The mixed acids are crystallised from methyl alcohol at -20° to separate the saturated acids as described in the preparatory work on low temperature

crystallisation. Both fractions are weighed and the iodine values determined.

The soluble acids are then crystallised at lower temperatures depending on the nature of the fat (the deer fat was crystallised at -50° in acetone) and each fraction is treated as before.

Overnight crystallisation was not possible at first at these lower temperatures and they were done in a lagged alcohol bath cooled to a few degrees below crystallisation temperature. The solution of acids is cooled slowly with shaking to crystallisation temperature, then kept at this for 5 - 6 hours to allow full crystallisation before filtering. In some cases the insoluble acids are recrystallised at the same temperature to get a more efficient separation. The python fat was crystallised in the reverse order to remove and treat the highly unsaturated fraction first; all crystallisations of this sample were done overnight using a large Dewar flask holding about 10 litres of cooled alcohol as the overnight bath. The temperature had risen only 5 - 6° by next morning and efficient separations were obtained.

The choice of crystallisation temperatures is not fixed and each case may differ according to its nature, the aim being to concentrate the saturated, monoethenoid and polyethenoid acids into separate fractions as far as possible

and fractions of similar iodine value may be combined, as with small fractions from the python fat, with this general scheme in view.

Esterification. The acid fractions are dissolved in methyl alcohol (5 ml. per g. of acids) containing 1% sulphuric acid and the solution is refluxed for 2 hours. About half of the alcohol is distilled off, water added and the precipitated esters extracted with ether, washed 3 times with cold 10% aqueous potassium hydroxide, avoiding emulsion, then with water etc., in the usual way. For highly unsaturated fractions, methyl alcohol containing 1% dry hydrochloric acid is used, leaving to esterify overnight at room temperature, then removing some of the solvent at room temperature and reduced pressure; the esters are obtained as above.

Fractional distillation of the esters. The esterified acid fractions are distilled as described in the preparatory work at 1.0 - 0.1 mms. in a Towers (T. 117) column fitted with a 12 fraction receiver or a Dixon 6" column with a 6-fraction receiver. The ester fractions, including the mixed esters, are weighed and analysed in the usual way and, if necessary, some selected ester fractions are spectrographically examined.

Non-saponifiable matter[19]. When the non-saponifiable matter is more than 1 or 2% of the whole fat it is best removed by

continuous ether extraction of the aqueous alcoholic soap solution after saponification of the fat. In the present cases, however, this was not required and the non-saponifiable matter concentrated in the residues during fractionation. After determination of the iodine value and saponification equivalent of the residues with non-saponifiable matter this is removed and the two estimations repeated. This is done after titration for the equivalent by making the solution alkaline, evaporating off most of the alcohol and extracting 2 or 3 times with ether to remove non-saponifiable matter. The solution is then acidified and the acids extracted in the usual way. Some of the indicator which remains with the acids may be removed by dissolving them in pet.-ether (40-60°) and filtering off undissolved indicator. Residue solutions are usually dark and this last precaution is hardly necessary due to masking of the end point.

When the residue is small or if the non-saponifiable matter is large, it can be directly determined by weight. After evaporation of alcohol from the alkaline saponification equivalent solution as above, the whole solution is washed into a separating funnel with water and extracted 3 times with ether, each extraction being washed with 20 ml. of water. The ether extracts are combined and washed with 20 ml. of half normal aqueous potassium hydroxide solution, then with

20 ml. of water, repeating this 3 times then finally with water till free of alkali. The solvent is removed and the non-saponifiable matter weighed. The necessary correction is then made to the residue fraction.

Spectrographic determination of the polyethenoid acids. The procedure was carried out as described by Hilditch, Morton and Riley[18]. Selective absorption takes place only when the double bonds are conjugated and the unisomerised acids are therefore checked first for the presence of conjugated acids. The method works best for acids or esters but not fats and since $E^{1\%}_{1\ cm.}$ has been determined for the acids[18b], samples from the acid fractions or acids from selected ester fractions were used. The latter were recovered in the usual way using pet.-ether (40-60°) for extraction since the indicator is less soluble in it than ether. Linolenic acid is determined by measuring $E^{1\%}_{1\ cm.}$ at 268 mμ after isomerisation at 170° for 15 minutes and linoleic acid by measuring $E^{1\%}_{1\ cm.}$ at 234 mμ after isomerisation at 180° for 60 minutes. Readings at 300 mu and 315 mμ were also taken and in some cases small maxima were observed, indicating the presence of tetraethenoid acids. The method is as follows:

Approximately 0.1 g. of acids is weighed accurately in a glass capsule and dropped into a pyrex test tube contain-

ing 10 ml. of a 7½% solution of potassium hydroxide in glycol kept at the appropriate temperature. The temperature is maintained for the necessary time (±0.3°). The tube is then cooled quickly, washed into a 250 ml. graduated flask and made up to the mark with rectified spirits. After standing at 0° overnight it is filtered to remove silica and diluted to a suitable concentration for spectroscopic analysis. The determinations are usually done in duplicate with a blank which is used in the solvent cell when measuring the absorption in a Unicam Spectrophotometer.

Identification of the acids.

Stearic, palmitic and myristic. These are identified as the acids themselves by isolation from selected ester fractions in the usual way and crystallisation from ethanol.

Oleic, hexadecenoic and tetradecenoic. These are isolated as their dihydroxy derivatives by oxidation in very dilute, cold alkaline solution with potassium permanganate[20].

2.5 g. of the fraction is hydrolysed with an equal weight of sodium hydroxide and made up to 2 litres with ice cold water. 200 ml. of 1% aqueous potassium permanganate is added quickly with stirring, left 5 minutes then decolourised with sulphur dioxide and acidified with 75 ml. concentrated hydrochloric acid. The product is filtered, etc., and crystallised from ethanol.

Linoleic and linolenic. The bromo-addition compounds are isolated[21]. (0.007 x I.V.)g. of bromine is added to 1 g. of the acids in 2 volumes pet.-ether (40-60°) at 0-10° over 20 minutes and stood overnight at 0°. Product is filtered, decolourised with charcoal and the tetrabromides dissolved in 8 volumes of ether to which, after filtering off the hexabromides, is added pet.-ether to crystallise the tetrabromides; the latter is recrystallised from pet.-ether (80-100°) and the hexabromide from dioxan.

Polybromide number. This was estimated in fraction D 7 of the python fat using the method described by Markley[22]. The bromination is done as above but in ether to keep the tetrabromides dissolved, excess bromine being removed by cyclohexene. After overnight at 0° the insoluble hexabromides are filtered off and weighed while the tetrabromides are recovered from the filtrate and weighed.

Method of Calculation.

The method of calculation is essentially that described by Hilditch[1] (p.505). No general formula can be used as each fraction must be considered on the basis of its iodine value, saponification equivalent and spectrographic analysis and those of adjacent fractions. To illustrate the method reference is made to the python fat analysis. Observation

is usually sufficient in the most saturated fraction (A) since the ester fractions contain only saturated and small amounts of monoethenoid esters and spectrographic data is not needed.

2 saturated and 1 unsaturated. A typical example is A9 (S.E. 285.6 and I.V. 5.0). No rise in iodine value corresponding to hexadecenoic ester is observed in earlier fractions and the unsaturation is assumed to be due to oleic ester throughout A fraction. The weight of oleic ester in A9 is calculated from the iodine value, the saponification equivalent of the remaining saturated esters is corrected to allow for removal of oleic, then the fraction is partitioned between palmitic and stearic esters according to the equivalent from tables of the equivalents of binary mixtures of saturated methyl esters. The stages are:

(1) Wt. of unsat. ester = $\frac{\text{wt. of fraction x I.V. of fraction}}{\text{I.V. of pure unsat. ester.}}$

(2) Subtract this wt. from total wt. of fraction to find wt. of sat. esters.

(3) Sat.moles. = total moles - unsat. moles

$$= \frac{\text{total wt.}}{\text{S.E. of fraction}} - \frac{\text{unsat.wt.}}{\text{S.E. of pure unsat.ester}}$$

Then corrected S.E. = $\frac{\text{Wt. of sat. ester}}{\text{No. of sat. moles.}}$

2 saturated and 2 unsaturated. If it is decided that a fraction (e.g., All) contains 2 saturated and 2 unsaturated esters of the same carbon content, then only a small error is entailed by taking the equivalent of the unsaturated part as the same as that of the fraction itself. Then, abbreviating "wt. of oleic ester" to ol and "wt. of C_{20} monoethenoic" to Ar', we have

$$\frac{\text{ol}}{\text{S.E. of oleic}} + \frac{\text{Ar}'}{\text{S.E. of Ar}'} = \frac{\text{ol} + \text{Ar}'}{\text{S.E. of fraction}} \quad \text{----- (I)}$$

and ol x I.V. of oleic + Ar' x I.V. of Ar'

= (ol + Ar') x I.V. of fraction ---------- (II)

By solving these equations for ol and Ar' the remaining part can be partitioned between the two saturated esters.

1 saturated and 2 unsaturated. Fraction B2 is calculated on this basis. Abbreviating as above, we have "wt. of palmitic" is p and "wt. of hexadecenoic ester" is h .

From wts. p + ol + h = wt. of fraction ---------- (i)

From S.E. $\frac{\text{p}}{\text{S.E. of p}} + \frac{\text{ol}}{\text{S.E. of ol}} + \frac{\text{h}}{\text{S.E. of h}}$

$= \frac{\text{wt. of fraction}}{\text{S.E. of fraction}}$ ---------- (II)

From I.V. ol x I.V. of ol. + h x I.V. of h

= wt. of fraction x I.V. of fraction ----- (III)

These 3 equations can be solved by the usual procedure.

Fractions C and D are more complicated due to the presence of linoleic and traces of linolenic esters, in which case spectrographic analysis gives the amount of these present.

Fraction C. C2, C8 and C13 fractions were examined spectrographically as well as the C mixed acids to determine the composition of the C_{16}, C_{18} and C_{20} unsaturated mixtures.

This data for C8 indicated the absence of saturated and triethenoid acids and fractions C6 to C11 were therefore calculated as mixtures of oleic and linoleic esters on the basis of iodine value, these fractions being fairly uniform in iodine value and equivalent. In earlier fractions (C2 to C5) the C_{18} esters were considered to have the same composition as in C6 and in later fractions (C12 and C13) the C_{18} is taken to have the same composition as in C11.

C2 isomerisation data showed that C_{18} content might account for all of the absorption. A trial calculation distributing between C_{16} and C_{18} (as in C6) by using the method of repeated approximations gave C_{18} as 9.3% which corresponded to $E^{1\%}_{1\ cm.} = 14.6$ (14.7 found). C2 was therefore calculated as C_{16} sat., C'_{16} and C_{18} as in C6 using the method for 1 saturated and 2 unsaturated esters. C3 and C4 were calculated as for C2, but C5 was taken as C_{16} saturated and C_{18} (as in C6) only.

C1 was calculated as C_{14} and C_{16} saturated and monoethenoid.

C12 and C13 undoubtedly contain some C_{20} esters and the C_{20} in fraction C12 was therefore calculated by the method of repeated approximations instead of graphically as described by Hilditch[1] (p.509). This procedure is resorted to when the iodine value and equivalent of the C_{20} esters are not known. Using w, I.V. and S.E. for the weight, iodine value and equivalent of the fraction and C_{18} and C_{20} for the weights of these esters we have:

From S.E. $$C_{18} \times \frac{1}{\text{S.E. of } C_{18}} + C_{20} \times \frac{1}{\text{S.E. of } C_{20}} = \underline{w} \times \frac{1}{\underline{\text{S.E.}}} \quad \text{---------- (I)}$$

From I.V. $$C_{18} \times \underline{\text{I.V. of } C_{18}} + C_{20} \times \text{I.V. of } C_{20} = \underline{w} \times \underline{\text{I.V.}} \quad \text{---------- (II)}$$

From wts. $$C_{18} + C_{20} = \underline{w} \quad \text{---------- (III)}$$

The underlined values are known in these equations and to solve them either the I.V. of C_{20} or S.E. of C_{20} can be approximated. In this case the S.E. of C_{20} was estimated mentally and used to solve for C_{18} in equation (I). Equation (II) then provided I.V. of C_{20} which corresponds to a definite S.E. in a C_{20} mixture. This new S.E. was then used in place of the approximate value and the calculation was repeated till the S.E. of C_{20} used first was the same as that obtained at

the end of the calculation. Having obtained the S.E. of C_{20}, C12 and C13 were then calculated as mixtures of C_{18} (as in C11) and C_{20} (as determined) and partitioned according to the S.E. of the fraction.

Fraction D. This was treated similarly to fraction C. Isomerisation data was obtained for D2, D7 and D11 as well as the mixed acids.

D7 was then calculated as C_{18} unsaturated esters only. Spectrographic data determined the linoleic and linolenic esters and the remainder of the iodine value was attributed to oleic ester. The composition of this fraction and also D5 to D9 (I.V. and S.E. fairly uniform) was found to be linolenic 5.7%, linoleic 67.0% and oleic 27.3% as esters.

D2 was considered to contain mostly C_{16} esters and a little C_{18} (as in D7). The method of repeated approximations was used to find the C_{16} composition and percentage of C_{18} present. Isomerisation data and iodine value then determined the composition of the whole fraction. Repeated approximation gave C_{18} (as in D7) = 11%, C_{16} = 89.0%.

Isomerisation data Absorption due to $C_{18} = \frac{11 \times 627.1}{100}$

$= 69.0$

$\therefore$ Contribution of $C''_{16} = 82.0 - 69.0 = 13.0$

$\therefore$ % $C''_{16} = \frac{13.0}{1006} = 1.3.$

Calculation by I.V.

	%	I.V.	Contribution
C_{18} (as in D7)	11.0	154.2	17.0
C''_{16}	1.3	190.5	2.5
			19.5 Total
C'_{16}	?	94.6	

$\therefore$ Contribution of C'_{16} = (95.2-19.5) = 75.7

$\therefore$ % $C'_{16} = \frac{75.7}{94.6} = 80.0$

$\therefore$ C_{16} sat = 7.7

$\therefore$ Accepted composition is C_{18} (as in D7) = 11%,

C_{16} (as below) = 89%

and C_{16} esters composition is C''_{16} = 1.5%, C'_{16} = 89.9%,

C_{16} sat. = 8.6%.

D3 was considered as a mixture of C_{16} and C_{18} of above composition and was calculated both on iodine value and equivalent; these differed slightly and the mean was used. D4 was calculated similarly on iodine value. D1 was taken as C_{14} saturated and monoethenoid accompanied by some C_{16} (as in D2).

D10 and D11 were calculated by the same procedure as C12 and C13.

The esters were totalled for each acid fraction. In fraction D the weights were partitioned according to the

composition of the C_{16} and C_{18} groups. The weights were then expressed as % esters of each acid fraction and then as % acids thus

$$\% \text{ acids} = \frac{\% \text{ esters} \times \frac{\text{S.E. acid}}{\text{S.E.ester}}}{\sum \left(\% \text{ esters} \times \frac{\text{S.E.acid}}{\text{S.E.esters}}\right)}$$

This was converted to increments of the total mixed acids expressed as % weight. These increments were totalled and then expressed as % weight and % moles excluding non-saponifiable matter.

DEER FAT.

Introduction.

Prior to the work of Baughman, Jamieson and McKinney[23] (1929) and Treadwell and Eckstein[24] (1939) information on deer fat was limited to reports of physical properties and the usual characteristics (summarised by Lewkowitsch[25], 1922). The first named authors studied five samples of American reindeer fat of iodine value 33.7 to 39.4. Treadwell and Eckstein studied fats from various parts of five Virginia white-tailed deer (Odocoileus virginianus borealis), the thiocyanogen values being used for estimation of the unsaturated acids. Although the analyses are not detailed, there is little variation in these samples apart from the mammary gland fat. These two sets of results are summarised on p.62.

The present sample of depot fat was obtained from a butcher in Perth and had come from a male, red deer (Cervus elaphus) reared in Scotland and presumably on its natural diet. The crude fat (230 g.) was not autoclaved and, being very hard on removal from the refrigerator, it was easily crumbled to a powder in a mortar. After extraction with pet.-ether (40-60°) 151 g. of fat was obtained, the characteristics of which are given with the analytical data.

Results.

Deer fat:- I.V. 35.5, S.E. 284.9, F.F.A. 2.0% (as oleic)

Mixed acids:- I.V. 32.4, S.E. 266.2.

Low temperature crystallisation of acids (weights corrected)

Mixed acids 97.88 g. I.V. 32.4

10 x MeOH | at -20°

Soluble 36.70 g. I.V. 91.5 — Insoluble 61.18 g. I.V. 5.9 (Fraction A)

10 x Acetone at -50°

Soluble 15.97 g. I.V. 122.7 (Fraction C) — Insoluble 20.73 g. I.V. 67.3 (Fraction B)

Further details of acid fractions.

		Mixed acids	A	B	C
Percentage		100	62.5	21.2	16.3
Iodine value		32.4	5.9	67.3	122.7
$E^{1\%}_{1cm.}$ Unisomerised	234 mμ		1.0	7.9	36.8 *
" "	268 mμ		0	0.5	5.6 *
" Isomerised	234 mμ	20.3	-	16.6	209.2
" "	268 mμ	6.9	-	2.0	80.9

Esters of acid fractions.

Iodine value	5.6	63.7	-
S.E.	287.5	-	-

*These values were neglected since they were not true bands.

Deer Fat. Fractional distillation of A esters (T.117 Press. 0.5 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{16}	C'_{18}	N.S.
A1	4.07	0.7	263.9	0.86	3.18	-	-	0.03	-	-
A2	4.55	0.6	270.3	0.01	4.51	-	-	0.03	-	-
A3	4.11	0.6	270.0	0.05	4.03	-	-	0.03	-	-
A4	4.03	0.9	271.9		3.76	0.23	-	0.03	0.01	-
A5	4.08	3.9	279.6		2.55	1.36	-	0.10	0.07	-
A6	2.08	8.3	293.6		0.32	1.56	-		0.20	-
A7	3.37	9.9	294.4		0.42	2.56	-		0.39	-
A8	3.99	9.6	298.5			3.54	-		0.45	-
A9	4.50	8.5	298.9			4.05	-		0.45	-
A10	4.15	7.4	298.0			3.79	-		0.36	-
A11	3.99	6.6	298.0			3.68	-		0.31	-
A12	4.27	5.9	298.6			3.93	0.05		0.29	-
A13	2.97	6.9	299.0			2.69	0.08		0.20	-
A14	2.23	16.1	320.3			1.02	0.99		0.15	0.07
Total/ (Mean)	52.39	(5.6)	(287.3)*	0.92	18.77	28.41	1.12	0.22	2.88	0.07
			% Esters	1.76	35.83	54.22	2.14	0.42	5.50	0.13
			% Acids	1.74	35.71	54.33	2.15	0.42	5.51	0.14

* Mean values are shown in brackets. A14 acids ex. N.S. S.E. 296.6, I.V. 15.4.

Notes on calculation. A13 and A14 probably contain some higher unsat.acid - this is included with the corresponding saturated acid and the I.V. of A13 and A14 is accordingly taken as 5.9.

Deer Fat Fractional distillation of B esters (T.117 Press. 0.4-0.5 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{14}	C'_{16}	C^{*}_{18}	N.S.
B1	1.683	2.0	245.6	1.439	0.211	-	-	0.026	0.007	-	-
B2	1.796	9.6	258.4	0.650	0.971	-	-	0.058	0.117	-	-
B3	1.848	30.8	277.3		0.907	0.324	-		0.406	0.211	-
B4	1.823	66.6	290.0		0.130	0.330	-		0.290	1.073	-
B5	1.747	82.8	294.3			0.096	-			1.651	-
B6	1.842	86.4	295.4			0.025	-			1.817	-
B7	1.949	87.5	295.5			0.002	-			1.947	-
B8	1.671	87.6	296.3				-			1.671	-
B9	1.703	87.6	295.9				-			1.703	-
B10	1.959	87.6	297.1				-			1.959	-
B11	1.349	73.5	328.4				0.144			1.101	0.104
Total/ (Mean)	19.370	(64.0)	(286.5)	2.089	2.219	0.777	0.144	0.084	0.820	13.133	0.104

		C_{14}	C_{16}	C_{18}	C_{20}	C'_{14}	C'_{16}	oleic	lin.	len.	N.S.
B11 Acids ex N.S.	% Esters	10.78	11.46	4.01	0.74	0.43	4.23	65.85	1.60	0.36	0.54
I.V. 81.3, S.E. 289.2	% Acids	10.68	11.43	4.02	0.75	0.42	4.22	65.95	1.60	0.36	0.57

* C_{18} composition:- oleic 98.04%, linoleic 1.60%, linolenic 0.36%

Notes on calculation C_{18} unsaturated taken as I.V. 87.6. Linoleic and linolenic calculated from isomerisation values and oleic by difference.

Deer Fat. Fractional distillation of C esters (Dixon 6" Press. 0.5 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{12}	C_{14}	C_{16}	C_{18}	Unsat.(g.) C_{14}	C_{16}	C_{18}*	N.S.
C1	1.026	24.4	241.7	0.007	0.782	-	-	0.237	-	-	-
C2	0.990	76.9	265.9		0.028	0.164	-	0.064	0.734	-	-
C3	1.145	108.7	282.3			0.063	-		0.498	0.584	-
C4	1.027	131.4	291.8			0.009	-			1.018	-
C5	1.056	132.6	294.4				-			1.056	-
C6	1.373	132.4	293.6				-			1.373	-
C7	1.041	133.1	294.6				-			1.041	-
C8	1.259	132.2	296.1				-			1.259	-
C9	1.180	129.6	294.4				0.026			1.154	-
C10	2.030	114.9	305.2				0.286			1.698	0.046
Total/ (Mean)	12.127	(113.3)	(287.0)	0.007	0.810	0.236	0.312	0.301	1.232	9.183	0.046

									oleic	lin	len	N.S.
C10 acids ex N.S.	% Esters	0.06	6.67	1.95	2.57	2.48	10.16	47.13	13.84	14.58	0.38
I.V. 119.0 S.E.284.3	% Acids	0.06	6.60	1.94	2.58	2.46	10.12	47.40	13.85	14.59	0.40

*C_{18} composition:- oleic 71.58%, linoleic 13.84%, linolenic 14.58%.

Notes on calculation. C_{18} unsaturated taken as I.V. 132.5, S.E.(calc.) 295.4 (Mean of C5-C8).

Deer Fat.

Component acids of fractions (increments % weight).

	A	B	C	Total	Excluding non-sap. %wt.	Equivs.	% mol.
Lauric	-	-	0.01	0.01	-	-	-
Myristic	1.09	2.26	1.08	4.43	4.4	.01944	5.3
Palmitic	22.32	2.42	0.32	25.06	25.1	.09801	26.7
Stearic	33.96	0.85	0.42	35.23	35.4	.12415	33.9
Arachidic	1.34	0.16	-	1.50	1.5	.00480	1.3
Tetradecenoic	-	0.09	0.40	0.49	0.5	.00216	0.6
Hexadecenoic	0.26	0.89	1.65	2.80	2.8	.01105	3.0
Octadecenoic	3.44	13.99	7.72	25.15	25.2	.08928	24.3
Octadecadienoic	-	0.34	2.26	2.60	2.6	.00931	2.5
Octadecatrienoic	-	0.08	2.38	2.46	2.5	.00887	2.4
Non-saponifiable	0.09	0.12	0.06	0.27	-	-	-

Calculated means (Including N.S. of I.V. zero)	S.E.	I.V.
Glycerides	285.9	35.7
Mixed acids	273.2	37.4

Deer Fat.

Identification of individual acids.

Stearic. Fraction A10 yielded a sample of m.p. and mixed m.p. with an authentic specimen 69-70°.

Palmitic. A3 yielded a sample of m.p. and mixed m.p. 60½-62°.

Oleic. B8, 9 and 10 combined yielded dihydroxystearic acid of m.p. and mixed m.p. 129½-130½°.

Hexadecenoic. C2 on oxidation, etc., yielded dihydroxy-palmitic acid which, after crystallisation from ethanol then ethyl acetate, had a m.p. of 116° (sharp). (Analysis: Found, C = 66.39%, H = 11.36%; theoretical, C = 66.63%, H = 11.19%). The normal deficative has m.p. 125°.

Tetradecenoic. C1 yielded a small amount only of the dihydroxy derivative. Repeated crystallisation and analysis of a specimen of m.p. 108-9° (usual derivative, m.p. 127-8°)[12] was not conclusive and too little was left for further treatment.

Linoleic and linolenic acids. C4 to C10 combined gave a tetrabromo derivative of m.p. and mixed m.p. 112½-113° and a hexabromo derivative of m.p. 179½-180½° and mixed m.p. 180-181° with authentic specimens.

CAMEL FAT.

Introduction.

Reports on the composition of camel fat seem to be limited to that of Armstrong and Allan[11] (1924) in which they have determined only the major component acids palmitic, stearic and oleic (see p.62).

The present sample was from the mesentery of a 7 year old male bactrian camel (Camelus bactrianus) and was sent from Belle Vue Zoo, Manchester. The animal's diet consisted of meadow and clover hays, supplemented by a daily feed of chopped vegetables and fruit with chopped hay, oats, bran and horse feed cubes together with a little cod liver oil; fresh grass was also available.

The crude fat was autoclaved at 120° for 40 minutes, crushed in a mortar and extracted with acetone to give a hard, pale yellow fat; the homogeniser was not available at this time. The characteristics of the fat are given with the other data.

Results.

Camel fat:- I.V. 35.1, S.E. 282.2, F.F.A. 2.1% (as oleic)

Mixed acids:- I.V. 36.8, S.E. 270.4.

Low temperature crystallisation of acids (weights corrected)

Mixed acids 69.07 g. I.V. 36.8

10 x MeOH at -20°

Soluble 30.37 g. I.V.79.3 — Insoluble 38.70g. I.V. 2.9 (Fraction A)

10 x acetone at -50°

Soluble 8.43g. I.V.107.5 (Fraction C) — Insoluble 21.94g. I.V.69.0 (Fraction B)

Further details of acid fractions.

	Mixed Acids	A	B	C
Percentage	100	56.03	31.76	12.21
Iodine value	36.8	2.9	69.0	107.5
$E^{1\%}_{1cm.}$ Unisomerised	No evidence of maxima			
" Isomerised 234 mμ	22.2	-	12.4	148.7
" " 268 mμ	4.6	-	1.5	37.5

Esters of acid fractions

Iodine value	2.8	65.8	102.4
S.E.	283.9	-	-

Camel Fat. Distillation of A esters (T.117 Press.0.5-1.0 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{16}	C'_{18}	C'_{20}*
A1	2.75	0.5	261.3	0.82	1.92	-		0.01		
A2	2.83	0.4	270.8		2.78	0.04		0.01		
A3	3.11	0.4	270.9		3.04	0.06		0.01		
A4	3.18	0.3	271.5		3.04	0.13		0.01		
A5	3.25	0.3	272.6		2.97	0.27		0.01		
A6	3.21	1.8	278.1		2.23	0.92		0.04	0.02	
A7	2.49	4.8	291.3		0.58	1.77			0.14	
A8	3.45	5.4	296.1		0.25	2.98			0.22	
A9	3.02	4.6	298.8			2.81	0.05		0.16	
A10	3.63	3.9	299.0			3.37	0.09		0.17	
A11	3.37	3.5	299.5			3.09	0.14		0.14	
A12	2.30	9.8	307.0			1.37	0.66		0.16	0.11
Total/ Mean	36.59	(2.9)	(284.0)	0.82	16.81	16.81	0.94	0.09	1.01	0.11
			% Esters	2.24	45.94	45.94	2.57	0.25	2.76	0.30
A12 Acids ex N.S. I.V. 9.9, S.E. 295.5.			% Acids	2.22	45.81	46.06	2.59	0.25	2.77	0.30

*C_{20} composition:- see totals on p.61.

Camel Fat. Distillation of B esters (T.117 Press.0.7-1.0 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	N.S.
B1	1.699	2.3	242.4	1.662	-	-	-	0.037	-	-	-	-
B2	1.545	8.8	251.2	0.932	0.479	-	-	0.079	0.055	-	-	-
B3	1.601	30.2	270.6		1.078	0.008	-		0.470	0.045	-	-
B4	1.523	59.8	288.4		0.165	0.322	-		0.276	0.760	-	-
B5	1.644	76.2	294.5			0.217	-			1.427	-	-
B6	1.660	82.1	295.9			0.108	-			1.552	-	-
B7	1.689	84.0	296.6			0.073	-			1.616	-	-
B8	1.705	85.0	296.5			0.054	-			1.651	-	-
B9	1.699	84.1	296.1			0.072	-			1.627	-	-
B10	1.580	84.3	295.9			0.063	-			1.517	-	-
B11	1.548	84.9	296.8			0.051	-			1.497	-	-
B12	1.660	85.0	298.2			-	-			1.546	0.114	-
B13	1.200	69.0	333.2			0.017	0.057			0.174	0.903	0.049
Total/ (Mean)	20.753	(64.4)	(286.4)	2.594	1.722	0.985	0.057	0.116	0.801	13.412	1.017	0.049

		Oleic	lin	len	C'_{20}	N.S.
B13 Acids ex N.S. I.V.77.7, S.E.305.6	% Esters	63.16	1.19	0.27	4.90	0.24
	% Acids	63.27	1.19	0.27	4.93	0.25

*Notes on calc^{n}. In B5-11 the composition of C_{18} unsat. esters is calculated by, isomerisation data gives C''_{18} and C'''_{18} for whole of C then contribution of C'_{14}, C'_{16} and C'_{20} subtracted from I.V. to find oleic. This gives I.V. 87.8 for C_{18} unsat. esters and the remainder in B5-11 is taken as stearic.
C_{18} = oleic 98.54%, linoleic 1.19%, linolenic 0.27%.

Camel Fat. Distillation of C esters (Dixon 6" Press.0.2-0.4 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	Sat.(g.) C_{16}	Sat.(g.) C_{18}	Unsat.(g.) C'_{14}	Unsat.(g.) C'_{16}	Unsat.(g.) C^{*}_{18}	Unsat.(g.) C^{+}_{20}	N.S.
C1	0.981	27.5	243.9	0.678	0.044	-	0.223	0.036	-	-	-
C2	1.062	86.9	271.2		0.104	-		0.850	0.108	-	-
C3	1.101	98.2	286.7		0.097	-		0.254	0.750	-	-
C4	1.129	112.5	295.4			-			1.129	-	-
C5	1.155	111.6	295.7			-			1.155	-	-
C6	1.145	109.5	299.5			0.027			1.118	-	-
C7	0.708	179.9	320.3						0.047	0.784	0.152
C8	0.275	-	-								
Total/ (Mean)	7.556	(100.3)	(291.5)	0.678	0.245	0.027	0.223	1.140	4.307	0.784	0.152

		oleic	lin	len	C^{+}_{20}	N.S.
C8 N.S. direct=0.152g.	% Esters	38.12	12.12	6.76	10.38	2.01
C7 acids ex N.S. I.V.164.8, S.E. 309.5	% Acids	38.16	12.13	6.76	10.43	2.11

*Notes on calcn. C4 and C5 taken as C^{*}_{18} unsat.only and I.V. 112.1 (mean) used for C_{18} unsat. in other fractions. Total C_{18} unsat. in C then divided using isomerisation data into oleic 81.12%, lin 12.12%, len 6.76%. Esters in C8 taken as same as in C7; S.E. of hydrogenated material calculated and partitioned between C_{18} and C_{20}; C_{20} then taken as unsaturated (about -4.5 H) and C_{18} taken as in C6 (stearate negligible).

†C_{20} composition:- see totals on p.61.

Camel Fat.

Component acids of fractions (increments % weight).

	A	B	C	Total	Excluding non-sap. %wt.	Equivs.	%mol.
Myristic	1.24	3.93	1.08	6.25	6.3	.02745	7.4
Palmitic	25.67	2.63	0.39	28.69	28.8	.11228	30.5
Stearic	25.81	1.51	0.04	27.36	27.4	.09649	26.2
Arachidic	1.45	0.09	-	1.54	1.6	.00496	1.3
Tetradecenoic	-	0.18	0.36	0.54	0.5	.00239	0.6
Hexadecenoic	0.14	1.22	1.83	3.19	3.2	.01258	3.4
Octadecenoic	1.55	20.08	4.67	26.30	26.4	.09342	25.3
Octadecadienoic	-	0.38	1.48	1.86	1.9	.00667	1.8
Octadecatrienoic	-	0.09	0.83	0.92	0.9	.00330	0.9
"Eicosenoic"*	0.17	1.57	1.27	3.01	3.0	.00976	2.6
Non-saponifiable	-	0.08	0.26	0.34	-	-	-

*Mixture of unsat. acids higher than C_{18}; approx. monoethenoid in B and more than -4·0 H in C; an average value of -3·0 H was used for calculation of % mol.

Calculated means (Including N.S. of I.V. zero)	S.E.	I.V.
Glycerides	284.4	35.3
Mixed acids	271.7	37.0

Component acids of some "stearic-rich" animal fats (% wt.).

	Deer			Camel		Sheep	Ox	Pig	Hippopotamus
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)
Myristic	4.4	7		6.3	-	2-4	3.0	ca 1.0	2.3
Palmitic	25.1	35	65-70	28.8	37	23-28	29.2	28-30	27.1
Stearic	35.4	20		27.4	16	15-31	21.0	12-16	22.2
Arachidic	1.5	1		1.6	-	-	0.4	-	1.1
Tetradecenoic	0.5	-	-	0.5	-	-	0.6	ca 0.2	0.4
Hexadecenoic	2.8	-	-	3.2	-	1.0-2.5	2.7	ca 2.5	2.2
Octadecenoic	25.2	37	27-31	26.4	47	36-46	41.1	41-48	39.3
Octadecadienoic	2.6	-	3-5	1.9	-	4-6	1.8	6-7	3.5
Octadecatrienoic	2.5	-	-	0.9	-	-	-	-	1.5
C_{20-22} unsaturated acids	-	-	-	3.0	-	ca 0.5	0.2	ca 2.0	0.4

(a) and (d) Present work.
(b) Baughman, Jamieson and McKinney[23] (1929).
(c) Treadwell and Eckstein[24] (1939)
(e) Armstrong and Allan[26] (1924)
(f), (g) and (h) quoted by Hilditch[1] (1947, p.105)
(j) Barker and Hilditch[2] (1950).

Camel Fat.

Identification of individual acids.

Stearic. A9 yielded a sample of m.p. and mixed m.p. 69-70°

Palmitic. A3 " " " " " " " " 62-63°

Myristic. B1 " " " " " " " " 52-52½°

Oleic. B7 & 8 yielded dihydroxystearic acid " " 129-130°

Hexadecenoic. C2 & B3 yielded dihydroxypalmitic acid of m.p. 123-124½°. No authentic specimen was available.

Linoleic and Linolenic. C3 to 6 combined yielded tetrabromostearic acid of m.p. and mixed m.p. 111-113° and hexabromostearic acid of m.p. and mixed m.p. 179-181°.

Discussion on Deer and Camel Fats.

The final results for these two fats are shown on p.62 with some others of the "stearic rich" group. Since the other deer and camel reports are not as detailed as the present work, the figures are not directly comparable, but if we group the saturated and unsaturated acids, the red deer figures (a) fall within the range for the Virginia white-tailed deer (c) and apart from the distribution between palmitic and stearic acids, the values for the American reindeer (b) are fairly

similar to those of the red deer. The camel fat analyses (d) and (e) show an appreciable difference, the content of palmitic acid reported in the earlier work being remarkably high (37%). These differences are not unexpected considering the variation in different samples of the same species and also the approximate nature of the older methods.

Deer and camel fats are seen to be in the "stearic rich" group of animal depot fats and are more saturated than the usual sheep, ox and pig fats. This is shown in the higher content of stearic acid (particularly in the deer fat, 35.4%, which is well above the usual limit of 30% for sheep and ox tallows); palmitic is about the same and there is a fall in oleic acid, the amount of which is considerably lower in both fats. Among the minor components myristic is most abundant in both, followed by small amounts of arachidic, hexadecenoic and C_{18} polyethenoid acids with a trace of tetradecenoic acid. Though the deer fat contains only traces of C_{20-22} unsaturated acids, the camel contains 3% and these are grouped together as "eicosenoic" (average -3.0 H); these are probably ingested from the cod liver oil added to its diet.

The C_{18} unsaturated acids in both fats have been shown by isolation of derivatives to be the usual linoleic and linolenic acids found in seed fats and the amounts in deer fat are appreciable (2.6% and 2.5% respectively) though less in the

camel fat (1.9% and 0.9% respectively). The use in the absorption data calculations of the constants worked out for acids isolated from seed oils[18b] which are wholly cis, has therefore been justified. Different constants would have been necessary had these animal polyethenoid acids been isomeric and given different products after isomerisation or isomerised at a different rate[31] to those of the seed oil acids. The octadecadienoic acid in crocodile fat (Gunstone and Russell)[8b] has been shown by a different means to be the usual linoleic acid.

The hexadecenoic acid in deer fat yielded a dihydroxy derivative melting sharply at 116° (the usual derivative has m.p. 125° and was obtained from both camel and the python fat); this derivative analysed correctly, but there was not sufficient material to investigate the acid further. A similar dihydroxy derivative (m.p. 115°) of a hexadecenoic acid isolated from sperm oil has been reported by Hilditch and Lovern[12], but the position of the double bond was not determined.

PYTHON FAT.

Introduction.

The only information available on python fat is given by Tsujimoto and Kobayaschi[26] (1920) who studied a python oil of iodine value 80.3, saponification value 194.1 and acid value 0.6 which yielded some ether insoluble polybromides (2.1%) indicating the presence of highly unsaturated acids, probably of the C_{20} and C_{22} series. Pollard and McLaughlin[27] (1950) have given the characteristics of some other snake oils and follow this by a component acid analysis of a cottonmouth moccasin depot fat[8a] (1952). A few detailed analyses of the depot fats of amphibia and reptilia have been published and these are tabulated on p.74.

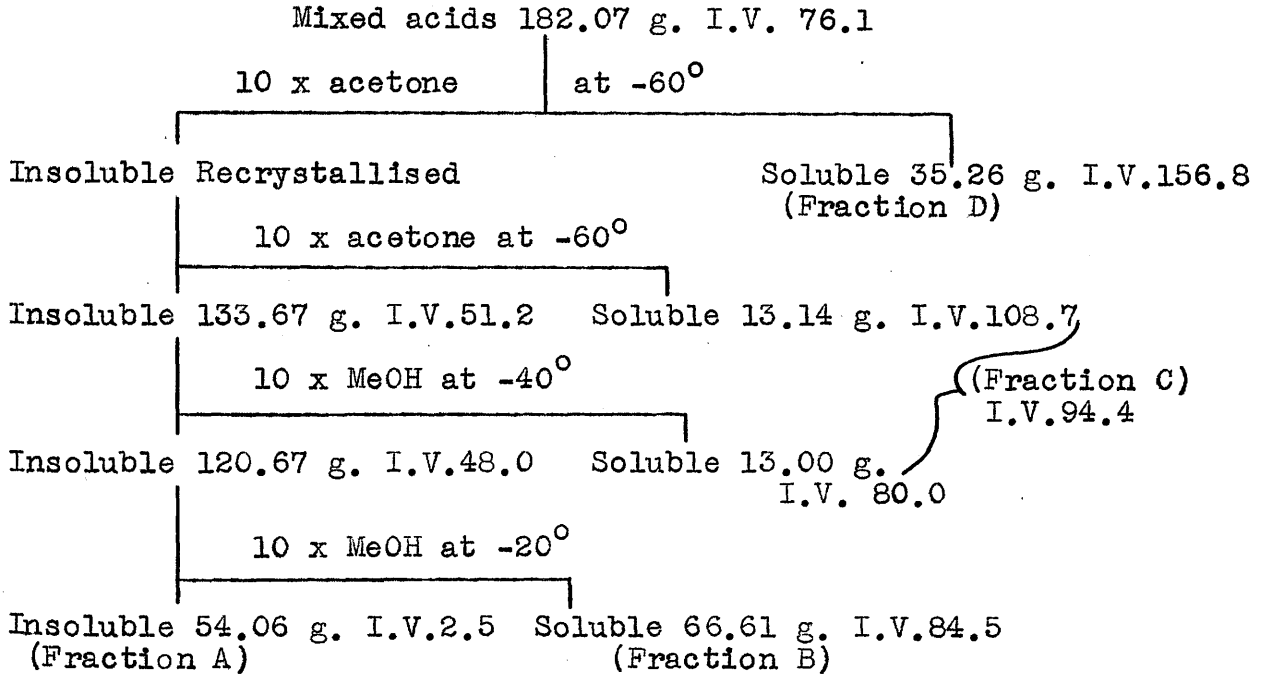
The sample of python depot fat sent from Belle Vue Zoo, Manchester, was from a female reticulated python (Python reticulatus) and was probably attached to the intestines. The snake, 19 feet long and 21 inches around its greatest girth, was fed on goats, ducks, hens and rabbits. The crude fat was in lobes and was autoclaved at 120° for 40 minutes, then the easily burst tissue was crushed by mortar and extracted with acetone to give 580 g. of white, semi-solid fat, the characteristics of which are given with the other data.

Results.

Python fat:- I.V. 73.0, S.E. 288.5, F.F.A. 0.3 (as oleic)

Mixed acids:- I.V. 76.1, S.E. 275.9.

Low temperature crystallisation of acids (weights corrected)



Further details of acid fractions

	Mixed	A	B	C	D
Percentage	100	29.69	36.58	14.36	19.37
Iodine value	76.1	2.5	84.5	94.4	156.8
$E^{1\%}_{1cm.}$ Unisomerised	-	-	-	-	-
" Isomerised 234 mμ	-	-	-	120.0	468.6
268 mμ	-	-	-	4.0	77.6
300 mμ	-	-	-	0	15.3
315 mμ	-	-	-	0	12.3

Esters of acid fractions

	A	B	C	D
Iodine value	2.7	80.6	88.9	150.2
S.E.	282.5	294.8	292.1	-

Python Fat. Distillation of A esters (T.117 Press. 0.4 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	C_{16}	C_{18}	C_{20}	Unsat.(g.) C'_{18}	C'_{20}	N.S.
A1	2.90	0.2	267.4	0.29	2.60	-	-	0.01	-	-
A2	2.87	0.2	270.1	0.04	2.82	-	-	0.01	-	-
A3	3.13	0.2	270.8		3.09	0.03	-	0.01	-	-
A4	3.04	0.2	271.1		2.96	0.07	-	0.01	-	-
A5	3.86	0.1	270.7		3.82	0.04	-	0.00	-	-
A6	3.89	0.2	270.7		3.85	0.03	-	0.01	-	-
A7	3.56	0.2	271.0		3.49	0.06	-	0.01	-	-
A8	3.47	0.6	272.6		3.17	0.28	-	0.02	-	-
A9	3.60	5.0	285.6		1.56	1.83	-	0.21	-	-
A10	3.04	7.3	298.3			2.78	-	0.26	-	-
A11	3.70	5.9	299.2			3.34	0.10	0.23	0.03	-
A12	3.34	4.5	300.2			2.95	0.21	0.15	0.03	-
A13	4.96	3.4	299.5			4.57	0.19	0.18	0.02	-
A14	2.96	9.7	324			1.20	1.35	0.12	0.19	0.10
Total or (mean)	48.32	(2.3)	(283.0)	0.33	27.36	17.18	1.85	1.23	0.27	0.10
A14 Acids ex N.S. I.V. 9.2, S.E. 298.7.			% Esters	0.68	56.62	35.55	3.83	2.55	0.56	0.21
			% Acids	0.67	56.48	35.64	3.86	2.56	0.57	0.22

Python Fat. Distillation of B esters (T.117 Press. 0.2 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	Sat.(g.) C_{16}	Sat.(g.) C_{18}	Unsat.(g.) C'_{16}	Unsat.(g.) C'_{18}	Unsat.(g.) C'_{20}	N.S.
B1	2.79	26.4	268.7	0.10	1.91		0.78	-		
B2	2.85	70.4	290.2		0.52		0.11	2.22		
B3	3.20	83.6	294.6		0.07			3.13		
B4	3.24	84.8	295.2		0.03			3.21		
B5	2.95	85.3	295.0		0.01			2.94		
B6	3.04	85.3	294.9		0.01			3.03		
B7	3.36	85.3	294.8		0.01			3.35		
B8	3.42	85.6	295.4					3.42		
B9	3.13	85.4	295.6			0.01		3.12		
B10	3.16	85.2	295.1			0.01		3.15		
B11	3.12	85.2	295.4			0.01		3.11		
B12	3.34	85.3	295.7			0.01		3.33		
B13	3.54	85.1	295.6			0.02		3.52		
B14	4.99	84.8	295.7			0.05		4.94		
B15	2.55	82.9	298.0			0.10		3.46	0.21	0.03
B16	1.25	67.5	300.5							
Total or (Mean)	49.93	(80.4)	(293.6)	0.10	2.56	0.21	0.89	45.93	0.21	0.03
B16 N.S. Direct = 0.03 g.			% Esters	0.20	5.13	0.42	1.78	91.99	0.42	0.06
			% Acids	0.20	5.11	0.42	1.77	92.02	0.42	0.06

Isomerisation:- B8 gave zero absorption at 234, 268, 300 and 315 mu.

Python Fat. Distillation of C esters (T.117 Press. < 0.1 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	Sat.(g.) C_{16}	Unsat.(g.) C'_{14}	Unsat.(g.) C'_{16}	Unsat.(g.) C'_{18}	Unsat.(g.) C''_{18}	Unsat.(g.) C^{*}_{20}	N.S.
C1	1.635	21.8	246.7	1.069	0.219	0.262	0.085	-	-	-	-
C2	1.548	59.1	271.7		0.591		0.805	0.126	0.026	-	-
C3	1.766	79.8	285.8		0.347		0.301	0.927	0.191	-	-
C4	1.931	93.6	292.8		0.129		0.096	1.414	0.292	-	-
C5	1.966	98.8	294.3		0.035			1.600	0.331	-	-
C6	1.822	100.6	294.7					1.510	0.312	-	-
C7	1.958	100.5	295.1					1.625	0.333	-	-
C8	2.029	100.8	295.1					1.677	0.352	-	-
C9	1.894	100.4	294.0					1.574	0.320	-	-
C10	1.861	100.1	294.5					1.553	0.308	-	-
C11	1.915	99.4	294.7					1.613	0.302	-	-
C12	2.276	98.6	301.5					1.525	0.286	0.465	-
C13	2.04	81.3	336					0.168	0.032	1.750	0.09
Total or (mean)	24.641	(88.7)	(292.0)	1.069	1.321	0.262	1.287	15.312	3.085	2.215	0.09
C13 N.S. direct = 0.09 g.			% Esters	4.34	5.36	1.06	5.22	62.14	12.52	8.99	0.37
			% Acids	4.29	5.34	1.05	5.20	62.18	12.52	9.03	0.39

Spectroscopic data ($E^{1\%}_{1\ cm.}$)

Fraction	I.V.	Unisomerised (mμ) 234	268	300	315	Isomerised (mμ)+ 234	268	300	315
C2	62.3	-	-	-	-	14.7	4.0	0	0
C8	105.8	9.9	(0.4)	0	0	152.1	0	0	0
C13	-	41.0	(16.1)	(6.9)	(5.1)	80.2	31.4	10.5	9.0
C acids	94.4	-	-	-	-	120.0	4.0	0	0

+ Isomerised at 170° for 15 minutes. See "Method of calculation" for calculation.

* C_{20} of S.E. 324.1, I.V. 95.4 (mainly monoethenoid + trace of triethenoid).

Python Fat. Distillation of D esters (T.117 Press. < 0.1 mms.)

Fract.	Wt.(g.)	I.V.	S.E.	Sat.(g.) C_{14}	Unsat.(g.) C'_{14}	"C_{16}"	"C_{18}"	C^{*}_{20}	N.S.
D1	2.65	68.0	255.0	0.71	0.55	1.39	-		
D2	2.73	95.2	271.5			2.43	0.30		
D3	3.11	128.9	285.8			1.09	2.02		
D4	3.70	151.9	294.6			0.13	3.57		
D5	2.80	154.8	294.6				2.80		
D6	2.97	154.7	294.4				2.97		
D7	3.23	153.9	295.2				3.23		
D8	3.35	152.7	294.7				3.35		
D9	3.18	152.8	295.6				3.18		
D10	3.17	177.8	306.4				1.72	1.45	
D11	3.63	196.5	341				0.38	2.86	0.39
Total or (mean)	34.52	(146.8)	-	0.71	0.55	5.04	23.52	4.31	0.39

D11 Acids ex N.S. I.V. 214.8, S.E. 304 N.S. direct = 0.39 g.

	C_{14}	C_{16}	C'_{14}	C'_{16}	C''_{16}	C'_{18}	C''_{18}	C'''_{18}	C^{*}_{20}	N.S.
Total(g)	0.71	0.43	0.55	4.53	0.08	6.42	15.57	1.34	4.31	0.39
% Esters	2.06	1.25	1.59	13.12	0.23	18.60	45.65	3.88	12.49	1.13
% Acids	2.04	1.24	1.57	13.06	0.23	18.61	45.64	3.88	12.54	1.19

Spectroscopic data ($E^{1\%}_{1\ cm.}$)

Fraction	I.V.	Unisomerised (mμ) 234	268	300	315	Isomerised (mμ) 234	268+	300+	315+
D2	100.8	6.5	(0.7)	-	-	82.0	2.9	-	-
D7	161.3	9.0	(0.7)	-	-	627.1	31.6	0	0
D11	216.3	89.7	52.7	(18.5)	(13.8)	350.0	319.8	113.3	99.2
D Acids	156.8	-	-	-	-	468.6	77.6	15.3	12.3

+ Isomerised at 170°/15 mins. *C_{20} esters taken as S.E. 321.3, I.V. 205.7. For calculation see "Method of calculation".

Python Fat.

Component acids of fractions (increments % weight).

	A	B	C	D	Total	Excluding non-sap. %(wt.)	Equivs.	%(mol.)
Myristic	0.19	0.07	0.62	0.40	1.28	1.3	.005604	1.6
Palmitic	16.77	1.87	0.77	0.24	19.65	19.7	.076947	21.2
Stearic	10.58	0.15	-	-	10.73	10.8	.037857	10.5
Arachidic	1.15	-	-	-	1.15	1.2	.003680	1.0
Tetradecenoic	-	-	0.15	0.30	0.45	0.5	.001988	0.5
Hexadecenoic	-	0.65	0.75	2.53	3.93	3.9	.015488	4.3
Hexadecadienoic	-	-	-	0.04	0.04	Tr	.000158	Tr
Octadecenoic	0.76	33.67	8.92	3.60	46.95	47.0	.166876	46.0
Octadecadienoic	-	-	1.79	8.85	10.64	10.7	.038085	10.5
Octadecatrienoic	-	-	-	0.75	0.75	0.8	.002694	0.7
As Eicosenoic*	0.17	0.15	1.30	2.43	4.05	4.1	.013199	3.7
Non-saponifiable	0.07	0.02	0.06	0.23	0.38	-	-	-

*Includes all unsaturated acids higher than C_{18} (average unsaturation -4.1H)

Calculated means (including N.S. of I.V. zero)	S.E.	I.V.
Glycerides	289.6	71.5
Mixed acids	276.9	74.8

Component acids of amphibian and reptile fats (% wt.).

	Python (a)	Frog (b)	(c)	Lizard (d)	Tortoise (e)	Moccasin (snake) (f)
Myristic	1	4	4	4	1	2
Palmitic	20	11	18	29	14	17
Stearic	11	3	7	10	4	9
Arachidic	1	-	-	-	-	-
Hexadecenoic	4	15	10	12	9	7
Octadecenoic	59 (-2.4H)	52 (ca -2.5H)	56 (-2.4H)	40 (-2.7H)	65 (-2.4H)	53 (-2.6H)
Higher unsaturated acids	4 (-4.1H)	15 (ca -6H)	5 (ca -5H)	5 (-5.5H)	7 (ca -4H)	12 (?)

(a) Present work.
(b) Klenk[28] (1933)
(c) and (e) Klenk, Ditt and Diebold[29] (1935)
(d) Hilditch and Paul[30] (1937)
(f) Pollard and McLaughlin[8a] (1952).

Python Fat.

Identification of individual acids.

Stearic. A10 yielded a sample of m.p. and mixed m.p. 69-71°

Palmitic. A4 " " " " " " " " 62-63°

Hexadecenoic. D2 yielded dihydroxypalmitic acid of m.p. 123½-125½°, undepressed by the sample of this acid from camel fat; no reference compound was available.

Oleic. B8 yielded dihydroxystearic acid of m.p. and mixed m.p. 130-131°.

Linoleic and Linolenic. D7 gave 2.1 g. of mixed acids. The tetrabromide number of this sample was found using the method described by Markley[22].

1.450 g. of tetrabromides were obtained from 2.102 g. of acids

$$\therefore \text{Tetrabromide number} = \frac{1.450}{2.102} \times 100$$

$$\text{percentage of linoleic acid} = \frac{1.450 \times 100}{2.102 \times 90.6} \times 100 = 77\%.$$

This was slightly higher than from isomerisation data (67%).

The tetrabromostearic acid had m.p. and mixed m.p. 112-113½°.

The hexabromostearic acid (small amount) had a m.p. of 175-178° which was raised to 181-182° when mixed with an authentic specimen.

Discussion.

The final python results are summarised on p.74 with other amphibian and reptile analyses. These results show that python fat is, like those of the others in this class, intermediate between marine and higher land animals in the nature of its component acids, a generalisation first pointed out by Klenk[28]. Comparing these values, we have (figures in parenthesis are average values for marine and higher land animal fats respectively); palmitic acid, 20% (12-15%, 25-30%); stearic acid, 11% (very small, values rising to over 30%); and also in the mean unsaturation of the C_{18} unsaturated acids, -2.4H (unsaturation more than -3.0 H, slightly greater than -2.0 H). The figures for hexadecenoic (4%) and C_{20-22} unsaturated acids (4%) are both closer to those of the higher land animals than other examples shown, but python fat is clearly similar in composition to those of the other reptiles and amphibians.

As in the deer and camel fats, the C_{18} unsaturated acids were estimated by spectrographic means using the seed acid constants, and again this has been justified by isolation of the tetrabromide (in high yield) and the hexabromide which were identical with those of the usual linoleic and linolenic acids. In the case of the linoleic acid the tetrabromide number was estimated and this confirmed within experimental

error the value found by spectrographic means. The C_{18} unsaturated acids (oleic 47.1%, linoleic 10.7% and linolenic 0.8%) vary somewhat from those given for the moccasin fat (oleic 37.2% and linoleic 16.6%). The spectrographic data also indicates small, though scarcely significant amounts (1-2% of the C_{18} diene acids) of isomeric octadecadienoic acids; relatively larger amounts of these isomers have been reported in hippopotamus fat[2]. Evidence of isomers of linoleic acid has been found in horse fat[4b].

Lack of information makes it still difficult to distinguish the individual acids in the C_{20-22} unsaturated group. These have therefore been grouped as "eicosenoic" acid (-4.1H) though spectrographic data indicates that some tetraethenoic acids are present (Fractions C13 and D11).

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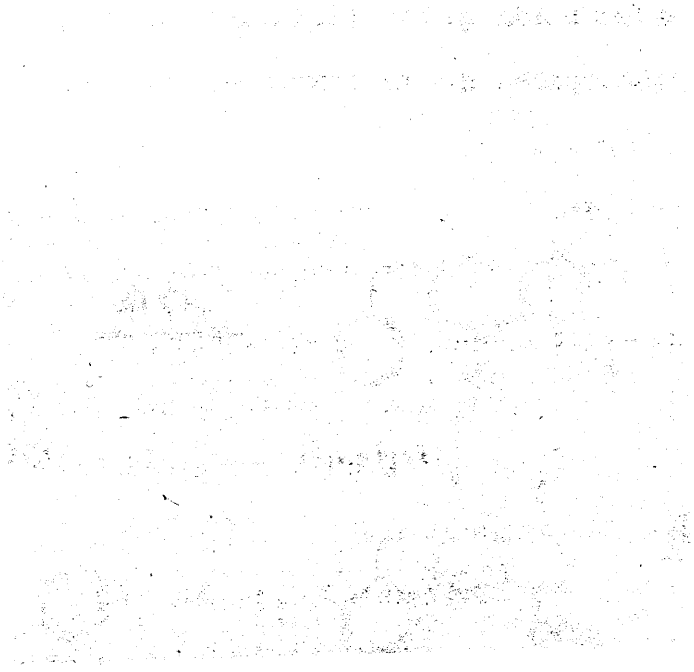
PART II.

Cyclisation in the Friedel-Crafts Ketone Synthesis.

SUMMARY.

Cyclisation in the Friedel-Crafts ketone synthesis. In an attempt to prepare 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone by the Friedel-Crafts interaction of 3-methoxy-2-naphthoyl chloride and 2-methylnaphthalene, previous workers obtained a dark red compound when carbon disulphide was the solvent. Under the same conditions but with methylene chloride as solvent a different, yellow compound was produced. In this research the factors controlling formation of each compound have been determined, the structure of the yellow compound has been shown to be that of the expected ketone and the nature of the red compound has been investigated. The yellow ketone has been pyrolysed in an attempt to prepare 4-methoxy-1:2-5:6-dibenzanthracene. The red compound is thought to be formed by demethylation and oxidative cyclisation, and the structure 4-hydroxy-5-methyl-1:2-8:9-dibenz-10-anthrone has been proposed. Comparison of its spectrum with that of the parent 1:2-8:9-dibenz-10-anthrone strongly supports this theory. The red compound has been degraded in stages to phthalic acid and water soluble acids. A lactonic structure consistent with its properties has been assigned to an intermediate product, but this has not been rigorously proved. A number of attempts to degrade the red

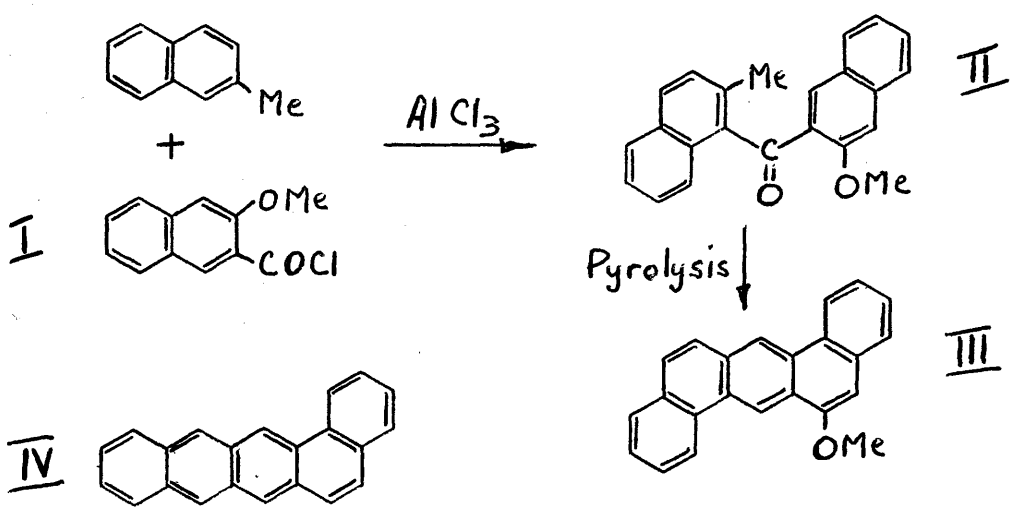
compound by other means have been unsuccessful; oxidation of its methyl ether gave the same lactone as did the red compound itself. The red compound has been prepared directly from the yellow ketone by the Scholl benzanthrone synthesis and at low temperature in methylene chloride. An attempt to prepare the parent 1:2-8:9-dibenz-10-anthrone by the Scholl synthesis failed.



INTRODUCTION

During the investigation of the metabolism of the carcinogen 1:2-5:6-dibenzanthracene in rabbits, Cook and Schoental[1] isolated through its methyl ether an oxidation metabolite of the compound. This was shown to be a monomethyl ether and of the 7 possible isomers, all but the 4 and 4'-methoxy-1:2-5:6-dibenzanthracenes have been eliminated by m.p. or considered unlikely. These two have not yet been synthesised.

A preliminary trial by the above authors[2] to synthesise the 4-methoxy compound (III) using the following route was unsuccessful, the methoxyl group being removed by



the high temperature of the last stage: the only product isolated was 1:2-benznaphthacene (IV).

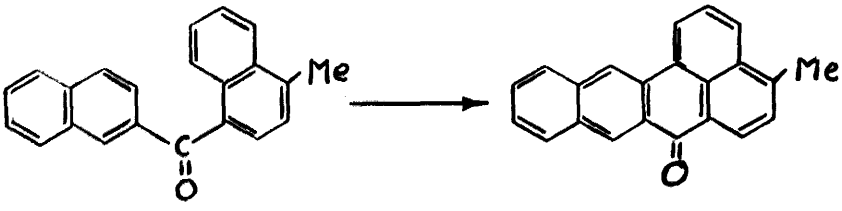
In the first attempt to obtain 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II) by means of the Friedel-Crafts reaction, carbon disulphide was used as a solvent with 2 mol. of aluminium chloride which was added to 1 mol. of each of the reactants in the solvent at 0°. The product from this reaction gave dark red needles (cpd. A) which melted with decomposition at 186° and which analysed to suit $C_{22}H_{14}O_2$ or $C_{23}H_{16}O_2$. A small amount of a colourless compound of m.p. 172-3° (cpd. B) was also isolated from the mother liquors; this analysed for $C_{23}H_{18}O_2$, fitting the ketone formula.

The Friedel-Crafts reaction was repeated under the same conditions and with the same proportion of reactants, but methylene chloride was used as solvent. The product in this case, after distillation, gave pale yellow prisms of m.p. 108-10° (cpd. C) which analysed to suit $C_{23}H_{18}O_2$ and was evidently an isomer of the colourless ketone. Pyrolysis of this yellow ketone (200 mg.) yielded a small amount of golden yellow leaflets which were shown to be 1:2-benznaphthacene (IV) by ultra violet spectrum and mixed m.p.

The present work set out to investigate the Friedel-Crafts reaction, identify the different products and repeat the final stage on a larger scale in the hope of isolating some of the required 4-methoxy-1:2-5:6-dibenzanthracene. In

view of the steric effect of the substituents, the yellow ketone could possibly be an isomer of the desired ketone (II).

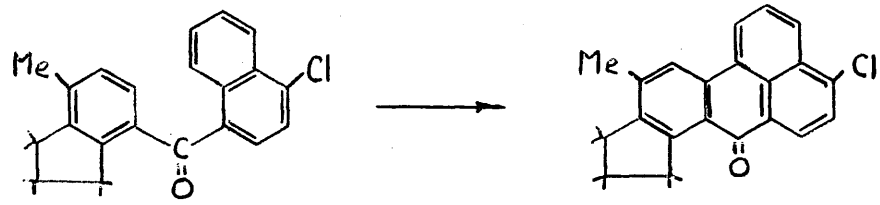
The dark red colour of the first Friedel-Crafts product (cpd. A) obtained in the above synthesis suggests ring closure of some kind. Oxidative cyclisation of certain aryl ketones to form benzanthrones in the attempted preparation of these ketones by the Friedel-Crafts reaction has been reported by Buckley[3]. He has found that in carbon disulphide the expected ketones are formed in presence of 1 mol. of aluminium chloride, while 2 mol. or more led in certain cases to this oxidative ring closure in good yield to form mesobenzanthrones. Interaction of 2-naphthoyl chloride with 1-methylnaphthalene gave this result, but not with 2-methylnaphthalene, naphthalene, 1-bromonaphthalene or 8-methylquinoline. The benzanthrone in poor yield was also obtained by treatment of the ketone itself in the solvent with aluminium chloride. This cyclisation took place in carbon disulphide solution but not in benzene, nitrobenzene or petroleum-ether.



V

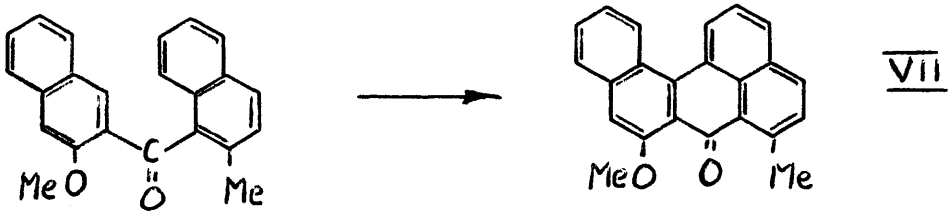
The cyclised product from 2-naphthoyl chloride and 1-methylnaphthalene was shown by Buckley, using degradation, to be 7-methyl-2:3-8:9-dibenz-10-anthrone (V).

Fieser and Desreux[4] have reported a similar cyclisation in the Friedel-Crafts preparation of 4-methyl-7-(4'-chloro-1'-naphthoyl)-hindrindene (VI) in tetrachloroethane using about $2\frac{1}{2}$ mol. of aluminium chloride with respect to the acid chloride. The product was yellow and behaved

VI 

like a benzanthrone, but the structure was not verified. These authors also obtained the cyclised material in poor yield by treatment of the ketone in tetrachloroethane at 25° with aluminium chloride.

The above cases are therefore examples of low temperature ring closure of the Scholl type[5] which usually requires temperatures of 100° or above.

II 

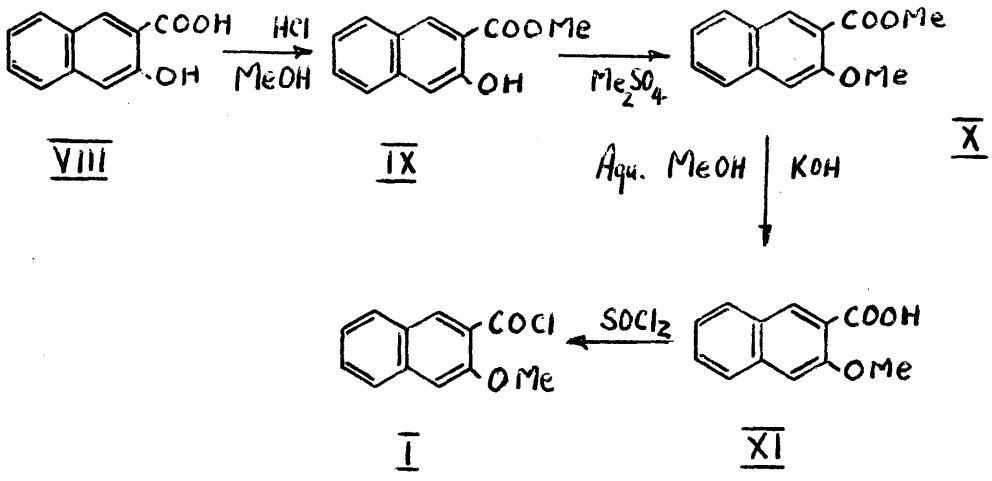
VII

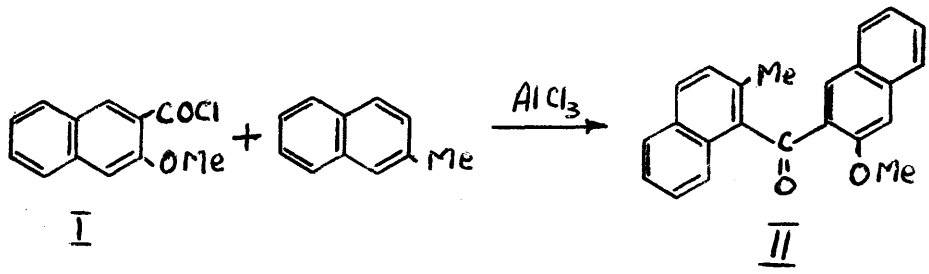
In the present case ring closure of 2-methyl-3'-methoxy-1:2'-dinaphyl ketone(II) would give 4-methoxy-5-methyl-1:2-8:9-dibenz-10-anthrone(VII).

Since Buckley found that the interaction of 2-naphthoyl chloride with 2-methylnaphthalene did not lead to cyclisation and since the solvent rather than the proportion of aluminium chloride seemed to be the controlling factor in the present case, it was decided to investigate the effect of varying the Friedel-Crafts conditions.

DISCUSSION

The starting material in this work was 3-hydroxy-2-naphthoic acid (VIII). The methoxy acid (XI) was obtained via the methoxy ester (X) which was prepared in good yield,





by first forming the methyl ester of 3-hydroxy-2-naphthoic acid (IX) and refluxing this in acetone with dimethyl sulphate in presence of potassium carbonate. A similar result in slightly lower yield was obtained by refluxing the potassium salt of the ester with methyl iodide in acetone

containing sufficient water to keep the salt in solution. Lesser et al.[6] obtained the compound from the hydroxy ester (IX) using methanolic potassium hydroxide and dimethyl sulphate at room temperature, but on trial this treatment yielded mainly starting material: possibly the temperature was too low to start the reaction since it was found necessary to reflux in the successful experiments. By forming the methoxy ester it was possible to obtain a pure product, removing any unchanged phenolic starting material by washing with cold alkali. The methoxy ester was then hydrolysed to 3-methoxy-2-naphthoic acid (XI) and converted to the acid chloride (I) by treatment with thionyl chloride in boiling ether.

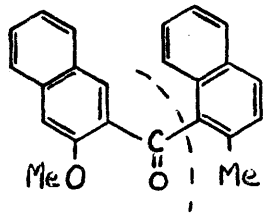
3-methoxy-2-naphthoyl chloride was made to react with 2-methylnaphthalene in a series of eight Friedel-Crafts experiments to find the effect of varying the solvent, the proportion of aluminium chloride and the order of adding the reactants. Nitrobenzene was avoided as a solvent in view of the work of Baddeley[7]. He has shown that in the Friedel-Crafts reaction of benzoyl chloride on naphthalene, considerable β-substitution takes place when the acid chloride-aluminium chloride complex is made more bulky. This is achieved by adding reagents capable of combining with the complex, such as nitrobenzene, nitromesitylene and excess of the acid chloride. In addition, the substituents in the present case are both

adjacent to the point of reaction and probably hinder attack at the α position. This became evident when a Stuart (Catalin) model of the ketone was constructed and was supported by later work on the Grignard reaction.

It will be seen from the summarised results (p.90) that cyclisation takes place in the presence of 2 mol. of aluminium chloride in methylene chloride as well as carbon disulphide while the yellow ketone (cpd. C) is formed by 1 mol. of the reagent; the order of addition of the reactants does not affect the results. This supports the conclusions of Buckley and extends this type of reaction to methylene chloride as well as carbon disulphide and tetrachloroethane[4].

The yield of the yellow compound C was rather low (6%) in all cases. The crude distilled gum was slow to crystallise and afforded only a small amount of pure ketone. In the light of later work this was probably due to the presence of some demethylated material and other isomers, one of which was isolated by Miss Schoental and confirmed here.

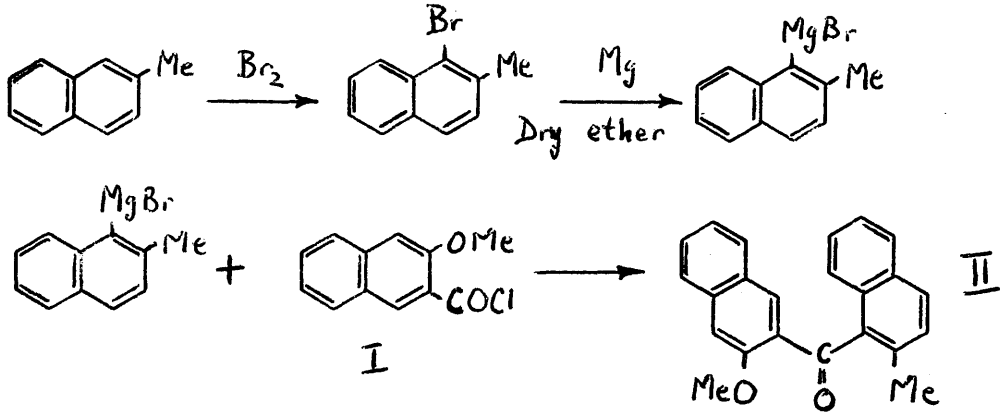
An attempt to check the structure of the yellow compound C by fusion in potassium hydroxide at 260° brought about



Expt.	Mol. $AlCl_3$	Solvent	Conditions	Result
1	2	CS_2	$AlCl_3$ added to solution of both reactants.	Red gum. Distillation gave some yellow compound D.
2	2	CH_2Cl_2	" " " "	Crystals of red compound A
3	1	"	Naphthalene complex soln. added to acid chloride soln.	Brown gum. Distillation gave yellow compound D.
4	2	"	Naphthalene soln. added to acid chloride complex.	Crystals of red compound A.
5	1	"	Acid chloride soln. added to naphthalene complex.	Yellow compound D.
6	1	CS_2	" " " "	" "
7	1	C_6H_6	" " " "	" "
8	1	$C_2H_2Cl_4$	" " " "	" "

scission mainly on one side and the acid fraction yielded a large proportion of 3-hydroxy-2-naphthoic acid. The residue was esterified and the phenolic fraction removed, but the remaining small amount of gum yielded only a few very impure crystals and the required 2-methyl-1-naphthoic acid was not obtained.

On account of the low yield from the Friedel-Crafts reaction and the failure of the fusion, an unambiguous synthesis was attempted via the Grignard compound. 2-methylnaphthalene was brominated in good yield in the absence of light at 0° using iodine and iron powder. The bromide reacted with magnesium without the use of a primer and part of the Grignard compound was converted to the acid with carbon dioxide: this had the correct m.p. for 2-methyl-1-



naphthoic acid.

The rest of the Grignard solution was added to the 3-methoxy-2-naphthoyl chloride in ether at -10°: the reaction

was completed by reflux. Distillation of the product gave a large amount of 2-methylnaphthalene and a small amount of a fairly pure material which formed colourless prism of m.p. 104-6°; this was considerably lower than that of the colourless ketone (m.p. 172-3°) from the Friedel-Crafts reaction. This compound analysed for $C_{23}H_{18}O_3$ and was shown by hydrolysis to be an ester of 3-methoxy-2-naphthoic acid with 2-methyl-1-naphthol. Some oxidation of Grignard compound had obviously taken place and a second attempt was made in an atmosphere of nitrogen; the reflux temperature was raised by running in dry benzene and distilling off the ether. A little of the same ester was obtained along with most of the starting material; no evidence of the ketone was found. The explanation of this negative result may be that the Grignard complex is sterically hindered by the substituents.

The less bulky lithium compound was an obvious next choice. Lithium reacted readily with 1-bromo-2-methylnaphthalene and the lithium compound solution was added slowly to an ethereal solution of the acid chloride at 0°. The whole reaction was carried out under nitrogen and again benzene replaced ether for the final reflux. The distilled product crystallised to give the same yellow ketone (cpd.C) as from the Friedel-Crafts reaction; the yield was higher (15%). The structure of the yellow ketone was thus shown to

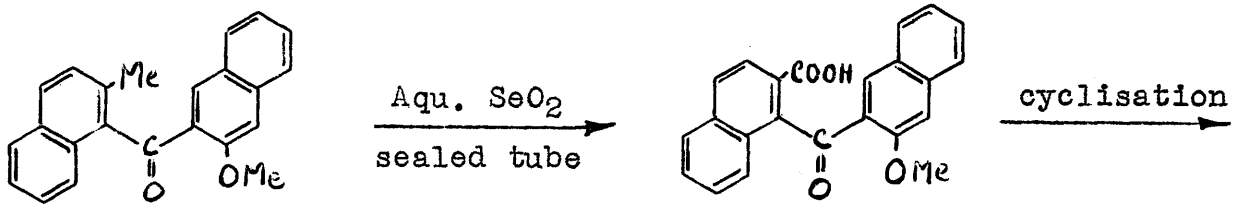
be the required 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II).

The yellow ketone (II) was pyrolysed with the precaution of avoiding too high a temperature in the hope of retaining the methoxyl group. Distillation of the product afforded a yellow crystalline material in 25% yield. Since 1:2-benznaphthacene (IV) was isolated from this reaction by Miss Schoental and it is capable of forming a maleic anhydride adduct, this technique was used to purify the product. The solution became colourless during adduct formation and the non-adduct fraction yielded colourless plates of 1:2-5:6-dibenzanthracene: no evidence of the methoxydibenzanthracene was found. The adduct was sublimed from potassium hydroxide to form yellow plates of 1:2-benznaphthacene. The Elbs pyrolysis had therefore removed the methoxyl group; this confirmed and extended Miss Schoental's results.

Loss of a methoxyl group in an α position of a ring involved in cyclisation during the Elbs pyrolysis was reported by Fieser and Desreux[4] in the preparation of 6-methoxy-20-methylcholanthrene. In the present case the methoxyl group is in a β position, but it is also in the ring involved in the cyclisation and is probably too near the point of reaction. The corresponding 2- and 3-methoxy-20-methylcholanthrenes have been obtained by the above authors using the Elbs pyrolysis, but in those cases the methoxyl groups are in the ring remote

from the site of ring closure.

An attempt to oxidise the methyl group to carboxyl using aqueous selenium dioxide[8] at 230-240° in a sealed tube



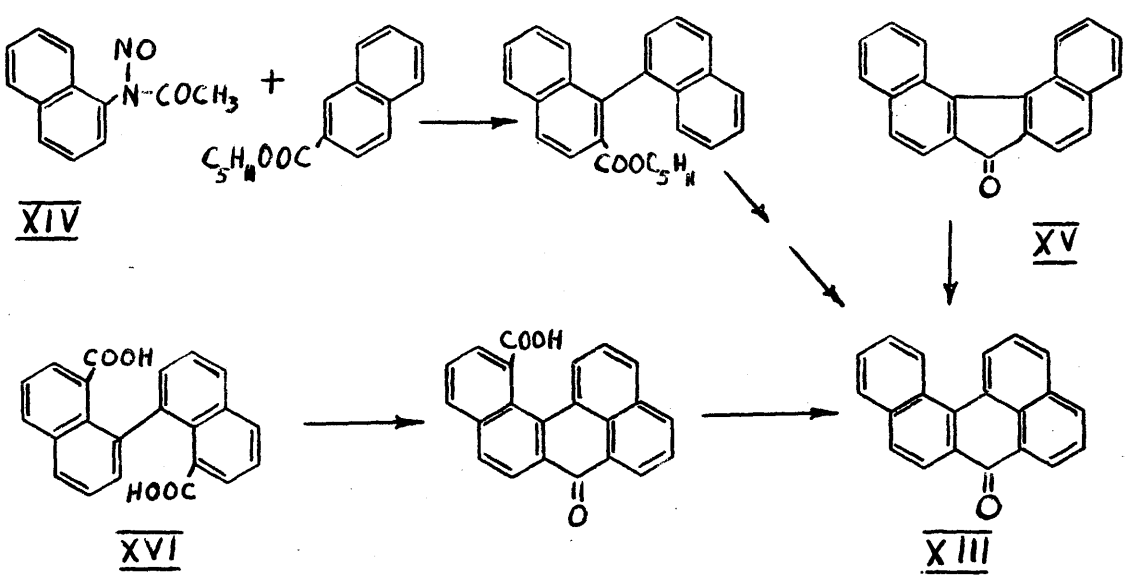
II XII

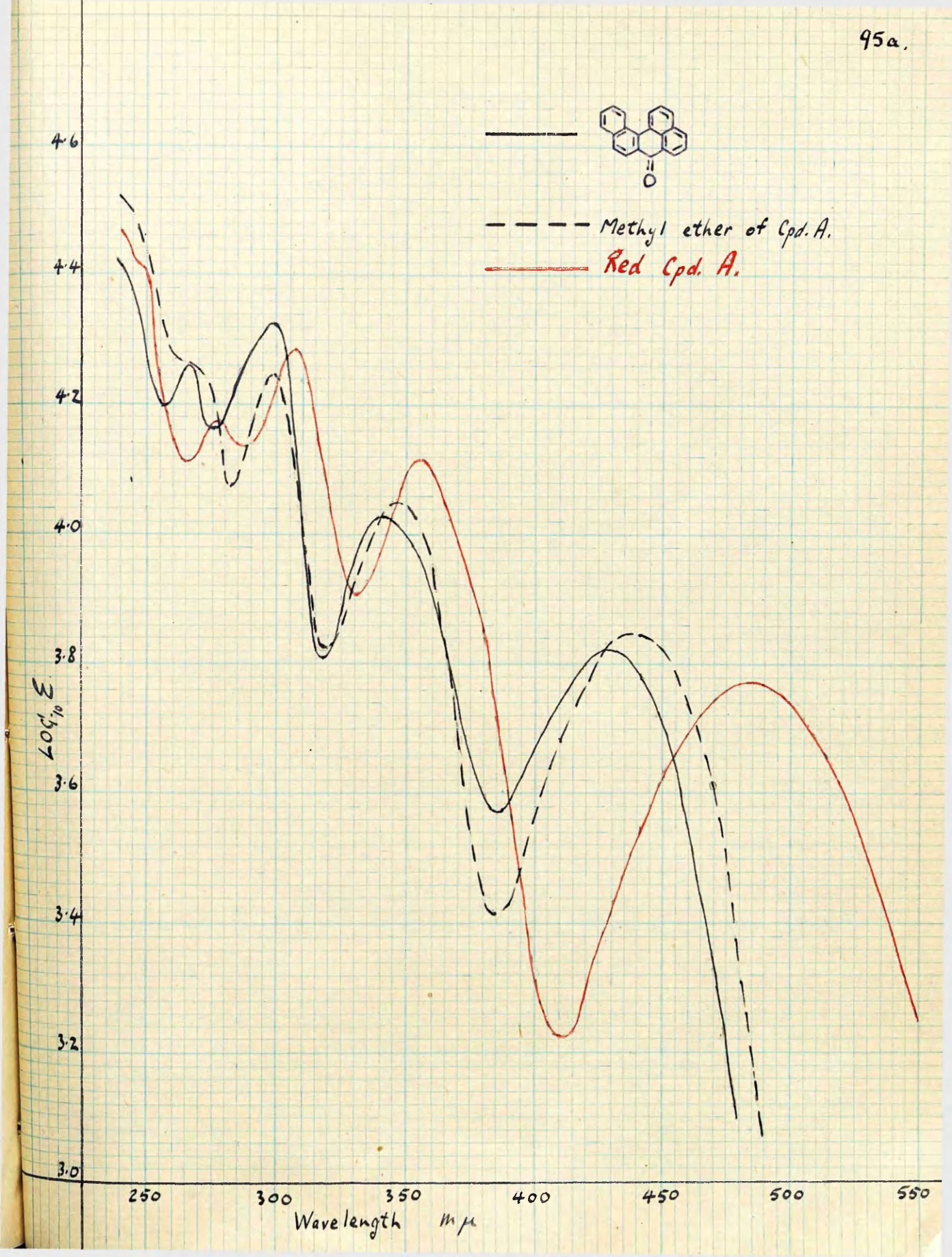
was unsuccessful. The product was almost wholly insoluble in boiling alkali and contained a substantial amount of unchanged ketone. If the acid (XII) could be obtained it should be possible to reduce the keto group, cyclise and aromatise to the 4-methoxy-1:2-5:6-dibenzanthracene. Lack of time prevented further work on this synthesis, though it was hoped by other means to oxidise the methyl group; an alternative method might be to brominate the methyl to $-CH_2Br$ using N-bromosuccinimide, followed by hydrolysis and oxidation to the acid.

Studies of the structure of the red Friedel-Crafts product (cpd. A).

Reference to compounds of similar structure showed that 4-methoxy-5-methyl-1:2-8:9-dibenz-10-anthrone (VII) should be yellow or orange. It seemed therefore that the red compound A might be a hydroxy compound resulting from demethylation by aluminium chloride during the Friedel-Crafts reaction and this has been proved by formation of both the acetate and the methyl ether which, as expected, are yellow. The hydroxyl group, however, is only feebly acidic; in the absence of moisture it forms a purple sodium salt, but this is quickly hydrolysed in moist air with return of the red colour.

1:2-8:9-dibenz-10-anthrone (XIII) which has the same



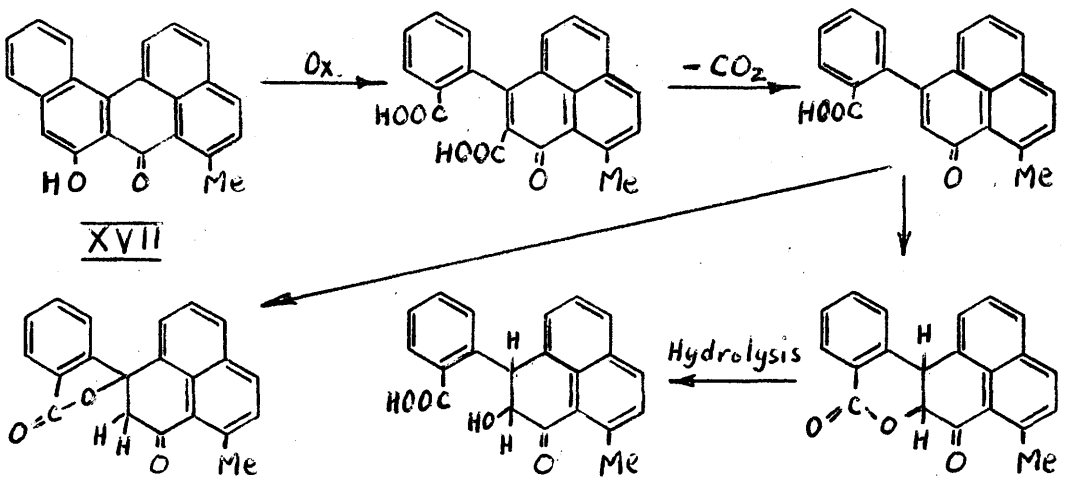

Methyl ether of Cpd. A.
Red Cpd. A.
O
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
Log10 ε
250
300
350
400
450
500
550
Wavelength mμ

ring system as the proposed structure for the red compound A, has been synthesised by three different methods. One route starts with amyl-2-naphthoate and N-nitrosoaceto-1-naphthalide (XIV) (Swain and Todd[9]): another involves aluminium chloride fusion of 3:4-5:6-dibenzfluorenone (XV) (Martin[10]) and a third is the unambiguous route consisting of cyclisation of one carboxyl group in 1:1'-dinaphthyl-8:8'-dicarboxylic acid (XVI)[11], followed by decarboxylation (Bradley and Sutcliffe[12]). A sample of 1:2-8:9-dibenz-10-anthrone (XIII) was kindly supplied by Professor A.R. Todd[9] and its ultra violet and visible absorption spectrum has been compared with those of the red compound A and its methyl ether (p.95a.). It is obvious from this that the structures of these three compounds are very similar and there is little doubt that the red compound is a benzanthrone.

To prove the structure conclusively, the present work has aimed at degrading the red compound by oxidation or other means to a compound of known structure.

Oxidation of the red compound A has yielded a colourless lactone (cpd. D) of molecular weight about 300 and which hydrolysed to an acid (cpd. E) with only one carboxyl group. From this information it is evident that the molecule of compound A has not been degraded to any great extent, but since the product is colourless, the chromophoric system has been

altered. The conclusions are that the hydroxyl containing ring has been ruptured, followed by decarboxylation and lactone formation by addition to the double bond of the keto-ring to give compound XVIII or the alternative XVIIIa. The analyses of the lactone, acid and ester fit this scheme.



XVII

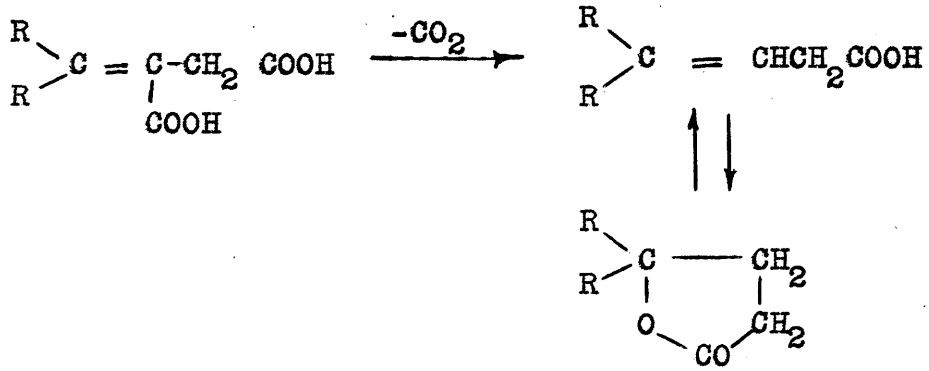
XVIII a

XVIII

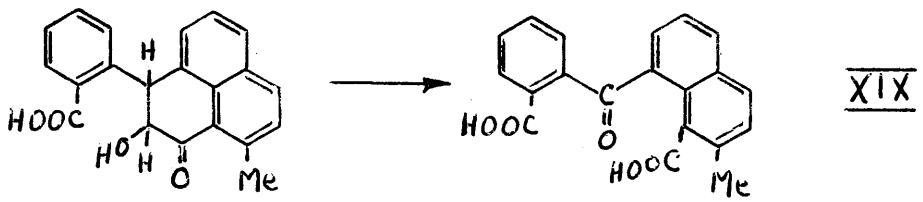
Compound		Lactone (cpd.D)	Acid (cpd.E)	Ester (cpd.F)
Suggested formula		$C_{21}H_{14}O_3$	$C_{21}H_{16}O_4$	$C_{22}H_{18}O_4$
Calculated	C	80.2	75.9	76.3
	H	4.49	4.85	5.24
Found	C	80.2	75.9	75.79
	H	4.45	4.38	4.99

Decarboxylation followed by addition of a carboxyl group to a double bond to form an ester (Johnson et al.[13]) does occur under conditions similar to those in the oxidation. These

authors used acetic acid containing a little water and a



mineral acid (hydrobromic). Oxidation of the acid (cpd. E) did not yield the expected benzoyl naphthoic acid XIX since



XIX

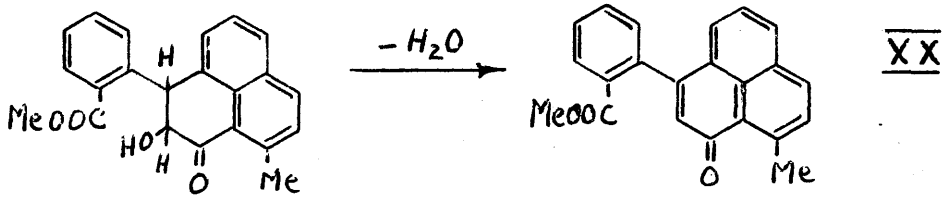
the molecule was degraded further to phthalic acid and a mixture of water soluble acids.

The oxidation of the red compound A was carried out in boiling acetic acid using sodium dichromate and a good yield of the crude product was obtained: the molecule resisted oxidation below the boiling point of the mixture. The crude material was mainly non-acidic and this yielded colourless prisms of m.p. 213-14° (cpd. D). The acidic fraction crystallised as the methyl ester in colourless prisms of m.p. 186-7° (cpd. F). Compound D dissolved in hydroxide solution from which was obtained the acid (cpd. E) which

forms colourless needles of m.p. 232-4°. This acid on esterification by both the Fischer and diazomethane methods gave the same methyl ester (cpd. F) as the acidic oxidation fraction above. This showed that compound D is not a keto-lactone which would form a pseudo ester by the Fischer method but a normal ester with diazomethane[14]. The oxidation had thus produced a mixture of the lactone (cpd. D) along with some of the acid (cpd. E) from the same lactone.

The possibility that compound D might be an anhydride led to an attempt to convert the acid (cpd. E) back to the anhydride by boiling in acetic anhydride. This yielded a non-acidic material of m.p. 226-8° which is neither compound D nor an isomer; its carbon content is 4% lower. An attempt to form the half ester by refluxing compound D in methanol left it unaffected, indicating that it is not an anhydride. A Rast determination of its molecular weight gave a value of 288. Estimation of the saponification equivalent was unsatisfactory due to an indefinite end point: there seemed to be a slow hydrolysis of a weakly acidic group at the pH of the end point with the result that the saponification equivalent rose from 203 to 260 and was still rising. The ester (cpd. F) has a methoxyl content (Zeisel) equal to one methoxyl group, while compound D gave a zero value: compound D was thus shown to be a lactone and there is only one carboxyl group in the acid. The correct saponification equivalent is therefore

equal to the molecular weight and prolonged titration in the above estimation would probably have given this value. That the keto group is still present in the molecule was demonstrated by formation of the 2:4-dinitrophenylhydrazone of compound D which analysed for one keto group. The ester (cpd. F) resisted dehydration by heating with potassium bisulphate; no colour change was observed and on sublimation the ester was recovered unchanged. It was hoped by this means to convert it to a derivative of perinaphthenone (XX), a yellow compound.



XX

Further degradation of the lactone or the acid was tried under various conditions. Alkaline potassium permanganate at room temperature was not effective, but at 80° the molecule was broken down to a mixture of acids. From this yellow gum phthalic anhydride was readily sublimed, but the residue failed to crystallise even as the methyl esters. Chromatography on silica and alumina of the esters resulted in several bands, but these yielded only traces of gum; partial hydrolysis took place on the alumina column. An

insufficient amount of permanganate was used in one trial to reduce the possibility of the oxidation's going beyond the naphthoic acid stage. Although some unchanged starting acid was recovered, a water insoluble fraction other than starting material was not formed and examination of the water soluble fraction yielded only phthalic anhydride and a gum as before. It is obvious from this work that conditions strong enough to oxidise the lactone or its acid are sufficient to oxidise further the intermediate naphthoic acid.

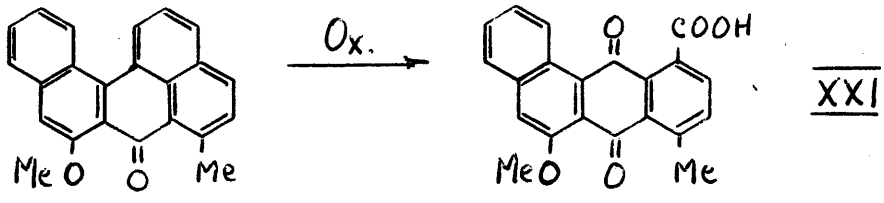
An alternative approach is to break the keto bonds by potassium hydroxide fusion of the lactone (cpd. D) whereby a derivative of naphthoic acid might be obtained. Treatment of the lactone under the same conditions as for compound II produced some insoluble amorphous material which was probably a polymer, along with a small amount of acidic gum. The latter was esterified and sublimed, but a crystalline product could not be isolated.

Isolation of phthalic anhydride agrees with the proposed structure for the lactone (cpd. D) but some other method of degrading the red compound A was desirable through which more proof of its structure could be found. By using alkaline or neutral oxidation conditions it was hoped to avoid formation of the lactone with the possibility of isolating a more suitable intermediate product. The chromic acid - pyridine complex (Sarett et al.[15]) provided the former conditions,

but this reagent proved to be too mild. The red compound also resisted oxidation by potassium permanganate in acetone even after 8 hours' reflux.

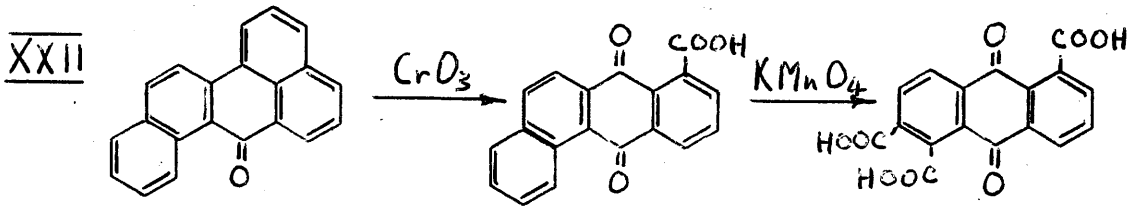
As pointed out above, the red colour of compound A is not consistent with the proposed methoxy - benzanthrone structure (VII). In spite of the fact that it is completely insoluble in aqueous alkali and no more soluble in alcoholic hydroxide than the original compound, a methoxyl determination gave a zero value and attempts to demethylate it failed. The presence of the hydroxyl group was clearly shown by the isolation of a purple sodium salt using sodium ethoxide in dry alcohol or benzene and by formation of the yellow acetate in boiling acetic anhydride in presence of a little anhydrous sodium acetate.

In view of the results from oxidation of the red compound A, it was decided to methylate the easily oxidised hydroxyl group. By protecting the ring, oxidation should take place in the peri-position with formation of an anthraquinone acid (XXI), similar to the type of degradation used

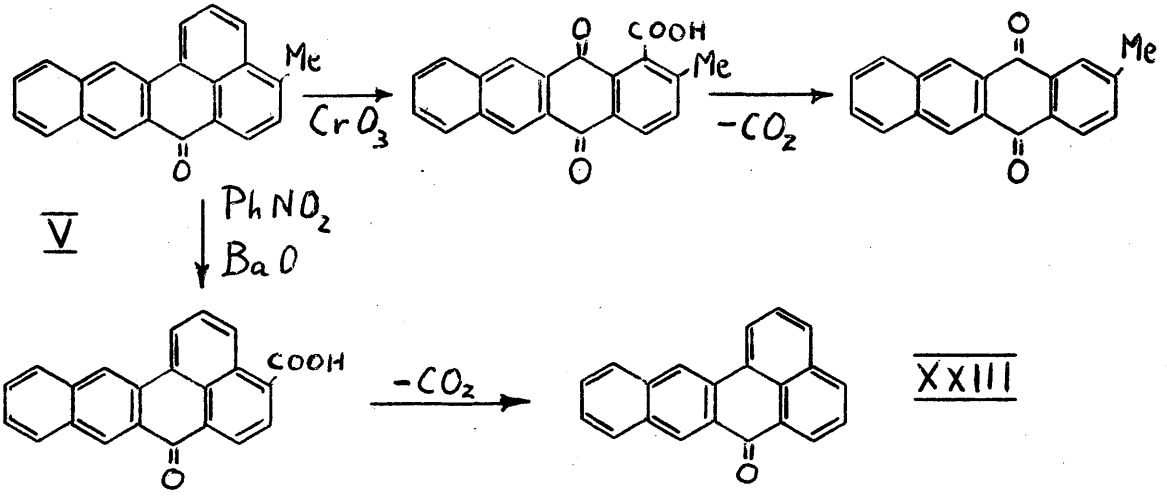


XXI

by Cook and de Worms[16] for 3:4-8:9-dibenz-10-anthrone XXII.



Buckley[3] verified the structure of 7-methyl-2:3-8:9-dibenz-10-anthrone (V) by oxidation to an anthraquinone. Contrary to expectation, methylation failed to stabilise the



ring and oxidation of the methoxy compound using the same conditions as for the red compound resulted in the same colourless lactone (cpd. D).

The methyl ether was prepared, after the usual methods failed, by forming the sodium salt of the red compound in dry benzene and refluxing with methyl iodide; the salt is more soluble in benzene than ethanol. Since the starting material

could not be removed by aqueous alkali, chromatography was used and on the large scale (10 g.) this was tedious. The yield was low but enough of the product was obtained to carry out an oxidation and isolate the pure lactone. The red compound was unaffected by boiling in acetone with potassium carbonate and dimethyl sulphate. Other methods tried included diazomethane, heating the sodium salt in dimethyl sulphate alone, and heating the sodium salt at 200° with potassium methyl sulphate (Graebe and Bernhard[17]). The last two were used with the difficultly methylated hydroxy-anthraquinones.

Another approach to determining the structure of the red compound is by reduction to the hydrocarbon and since it is a phenol, this was tried on compound A by means of the zinc dust melt (Clar[18]). The product was a red gum which had a marked green fluorescence in solution, showing that reduction had taken place, but this did not yield a crystalline product even after repeated chromatography and no evidence of a separation was seen on the column.

Potassium hydroxide fusion of the red compound afforded a large amount of an almost black, non-acidic solid which was insoluble in the usual solvents. This result is not surprising since a benzanthrone would be expected to form a polymer in the same way as dibenzanthrone is prepared by hydroxide

fusion of benzanthrone itself (Bally[19]).

Further evidence in favour of the hydroxybenzanthrone structure was provided by applying the Scholl benzanthrone synthesis to both 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II) and the corresponding phenol. In both cases the red compound A was obtained by fusion in a sodium chloride - aluminium chloride mixture. The product was isolated by chromatography.

Additional confirmation for the proposed structure would be given if 1:2'-dinaphthyl ketone itself cyclised by Scholl treatment to form 1:2-8:9-dibenz-10-anthrone (XIII), a sample of this being available. This reaction, however, did not proceed as in the above cases of the methoxy and hydroxy ketones, and very impure products resulted. Dry oxygen was bubbled through the melt (Vollmann et al.[20]) and in three experiments the time and temperature were varied. A small amount of a compound forming yellow prisms of m.p. 162-5° was isolated after chromatography and distillation in vacuum; 1:2-8:9-dibenz-10-anthrone (XIII) forms yellow needles of m.p. 186-7°. The product is not 2:3-8:9-dibenz-10-anthrone (XXIII) (m.p. 199-200°) which could result from ring closure in the β -position instead of the expected α -position. The nature of this product was not investigated due to lack of time.

As referred to in the introduction, Buckley[3] found that in cases where oxidative cyclisation took place in the Friedel-Crafts reaction to form a mesobenzanthrone, the same benzanthrone could be formed in poor yield by treating the corresponding ketone with aluminium chloride in carbon disulphide. In the present case the red compound A was also obtained by treating 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone with aluminium chloride in methylene chloride at room temperature.

Summarising the results of this work, we have a great deal of evidence for the proposed benzanthrone structure for the red compound A. The similarity in the behaviour of the compound to that of the cyclised products obtained by Buckley is marked. The factors controlling its formation are the same. The presence of the hydroxyl group no doubt accounts for the fact that cyclisation takes place in the present case in spite of the fact that Buckley did not obtain a benzanthrone in the Friedel-Crafts reaction of 2-naphthoyl chloride with β-methylnaphthalene. Its formation from 2-methyl-3'-hydroxy-1:2'-dinaphthyl ketone (II) by aluminium chloride in methylene chloride as well as by the Scholl reaction suggests that no rearrangement has taken place and that the red compound results from the straightforward cyclisation to form 4-hydroxy-5-methyl-1:2-8:9-dibenz-10-anthrone. Its poly-

merisation in fused potassium hydroxide is typical of a benzanthrone. There is a strong resemblance between the spectrum of 1:2-8:9-dibenz-10-anthrone (XIII) and that of the red compound and more especially of its methyl ether: in the latter case the positions of the maxima are almost coincident. The degradative results are consistent with the proposed structure though they do not provide certain proof.

EXPERIMENTAL

Methyl 3-hydroxy-2-naphthoate (IX). 3-hydroxy-2-naphthoic acid (150 g.) was refluxed in methanol (600 ml.) containing 2% dry hydrogen chloride for 2 hours and left overnight. A crop of the crystalline ester was then filtered off but the mother liquor contained some unchanged acid and this was refluxed for another 2 hours to yield a second crop of ester. The product (143 g.; 89% yield) was obtained as yellow needles from methanol, m.p. 75-6°.

Methyl 3-methoxy-2-naphthoate (X).

Expt.1. This was the method finally used for the large scale preparation. Methyl 3-hydroxy-2-naphthoate (66 g.), potassium carbonate (92 g.) and dimethyl sulphate (82 g.) were refluxed in acetone (540 ml.) with stirring till the yellow colour of the hydroxy ester was discharged (2 hrs.). ¾ of the acetone was distilled off, water and benzene added and the extract washed with dilute sodium hydroxide solution till the yellow washings became colourless. The benzene layer was concentrated and the product in almost quantitative yield formed colourless prisms of m.p. 61-63°. The benzene concentrate did not crystallise at 0° after long standing, but when a seed was obtained by cooling a sample to -50°, the rest crystallised readily.

Expt.2. A modification of expt.1 also gave good results with the ester. The potassium salt of methyl 3-hydroxy-2-naphthoate (1 g.) was formed with the calculated amount of hydroxide in methanol. The solvent was removed and the salt was taken up in acetone (5 ml.) containing enough water to dissolve it. Methyl iodide (1.4 g.) was run in and the clear solution left at room temperature; samples were withdrawn at intervals to follow the reaction. After standing overnight, boiling for 1 hour was required to complete the reaction. The product was isolated in 70% yield; longer boiling should increase this.

Expt.3. As described by Lesser, Kranapuhl and Gad[6]. This method starts with methyl 3-hydroxy-2-naphthoate (2 g.), the potassium salt of which was formed in methanol (7 ml.) using the calculated amount of hydroxide with ice cooling and stirring followed by drop-wise addition of dimethyl sulphate (1.6 g.). After stirring at room temperature for 1½ hours it was warmed to 25-30° and cooled, then left overnight. Next day some of the methanol was removed and water and benzene added, etc. The benzene layer crystallised on concentration to give fairly pure starting material.

3-Methoxy-2-naphthoic acid (XI). Methyl 3-methoxy-2-naphthoate was hydrolysed by boiling for 2 hours in aqueous methanolic (1:1) potassium hydroxide solution in the usual way.

Most of the methanol was removed, water added, the solution acidified and the acid extracted with chloroform, washed with alkali, then water and dried. The product (90% yield from the hydroxy ester; 2 stages) formed colourless prisms from aqueous methanol (30% water) as colourless prisms of m.p. 132½-133½°.

3-Methoxy-2-naphthoyl chloride (I). Jambuserwala, Halt and Mason[21]. Dry 3-methoxy-2-naphthoic acid (20 g.) was boiled with thionyl chloride (22 ml.; purified by distillation from quinoline then from raw linseed oil[22]) in ether (70 ml.) till all the acid had dissolved (4 hours). The ether and excess of thionyl chloride were distilled off under vacuum on the steam bath and the yellow gum crystallised on cooling. The product was obtained as yellow needles from petroleum-ether (40-60°) in almost quantitative yield; m.p. 58-9°.

2-Methyl-3'-methoxy-1:2'-dinaphthyl ketone (II; cpd. C)(see pp. 112 and 115).

Friedel-Crafts reaction.

Expt.1. As described by Miss R. Schoental[2]. 3-methoxy-2-naphthoyl chloride (1 g.) was added with constant stirring to 2-methylnaphthalene (0.71 g.) in carbon disulphide (10 ml.), then small portions of powdered anhydrous aluminium chloride (1.3 g.; 2 mol.) were added over an hour on an ice bath.

The mixture was left at 0° for 5 hours then overnight at room temperature. The complex was decomposed with ice and dilute hydrochloric acid, steam distilled, extracted with benzene and the extract washed with dilute alkali, then water. The product was a dark red gum which did not crystallise but was shown by distillation at 0.3 mms. and 220° to contain about 30% of yellow gum along with the red compound A obtained by Miss Schoental. The gum slowly crystallised to form the yellow ketone of m.p. 112-114° (cpd. C).

Expt.2. By repeating expt.1 using methylene chloride as solvent, a good yield of the red compound A crystallised from the concentrated benzene extract. 6 g. of the acid chloride yielded 3.7 g. of red compound (33% yield) as dark red needles of m.p. 194-6°, with no evidence of decomposition. (Found: C, 85.31; H, 4.69. $C_{22}H_{14}O_2$ requires C, 85.13; H, 4.55%).

Expt.3. Expt.2 was repeated using the same amounts of reactants but only 0.65 g. of aluminium chloride (1 mol.). This time the solution of the aluminium chloride complex of the 2-methylnaphthalene was added to the acid chloride solution. A dark brown gum was obtained with no evidence of the red compound. Distillation at 0.2 mms. and 220° yielded a pale yellow gum (0.59 g.) which only crystallised with difficulty from a little ethyl acetate after long standing at -20°. Chromatography had failed to give a crystallisable fraction

from alumina. This product (0.1 g.; 6% yield) was the yellow ketone (cpd. C) which formed yellow prisms from ethyl acetate of m.p. 112-114°. (Found: C, 84.4; H, 5.4. $C_{23}H_{18}O_2$ requires C, 84.6; H, 5.6%). The mother liquors yielded a small amount of colourless prisms of m.p. 171-3° which were not analysed but were assumed to be the same isomeric ketone as isolated by Miss Schoental (cpd. B).

Expt.4. A repeat of expt. 2 (2 mol. of aluminium chloride) in which the solution of the complex of the acid chloride was added to the 2-methylnaphthalene solution, resulted in a good yield of the red compound A.

Expt.5. 1 mol. of aluminium chloride was used with methylene chloride as solvent. The acid chloride solution was added to the solution of the complex of the 2-methylnaphthalene at 0°. This produced a brown gum which gave the yellow ketone (cpd. C) in about the same yield as expt.3.

Expts. 6, 7 and 8. Expt.5 was repeated using carbon disulphide, benzene and tetrachloroethane as solvent, but the product in each case was the same yellow ketone and the yields varied little.

Larger scale preparation of 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone. 3-Methoxy-2-naphthoyl chloride (22 g.) in methylene chloride (50 ml.) was added dropwise to the complex of aluminium chloride (13.4 g.) and 2-methylnaphthalene (14.2 g.)

in methylene chloride (50 ml.) during 1½ hours with constant stirring at 0°. The reaction mixture was left at 0° for another 5 hours then overnight at room temperature and decomposed with ice and dilute hydrochloric acid, made alkaline and steam distilled for 2 hours. The product after extraction with benzene, washing with alkali and water, was distilled in two parts to reduce decomposition due to prolonged distillation. The main fraction was 10 g. of yellow gum at 2 to 4×10^{-3} mms. and 190-205° which gave 4 g. of crude ketone and 2 g. (6% yield) of pure ketone as yellow prisms of m.p. 112-114° from ethyl acetate.

Red compound A. This was prepared on the large scale as in expt.1 (p.110), but with methylene chloride as solvent. 110 g. of 3-methoxy-2-naphthoyl chloride yielded 50 g. (30%) of pure red compound A as dark red needles of m.p. 194-6°.

Potassium hydroxide fusion of 2-methyl-3-methoxy-1:2'-dinaphthyl ketone (II). As described by Cook[23]. 0.7 g. of pure yellow ketone (cpd. C; m.p. 112-114°) was gradually stirred into molten potassium hydroxide (5 g.) at 260°. After 10 minutes at 260-280° the melt was cooled, extracted with water, filtered and the filtrate acidified. The precipitate was extracted with ether, from which the acids were extracted with sodium carbonate solution, yielding a dark

brown gum (300 mg.) which formed yellow prisms from ethyl acetate. These were sublimed at 0.6 mms. and 150° and recrystallised; m.p. 128-130° undepressed by 3-hydroxy-2-naphthoic acid. The mother liquors yielded further crops of the same acid. The remaining gum failed to crystallise. It was sublimed and esterified, the phenolic part removed, but the remainder (20 mg.) did not crystallise.

Unambiguous synthesis of 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II; cpd. C.). 1-Bromo-2-methylnaphthalene. Adams and Binder[24]. A pinch of iron powder and a crystal of iodine were added to a solution of 2-methylnaphthalene (71 g.) in carbon tetrachloride (150 ml.). Protecting the reaction from light, bromine (80 g.) in carbon tetrachloride (150 ml.) was added dropwise over 8 hours with stirring, keeping the temperature below 5°. After standing overnight the solution was washed with 10% aqueous sodium hydroxide, then water and dried. The product (101 g.; 91% yield) distilled at 149-153° at 11 mms.

Attempt to form ketone via Grignard reagent. Reaction between 1-bromo-2-methylnaphthalene (0.7 g.) and magnesium (0.61 g.) in dry ether (5 ml.) was started by warming and scratching with a glass rod. The rest of the 2-methylnaphthalene (up to 5.5 g.) in dry ether (40 ml.) was added

slowly (1 hour) with stirring and the solution was boiled till the magnesium had dissolved. The solution was added dropwise with stirring to an ether solution (150 ml.) of 3-methoxy-2-naphthoyl chloride (5.5 g.) at -10°. After warming to room temperature (1 hour), the solution was boiled for 1½ hours then decomposed with dilute acid, made alkaline and steam distilled. Distillation of the crude product yielded a large amount of 2-methylnaphthalene and a small amount of yellow gum (0.2 mm. at 215-20°), most of which crystallised to colourless prisms (5%) from ethyl acetate; m.p. 104-6° (micro). (Found: C, 80.79; H, 5.11. $C_{23}H_{18}O_3$ requires C, 80.7; H, 5.3%).

This experiment was repeated replacing ether with dry benzene for the last stage to raise the reflux temperature and boiling was continued for 3 hours in an atmosphere of nitrogen. Only 2-methylnaphthalene and a little of the same product as before were obtained. This compound was hydrolysed and an acid of m.p. 131-3°, undepressed by 3-methoxy-2-naphthoic acid and a phenol which sublimed and crystallised to form needles from petroleum ether of m.p. 64-5° undepressed by 2-methyl-1-naphthol were isolated. The product was therefore a phenolic ester due to some oxidation of the Grignard compound. No evidence of the required ketone was found.

Synthesis of compound C (II) via the lithium compound. Fieser

and Desreux[4]. 1-Bromo-2-methylnaphthalene (2.75 g.) in dry ether (20 ml.) was added slowly with warming to lithium (0.19 g., cut in thin strips) in 5 ml. of dry ether under nitrogen. When all the lithium had dissolved (3 hrs.) the solution was filtered through sintered glass and added dropwise (1 hour) to a solution of 3-methoxy-2-naphthoyl chloride (2.8 g.) in dry ether (100 ml.) at 0° with stirring and under nitrogen, then left overnight at room temperature. Next day it was boiled for ½ hour, then dry benzene (100 ml.) was run in while the ether was distilled off and the boiling was continued for 2 hours. Dilute sulphuric acid was added, then the solution was steam distilled from alkali. The resulting gum was extracted with benzene and washed with water. The product was distilled at 0.2 mm. to give a main fraction at 210-220°. After removal of some starting acid with alkali this gum (0.75 g.) formed yellow prisms (15% yield) of the same ketone (II) as was formed in the Friedel-Crafts reaction.

Elbs pyrolysis of 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II). Fieser and Desreux[4].

0.9 g. of 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone was heated alone in a 5 ml. vacuum distilling flask, raising the temperature slowly from 350°. At 455° water distilled over. After 15 minutes at this the temperature was raised

to 470° over 4 minutes and cooled*. The melt was distilled at 0.2 mm., giving 0.22 g. of yellow crystalline solid at 280-295° and a large tarry residue. Repeated crystallisation from xylene failed to purify it even after chromatography through alumina. The product after chromatography was refluxed for 5 minutes in 5 ml. of xylene with excess maleic anhydride, steam distilled in alkali and the insoluble colourless crystals filtered, washed and crystallised from toluene; m.p. 266-7° (micro), undepressed by an authentic specimen of 1:2:5:6-dibenzanthracene. The adduct was obtained from the filtrate by acidification and extraction with ether. The product was sublimed from potassium hydroxide at 0.2 mm. and the sublimate gave yellow plates from toluene; m.p.273-5° (micro), undepressed by an authentic specimen of 1:2-benztetracene. No evidence of 4-methoxy-1:2-5:6-dibenzanthracene was found in any of the fractions from crystallisation.

Attempted oxidation of methyl group in 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II). Cook[8]. 1 g. of 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II), selenium dioxide (2 g., sublimed) and water (5 ml.) were heated in a sealed tube to 230-40° for 4 hours. The product was almost wholly insoluble in sodium hydroxide solution. Chromatography of 100 mg. yielded about a large amount of unchanged ketone and a small amount of non-crystallisable gum.

*These temperatures are uncorrected and are probably about 20° high.

Studies of the structure of the red Friedel-Crafts product (Cpd. A).

Oxidation of red compound A.

Expt.1. Cook and de Worms[16]. Compound A (1 g.) was added to chromic anhydride (4 g.) in 80% aqueous acetic acid and boiled for 1 hour. The solvent was removed under reduced pressure and the acids extracted (carbonate); these were water soluble (0.12 g.). Sublimation (0.2 mm.) yielded a few mg. of colourless needles (m.p. 120-140°).

Expt.2. Oxidation to the lactone (cpd. D). The red compound A (10 g.), sodium dichromate (60 g.) and acetic acid (200 ml.) containing water (10 ml.) were boiled for 2½ hours, then poured into water and extracted 3 times with boiling sodium carbonate solution. The undissolved material (4.7 g.) was crystallised from ethyl acetate then toluene to form colourless prisms (2.5 g.) of m.p. 213-14° (micro). (Found: 1st sample: C, 79.56; H, 4.12. 2nd sample: C, 80.19; H, 4.45%).

The crude acid fraction (1.8 g.) was esterified (6 hours) in methanol containing 2% sulphuric acid. Water was added and the ester extracted with ether, etc., yielding a yellow gum which formed colourless prisms (cpd. F) from a toluene-ethyl acetate mixture; m.p. 186-7° (micro). (Found: 1st sample: C, 75.54; H, 5.18; -OMe, 9.68.

2nd sample: C, 75.79; H, 4.99; -OMe, 9.31%).

Expt.3. Conversion of lactone (cpd. D) to the free acid (cpd. E). Compound D (0.2 g.) was hydrolysed in 0.5 N alcoholic potassium hydroxide solution and the acid obtained in the usual way. It formed fine colourless needles from toluene; m.p. 232-4° (micro). (Found: C, 75.92; H, 4.38%).

Expt.4. Esterification of acid (cpd. E). (1) Fischer method: Compound E (0.1 g.) was boiled in methanol containing 2% sulphuric acid (6 hours). The product formed colourless prisms of m.p. 186-7° undepressed by the ester from the acid fraction of the oxidation (cpd. F). (2) Diazomethane: Excess diazomethane in ether was added to the acid (0.1 g.) suspended in ether at 0° and left overnight. The product was again the same ester.

Expt.5. Saponification equivalent. Standard method used. 0.5011 g. of compound D was hydrolysed and the excess alkali titrated with standard hydrochloric acid (normality 0.5798). (Blank - titration of sample) = 4.25 ml.

$$\therefore \text{Equivalent} = \frac{0.5011 \times 1000}{4.25 \times 0.5798} = 203.3.$$

The end poind was very indefinite since the indicator colour kept returning quickly and continued titration raised the equivalent to 260 and it was still rising slowly.

Expt.6. Molecular weight (Rast). 0.0542 g. of compound D was dissolved in 0.4968 g. of camphor and this lowered the m.p. by 15°.

$$M = \frac{1000K\ w}{\Delta T\ W} = \frac{39.7 \times 1000 \times 0.054}{15 \times 0.497} = 288.$$

Expt.7. Test for ketonic group. The 2:4-dinitrophenylhydrazone of compound D crystallised from the reaction solution in ethanol after standing overnight. Chromatography on alumina using benzene gave a band which was extruded and extracted by Soxhlet (10 hours). The product formed orange rosettes from toluene which slowly decomposed above 300°. (Found: C, 65.94; H, 3.92; N, 12.29. $C_{27}H_{18}O_6N_4$ requires C, 65.58; H, 3.67; N, 11.33%). The ester (cpd. F) did not react with the above reagent and was recovered unchanged after several trials.

Expt.8. Attempt to form half ester to test for anhydride group. Compound D (0.1 g.) was boiled in methanol (5 ml.) for 6 hours. On cooling most of the starting material was recovered with a small amount of non-acidic colourless prisms (m.p. 227-40°) which were not further investigated.

Expt.9. Attempt to convert compound E (free acid) back to compound D. Compound E (0.1 g.) was boiled in acetic anhydride (3 ml.) for 6 hours. Removal of the solvent yielded a non-acidic gum from which traces of compound E were removed (carbonate). The product formed colourless prisms from

toluene of m.p. 226-8° (Found: C, 76.04; H, 3.9%).

Expt.10. Attempt to dehydrate compound F (ester). 50 mg. of compound F was heated with 1/5 of its weight of anhydrous potassium hydrogen sulphate at 180-200° for 1 hour. Under vacuum (0.2 mm.) the unchanged colourless ester sublimed off.

Expt.11. Hydroxide fusion of compound D. 0.5 g. of compound D was fused in 2.5 g. of potassium hydroxide as with the yellow ketone (cpd. C). The acidic fraction (0.3 g.) yielded a large amount of amorphous insoluble material and a small amount of almost colourless gum which was esterified. The esters slowly crystallised on long standing, but these were very impure and would not crystallise from any solvent even after sublimation.

Expt.12. Oxidation of the free acid (compound E). 0.1 g. of compound E in 3 ml. of dilute aqueous sodium hydroxide solution were warmed to 80° and 0.5 g. of potassium permanganate in 10 ml. of water were added slowly with shaking. The violet colour persisted when it had all been added and the temperature was kept at 80° for another ½ hour. The solution was then decolourised with a few drops of ethanol and the manganese dioxide was filtered off, washing with warm water. The filtrate was acidified and extracted with ether and the extract washed with water and dried. The product was an almost colourless gum which would not crystallise, but sublimation at atmospheric pressure and 140° yielded long colourless

needles of m.p. 129-132°, undepressed by an authentic specimen of phthalic anhydride.

The residue after sublimation was further sublimed at 0.2 mm. and 200-210° to give 30 mg. of colourless glass which was still acidic but would not crystallise. The glass was esterified using diazomethane in ether, leaving overnight to complete the reaction. The esters were obtained in the usual way but no crystals formed. Chromatography on silica failed to yield a crystallisable fraction.

The above oxidation was repeated on 0.65 g. of free acid (compound E) using proportionately less permanganate (1.5 g.) but otherwise the same conditions. About 80 mg. of water insoluble acids were obtained and this was unchanged starting material: this was proved by conversion to the methyl ester which crystallises more readily than the acid. The water soluble acids were extracted with ether yielding 0.2 g. of gum which gave phthalic anhydride as before, but esterification and chromatography of the remainder failed to give a crystalline material.

The oxidation was repeated at room temperature on 0.1 g. of compound E using 0.3 g. of potassium permanganate. The solution took 3 hours to change to green, then it was decolourised, etc., as above. On acidification of the filtrate a substantial water insoluble fraction was obtained and this proved to be fairly pure starting material; one crystallisation

from toluene yielded pure needles of m.p. 232-4°.

Acetate of red compound A. 0.2 g. of compound A was boiled in 5 ml. of acetic acid containing a few crystals of anhydrous sodium acetate for 15 minutes. The colour changed from red to orange and on cooling golden yellow needles formed. After filtration, washing with water and drying, these formed yellow needles from ethyl acetate, m.p. 219-222° (micro). (Found: C, 81.77; H, 4.69. $C_{24}H_{13}O_3$ requires C, 81.81; H, 4.58%).

Methyl ether of red compound A (cpd. G). Several trials using the usual methods failed (see later). The purple sodium salt of the red compound A quickly hydrolysed in moist air and it was more soluble in benzene than ethanol. Moisture was therefore avoided and benzene used as solvent in the following method.

Sodium (1.5 g.) was dissolved in ethanol (20 ml. dried with sodium then with ethyl phthalate[22], p.359) and the solution was added to a warm benzene solution (1 litre, sodium dried) of the red compound A (10 g.). The solution was boiled using a calcium chloride tube for 1 hour to complete formation of the sodium salt. Methyl iodide (20 ml.) was added and boiling continued till the purple colour of the salt was discharged (17 hours). When cool the solution was washed free of sodium iodide (water) and the benzene distilled

off. The product, a dark red gum containing a large amount of starting material, was chromatographed on alumina (800 g.) with benzene and protected from light. 11 days using about 60 litres per day were required to elute the orange band (below the purple band of compound A) to give 1 g. of brown gum which yielded 0.5 g. (5%) of orange-yellow plates, m.p. 169-171° (micro). (Found: C, 86.41; H, 5.30; -OMe, 9.12. $C_{23}H_{16}O_2$ requires C, 85.15; H, 4.97; -OMe, 9.57%). Pronounced coning on the column and the time the material was on the column probably reduced the yield considerably; a small scale experiment gave 10% yield. The product was readily converted back to compound A by boiling in hydrogen iodide solution.

Unsuccessful attempts to methylate compound A.

Expt.1. Compound A (0.5 g.) was boiled with excess dimethyl sulphate in acetone with excess potassium carbonate as in the preparation of methyl 3-methoxy-2-naphthoate. Almost pure (m.p. 192-4°) compound A was recovered.

Expt.2. The dry sodium salt of 0.1 g. of compound A was heated (steam bath) with 3 ml. dimethyl sulphate (neutral) for 2 hours (calcium chloride tube). The excess dimethyl sulphate was decomposed (alkali) and the mixture extracted (benzene), etc. Unchanged compound A was recovered.

Expt.3. (Graebe and Bernhard[17]). Potassium methyl sulphate

(1 g. well dried) was heated with the sodium salt of compound A (0.3 g.) using a calcium chloride tube, at 200° for 5 hours. The product was shaken up with benzene and water, etc. Charring occurred here; the product was almost black and was largely insoluble. A crystalline material could not be isolated.

Expt.4. Compound A was unaffected by treatment with diazomethane in ether at 0° then leaving overnight at room temperature.

Oxidation of methyl ether of compound A (cpd. G). 0.2 g. of compound G was oxidised under the same conditions as compound A (p.118) using 1 g. of sodium dichromate. The crude product (0.15 g.) was isolated in the same way and repeated crystallisation from ethyl acetate yielded colourless prisms of m.p. 212-13° (micro) undepressed by compound D, the oxidation product of compound A.

Zinc dust melt of red compound A. Clar[18]. Compound A (0.5 g.), zinc dust (0.5 g.), sodium chloride (0.5 g.) and moist zinc chloride (2.5 g.) were melted together with stirring at about 210°. The temperature was then raised quickly to 290° and kept there for 2 minutes and cooled. The mixture was treated with water, ~~and~~ extracted with benzene, and washed free of salts. A red gum (0.2 g.) with a green fluorescence

in solution was obtained and this was twice chromatographed, but no indication of a separation was seen and a crystalline product was not isolated. The red compound A had evidently been reduced, but the hydrocarbon was probably unstable.

Attempted oxidation of red compound A in acetone. Compound A (0.1 g.) was boiled in acetone (10 ml.) with powdered potassium permanganate (0.2 g.) for 8 hours. No colour change was observed and the product, isolated by evaporation of the solvent, addition of water, decolourisation by sulphur dioxide and filtration, was mainly starting material with some amorphous powder.

Compounds D and E also resisted this treatment.

Hydroxide fusion of red compound A. Compound A (2 g.) was fused in potassium hydroxide (10 g.) as with compound II (p. 113). The product (1.5 g.) was a non-acidic, amorphous, almost black powder, insoluble in the usual solvents.

Attempted oxidation of red compound A using chromic anhydride in pyridine. Sarett et al.[15]. Compound A (0.2 g.) was added to the complex of chromic anhydride (0.5 g.) in pyridine (5 ml.) and left overnight at room temperature, decomposed, etc. The product was extracted with benzene to give 0.1 g. of fairly pure compound A. The residue, a brown

powder, yielded almost colourless needles from acetic acid, but these were soluble in water, did not melt and left a large residue on combustion.

Preparation of red compound A by Scholl reaction.

2-Methyl-3'-hydroxy-1:2'-dinaphthyl ketone. 2-Methyl-3'-methoxy-1:2-dinaphthyl ketone (0.5 g.) was refluxed in constant boiling hydrogen iodide (25 ml., analar) for 35 minutes using an air condenser. When cool, water and benzene were added and the extract washed with water, sodium thiosulphate solution to remove iodine, water, then dilute sodium hydroxide solution to extract the phenol, but this layer on acidification gave no precipitate. The benzene layer was washed free of alkali and evaporated to yield 0.45 g. of yellow gum which formed yellow prisms (85% yield) from ethyl acetate of m.p. 162-4° (micro) (Found: C, 84.3; H, 5.8. $C_{22}H_{16}O_2$ requires C, 84.6; H, 5.2%). This compound gave a blue-green colour with ferric chloride solution in ethanol.

Scholl reaction on 2-methyl-3'-hydroxy-1:2'-dinaphthyl ketone. 0.3 g. of the ketone were added and stirred into a sodium chloride (0.5 g.), aluminium chloride (2.5 g.) melt at 120°. The temperature was slowly raised to 150-160° and kept at this for 1 hour using a calcium chloride tube. The dark red melt was poured into water, the product extracted with

benzene and the extract washed with water, alkali, then water. The product, a red gum, was chromatographed on alumina using benzene and protecting from light. A brown band was formed at the top followed by a purple band, then a yellow band which was probably unchanged ketone. The column was extruded and the purple band extracted and rechromatographed. Crystallisation from benzene yielded about 5 mg. of dark red needles, m.p. 188-192°; mixed m.p. with red compound A (m.p. 192-4°) was 190-194° (micro). The yield in this reaction was not estimated but it was rather low and the product was difficult to remove from the alumina.

Scholl reaction on 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone (II). The above experiment was carried out on 0.3 g. of the methoxy ketone (II) but the temperature was kept at 150-160° for 2½ hours in an attempt to increase the yield. About 0.25 g. of red gum was obtained but after chromatography and repeated crystallisation only a few mg. of the same red compound in a pure state were isolated.

Attempted synthesis of 1:2-8:9-dibenz-10-anthrone (XIII). 1:2'-dinaphthyl ketone. 2-naphthoic acid (10 g.) was converted to the acid chloride and coupled with naphthalene by the Friedel-Crafts reaction as described for 2-methyl-3'-methoxy-1:2'-dinaphthyl ketone. The product contained some

of the 2:2'-isomer which required repeated crystallisation to remove, reducing the yield (1.5 g., 9%): colourless prisms, m.p. 136-7°.

The preparation was repeated using the lithium compound method (p.116). The presence of a yellow impurity again reduced the already low yield (1.1 g., 7%).

Scholl reaction on 1:2'-dinaphthyl ketone. Vollmann et al.[20]. 1:2'-dinaphthyl ketone (0.5 g.) was stirred into an aluminium chloride (2.5 g.), sodium chloride (0.5 g.) melt at 120°. The temperature was slowly raised to 150-160° and kept there for 1 hour while dry oxygen was bubbled through the melt, which was then poured into water, extracted with benzene, etc. The crude product (150 mg.) was chromatographed on alumina using benzene-petroleum ether mixture. A yellow band was eluted, but this would not crystallise.

The fusion was repeated on 1.5 g. of ketone for ½ hour at 150-160°. Chromatography followed by sublimation yielded a fraction which yielded a few mg. of impure yellow prisms of m.p. 172-7°.

A repeat of the fusion on 0.6 g. of ketone for 10 minutes at 140-5° yielded after chromatography a crude crystalline product (200 mg.) which after repeated crystallisation afforded about 10 mg. of yellow prisms of m.p. 162-5° which

was depressed by the sample of m.p. 172-7° and by 1:2-8:9-dibenz-10-anthrone (yellow needles, m.p. 186-7°).

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ADDENDUM.

The Structure of Byssochlamic Acid.

SUMMARY

The structure of byssochlamic acid. In spite of considerable work by previous investigators, little is known of the structure of the mould metabolite byssochlamic acid, beyond that it is a tetrabasic acid of formula $C_{18}H_{20}O_6$, has its acid groups masked, probably as a double anhydride, does not form a free acid and is extremely stable to degradation. In the present short study, degradative experiments have been continued. Attempts to split the molecule thermally have afforded instead an isomer of byssochlamic acid. This compound forms a stable free acid, $C_{18}H_{22}O_7$, which can be catalytically hydrogenated, unlike byssochlamic acid or the isomer, to form an acid, $C_{18}H_{24}O_7$. Further oxidation has not been tried but their properties suggest that the free acid $C_{18}H_{20}O_7$ or the isomer should be more amenable to attack than byssochlamic acid. Another acid, $C_{18}H_{24}O_8$, was isolated from the mother liquor of the isomer. Oxidation of byssochlamic acid by permanganate in boiling acetone gave promising results, but time has curtailed this work. A potentiometric titration of byssochlamic acid confirmed tetrabasicity and the pH graph shows no marked difference in the strengths of the acid groups. The ultraviolet spectrum shows only general absorption with no evidence of conjugation.

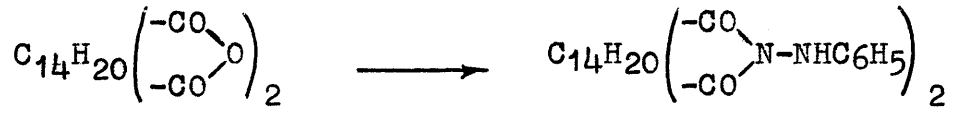
Introduction.

The ascomycete, Byssochlamys fulva, a mould which is a common cause of spoilage in processed fruits, was first described in 1933 (Olliver and Smith)[1]. In the same year its metabolic products were investigated by Raistrick and Smith[2], who isolated along with mannitol the main product, a new compound, byssochlamic acid, in yields of about 0.5% of the glucose consumed. They showed that it is a tetrabasic acid of formula $C_{18}H_{20}O_6$ in which the acidic groups are masked as anhydride or lactonic groups. The compound could not be isolated as the free acid. It was shown to be toxic to mice.

Ashley, Clutterbuck and Raistrick (1940)[3] carried out a great deal of work on byssochlamic acid, but it was inconclusive and added no major contribution to the molecular structure. The following summarises their results and conclusions.

They noted the strong similarity in the properties of the acid and those of another mould metabolite, glaucanic acid (Wijkmann et al.)[4]; they both have the same formula, but were shown to be different by mixed m.p. Byssochlamic acid contains no active hydrogen. Prolonged methylation by diazomethane produced an oil having methoxyl content equal to 3.6 methoxyl groups. Byssochlamic acid forms a fluorescein

and an eosine and reacts with phenylhydrazine and p-toluidine in the same way as phthalic anhydride. This suggests a double anhydride structure which would form an imide with phenylhydrazine as shown; titration with sodium hydroxide solution of the imide of both byssochlamic acid and phthalic acid causes opening of the ring to form a phenylhydrazide



carboxylic acid.

Byssochlamic acid is very stable and degradation experiments have not been fruitful. It is unchanged by heating in nitrogen at 230-245° for 45 minutes. It crystallises unchanged from concentrated nitric acid. It is soluble in warm concentrated sulphuric acid and is reprecipitated on addition of water. Alkaline permanganate reacts very slowly in the cold and on heating converts it entirely to carbon dioxide, no intermediate product being isolated. Sodium hypochlorite does not react with the acid. Both chromic acid - phosphoric acid mixture and boiling Beckmann mixture react extremely slowly. Neither ozone nor perbenzoic acid have any effect on byssochlamic acid in chloroform. Bromine does not react with byssochlamic acid in acetic acid solution, but heating with bromine in a sealed tube at 200°

for 2 hours converts it completely to carbon and hydrogen bromide.

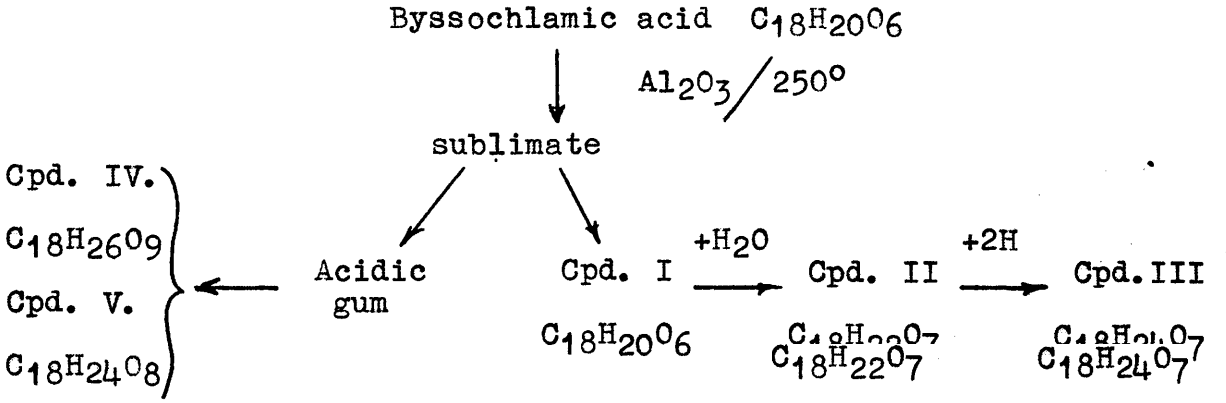
Byssochlamic acid is not reduced by sodium amalgam in alkaline solution nor by hydrogen with palladium charcoal either in alcohol or as the salt in aqueous solution, but it is reduced by zinc and acetic acid to form an acid, $C_{18}H_{24}O_7$, and another product which could not be crystallised. The former titrates as a tribasic acid, contains 2 active hydrogens and gives a dimethyl ester with diazomethane. The conclusions made from this are that two hydrogen atoms have added to a carbonyl group of an anhydride ring and 1 mol. of water has added to the other to form 2 free carboxyl groups.

The main product from decarboxylation by potassium hydroxide fusion of byssochlamic acid is a hydrocarbon $C_{14}H_{24}$ which seems to be fully saturated; it does not add bromine or hydrogen and is probably therefore tricyclic. This compound is not dehydrogenated by platinum black (COOK)[5], but byssochlamic acid itself when treated[3] with platinum black at 310-320° affords a small amount of a crystalline compound, $C_{16}H_{18}O_3$, which titrates as a monobasic acid but contains no active hydrogen. This compound can be oxidised by dilute nitric acid in a sealed tube to benzene pentacarboxylic acid[5].

The present short study continues the investigation of the structure of byssochlamic acid. Since the amount of the acid was limited, most of this work has been on the small scale.

Discussion.

Although byssochlamic acid is unstable as the free acid and immediately loses water to form anhydride or lactonic groupings, it has been possible by sublimation from alumina to convert it to an isomer (Cpd.I) which adds 1 mol. of water to form a stable free acid (Cpd.II). Accompanying this isomer was a mixture of free acids from which an acid (Cpd.V), $C_{18}H_{24}O_8$, was separated and corresponds to byssochlamic acid with 2 additional molecules of water. The isomeric free acid (Cpd.II) is interesting because it is capable of adding hydrogen in presence of palladium black in acetic acid to form a dihydro derivative (Cpd.III); neither byssochlamic acid nor the isomeric compound I shows this property.



The isomer (Cpd.I) was obtained during attempts by various means to degrade byssochlamic acid thermally. The latter sublimed unchanged alone and also from copper bronze, but from alumina at 250° under reduced pressure the isomer

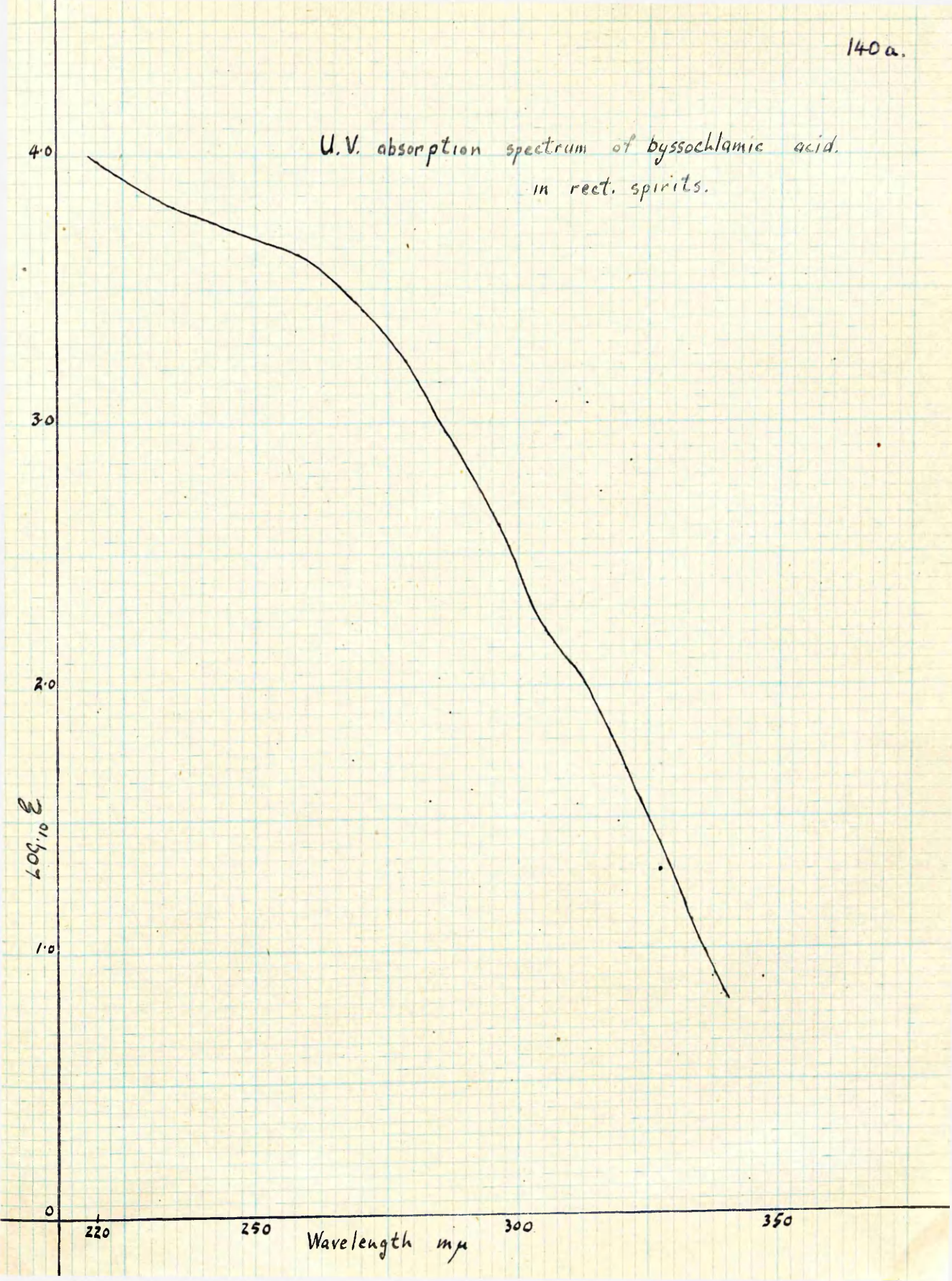
was formed in about 30% yield, but this varied appreciably; it forms colourless needles of m.p. 149-150°. A large number of sublimations were carried out to find the optimum conditions. The free acid (Cpd.II) formed colourless plates of m.p. 160-175° with loss of water to reform the "anhydride" (Cpd.I). On hydrogenation of the free acid it absorbed the theoretical amount of hydrogen, but only about 30% of the product (Cpd.III) crystallised as prisms of m.p. 185-200° (decomp.); the remainder was left as a gum. It seemed likely that the free acid would form a crystalline ester which might determine the number of carboxyl groups by a methoxyl estimation, but diazomethane treatment produced a gum which could not be crystallised: this is similar to the result from esterification of byssochlamic acid itself[3].

The mother liquor from the crystallisation of the isomer (Cpd.I) yielded, on evaporation, a gum which was entirely free acid. Crystallisation of a sample of this from an ethyl acetate - chloroform mixture afforded colourless needles (Cpd.IV) which melted at 113-115° and analysed for $C_{18}H_{26}O_9$: this corresponds to an addition of 3 mol. of water to byssochlamic acid. This compound also gave a higher melting form (Cpd.V, m.p. 160-180°, decomp.) from hot ethyl acetate of formula $C_{18}H_{24}O_8$, equal to byssochlamic acid plus 2 mol. of water. This suggests that the lower melting form

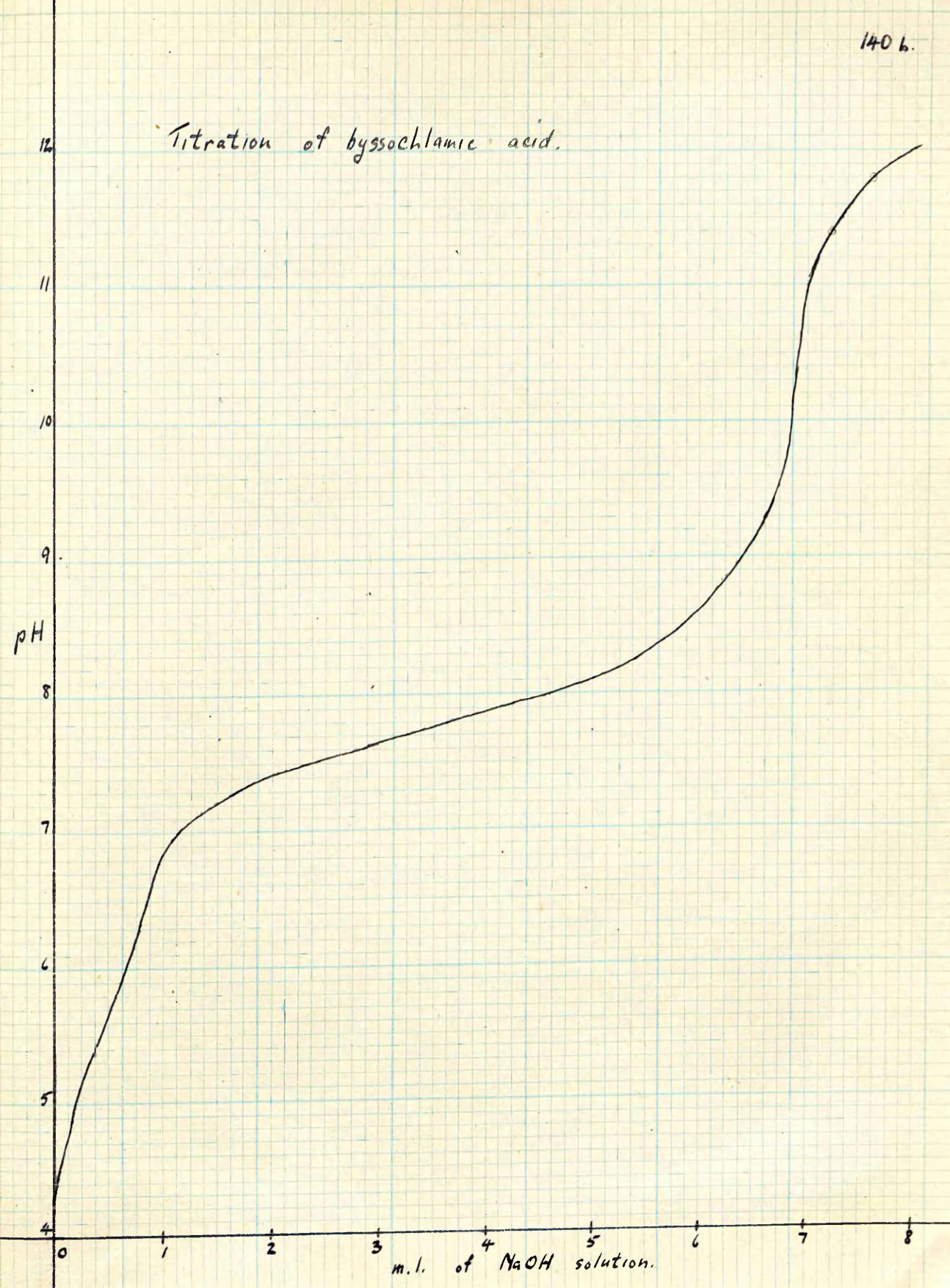
has 1 mol. of water of crystallisation. The main bulk of the free acidic gum from the isomer mother liquor had in the meantime partly crystallised on standing as prisms (10% of the original byssochlamic acid) of m.p. 140-160° (decomp.), but repeated crystallisation did not alter the m.p. and all attempts to obtain either of the above forms (m.p. 113-115° and 160-180°) from the crystalline fraction or the residual gum failed. A mixed m.p. of compounds V (m.p. 160-180°) and II (m.p. 160-175°) was lower than either (153-165°) and their analyses were different by 3% in carbon. Compound II did not give a lower melting form as did compound V. The crystalline fraction (m.p. 140-160°) from the main bulk of the gum probably contained some of compound II, but the almost zero solubility gradient of these acids, the indefinite m.ps. and the unsatisfactory results from esterification made separation difficult. Conversion to the "anhydrides" should give more readily crystallised material, but this was not tried in the present work.

Since the alumina sublimation had not degraded byssochlamic acid, further attempts to achieve this by oxidation were made. It was unaffected by hydrogen peroxide in both alkali and concentrated sulphuric acid at room temperature. In view of the excessive degradation by hot alkaline potassium permanganate, this reagent was used in boiling acetone. A

U.V. absorption spectrum of byssochlamic acid.
in rect. spirits.



Titration of byssochlamic acid.



38% yield of free acids, non-volatile in steam, was obtained as a gum which did not crystallise. Lack of time has cut short further work on this oxidation and on oxidation of isobyssochlamic acid (Cpd.I).

The ultra-violet absorption spectrum of byssochlamic acid shows no maxima or minima and is similar in shape to those of glauconic and glaucanic acids (glauconic acids I and II)[4]; it does not provide direct aid in the study of its structure. Its infra-red spectrum may be of value but an instrument was not available at the time of working. A potentiometric titration of byssochlamic acid in aqueous acetone confirmed that the acid is tetrabasic, but no distinction between the strengths of the different acid groups was shown (p. 140a and b).

While the present work has provided little direct information on the structure of byssochlamic acid, the results indicate that in isobyssochlamic acid the double bond is in a different, more accessible, position and that the isomer should be more amenable to attack by oxidising agents than byssochlamic acid has proved to be. A method of isomerising the acid in high yield would be of value in this connection. The permanganate in acetone oxidation looks more promising than any of the others tried and should be studied further.

Experimental.

Isobyssochlamic acid (Cpd.I). Byssochlamic acid (100 mg.) and alumina (400 mg.) were intimately mixed in a mortar and preheated in a small sublimation apparatus for 15 minutes at atmospheric pressure at 180-190°. The pressure was then reduced to 1 mm. and the temperature was raised quickly to 250° and kept at this till no more sublimate formed (1 hour). The sublimate contained some alumina and this was removed by dissolving the product in acetone and centrifuging. The product formed clusters of fine, colourless needles (about (30% yield) from benzene, m.p. 149-150°. (Found: C, 64.93; H, 5.67. $C_{18}H_{20}O_6$ requires C, 65.03; H, 6.07%). Mixed m.p. with byssochlamic acid (m.p. 163°) was 122-135°. The product is insoluble in sodium carbonate, but slowly soluble in sodium hydroxide solution. The alumina used in this sublimation had been prepared for chromatography (acid washed and heated to 500°) but had stood for some time and had absorbed a little moisture. Experiments using freshly prepared alumina gave poor results. It was also found that small scale sublimations (500 mg. or less) gave better results than those on the larger scale.

Free acid from isobyssochlamic acid (Cpd.II). Isobyssochlamic acid was dissolved in 5N aqueous sodium hydroxide solution.

The free acid was obtained by acidification and extraction in the usual way (ether) and crystallised from aqueous acetic acid or nitromethane. The solubility gradient was poor and the yield was about 16% from the byssochlamic acid; colourless plates, m.p. 160-175° (decomp.) (Found: C, 61.63; H, 5.88. $C_{18}H_{22}O_7$ requires C, 61.69; H, 6.33%. Micro-hydrogenation, (Pd./acetic acid) equivalent to 1.2 double bonds).

Dihydro-isobyssochlamic acid (Cpd.III). Isobyssochlamic acid (200 mg.) in acetic acid (30 ml., stood over chromic anhydride overnight and distilled) containing palladium black (160 mg., formic acid reduction method) was agitated with hydrogen at room temperature. In 10 minutes 15 ml. of hydrogen had been taken up by the catalyst and another 15 ml. was slowly absorbed during 2 hours. (Theoretical for 1 double bond is about 14 ml.). The catalyst was filtered off, the solvent removed and the product crystallised from nitromethane as colourless prisms (30%) of m.p. 185-200° with decomposition to the "anhydride"; the melt was insoluble in sodium carbonate (Found: C, 61.55; H, 6.75. $C_{18}H_{24}O_7$ requires C, 61.37; H, 6.86%). The product gave no colour reaction with ferric chloride. The residual gum did not crystallise.

Dehydration of Compound II. Compound II (50 mg.) was melted

and kept just above the melting point till the evolution of gas ceased (10 mins.). The melt was cooled and crystallised from benzene to form colourless needles of m.p. 147-149° undepressed by isobyssochlamic acid (Cpd.I). About 40% of compound II was recovered unchanged; it is almost insoluble in hot benzene.

Esterification of Compound II. Compound II (100 mg.) was treated with excess diazomethane in ether, to which a few drops of methanol were added, at 0°. After standing overnight at room temperature the ether was removed, the gum taken up in fresh ether and washed with sodium carbonate solution, then water. The ether layer yielded a faintly acidic gum (remaining anhydride groups) which would not crystallise. The gum (90 mg.) was slowly distilled (3 hours) at 1.25 x 10^{-4} mm. and the main fraction (80 mg.) was obtained at 120-123°. This fraction did not crystallise.

Compounds IV and V. The mother liquor from the crystallisation of the sublimate was evaporated down and the gum (2 g. from 5 g. of byssochlamic acid) taken up in sodium carbonate solution and filtered. The free acids were obtained by acidification of the carbonate solution, etc. A portion of the resulting gum was dissolved in an ethyl acetate - chloroform mixture (1:1) and small amount of colourless needles

(about 30% of the gum) formed on standing: m.p. 109-113°. The crystals had no appreciable solubility gradient and they were first converted to gum by dissolving in acetone and evaporating this off. The gum readily dissolved in the mixed solvent and quickly deposited needles, m.p. 113-115° (Cpd.IV) (Found: C, 55.75; H, 6.69. $C_{18}H_{26}O_9$ requires C, 55.95; H, 6.78%).

When compound IV was converted to a gum as above and taken up in hot ethyl acetate, the hot solution slowly deposited colourless prisms (Cpd.V) of m.p. 160-180° with decomposition; the melt was insoluble in sodium carbonate solution (Found: C, 58.78; H, 6.33. $C_{18}H_{24}O_8$ requires C, 58.68; H, 6.57%).

The main bulk of the gum from the sublimate mother liquor (about 2 g.) had slowly crystallised on standing and the colourless prisms (0.5 g. or 10% of the original byssochlamic acid) were washed with ether and filtered; m.p. 140-160°, decomp.). Further crystallisation from ethyl acetate - chloroform mixture yielded only a trace of compound IV and the m.p. was unchanged after several crystallisations. This material may have been a mixture, but the indefinite m.ps. of this series made it difficult to decide.

Hydrogen peroxide treatment of byssochlamic acid. The acid (100 mg.) in 2 ml. of 5N sodium hydroxide was treated with

4 drops of 30% hydrogen peroxide, shaken up and stood overnight at room temperature. The starting material was recovered unchanged next day. A similar treatment of 100 mg. of byssochlamic acid in 5 ml. of concentrated sulphuric acid afforded only starting material.

Sublimation of byssochlamic acid from copper-bronze. The acid (100 mg.) was intimately ground with copper-bronze powder (500 mg.) using ether. The dry mixture was heated to 250° for ½ hour then the pressure was reduced to 1 mm. and unchanged starting material sublimed off.

Permanganate oxidation of byssochlamic acid in acetone. Finely powdered potassium permanganate (1 g.) was added in portions to a boiling solution of byssochlamic acid (200 mg.) in distilled, Analar acetone (8 ml.) and the boiling continued for 1 hour. The acetone was evaporated off, water added and sulphur dioxide passed to decolourise. The solution was acidified with dilute sulphuric acid and extracted with ether, which was then extracted with sodium carbonate. The carbonate extract was acidified and steam distilled. The volatile acids were negligible and the non-volatile part (77 mg.) could not be crystallised. The carbonate insoluble fraction was also negligible, showing that the whole of the byssochlamic acid had been oxidised. By

varying the conditions of this oxidation a crystalline product may be obtained.

Potentiometric titration of byssochlamic acid. The solvent used was aqueous acetone (48 of water to 52 of acetone by volume). The acetone was purified as described by Åkerlöf[6]. 0.1644 g. of byssochlamic acid was dissolved in 200 ml. of the mixed solvent and 40 ml. of the solution was titrated with 0.060N sodium hydroxide solution using a glass electrode. The E.M.F. readings were converted to pH by reference to two standard buffer solutions of known pH, 0.01N borate (pH 9.20) and acetate buffer (pH 4.74).

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