A STUDY OF SOME SOLVENTS FOR THE EXTRACTION OF STEROLS FROM ALFALFA LEAF MEAL

Thesis for the Degree of M. S.

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Jean-Jacques Rosier

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This is to certify that the

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A STUDY OF SOME SOLVENTS FOR THE EXTRACTION OF STEROLS FROM ALFALFA LEAF MEAL

by

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INTRODUCTION

Different solvents have been used for the extraction of sterols. They have been: ethanol, ethyl ether, petroleum ether, chloroform, benzene, acetone, methanol. They have been used separately or in combination. Almost all of them have a great disadvantage because of their inflammability, their lack of specificity and their ability to extract substances other than sterol. However, ethanol and ethyl ether have been commonly used for this extraction.

In this work it was decided to try to use a noninflammable solvent such as ethylene dichloride and to compare the yield and properties of the preparations with those obtained by ethanol and ethyl ether.

The chief advantage of a chlorinated hydrocarbon is its noninflammability; a disadvantage is its higher cost. Its specificity is not too good for it tends to dissolve nonglyceride material thus interfering in the refining process of the oil and, therefore, of the sterol.

The purpose of this study is to compare the different amounts of sterols extracted from alfalfa leaf meal with two different solvents, namely the pair (a) ethyl alcohol-ethyl ether and (b) ethylene dichloride, and in addition to study the specificity of each solvent.

HISTORICAL

The sterols occur in three main forms: free sterols, steryl esters, steryl glycosides. The last ones are nearly insoluble in almost all of the solvents, little is known about their solubility and, therefore, will not be considered. On the contrary, the other two are very soluble in several solvents and are the object of this study.

In the past few years, different methods for the separation of sterols from biological material have involved distinct operations; (1) the separation and isolation of the lipids and, (2) the separation of the sterols from the lipid materials. A survey of these extractions, before and after saponification, is listed below.

A. Separation or Isolation of Lipids.

Beneke (1) in Germany in 1872 was one of the first who definitely studied the extraction of sterols, substances at that time called "cholesterin". He extracted egg yolk, brain material and calves eyes with ethanol in the cold.

The next one working with ethanol was Tanret (2) in 1879 in France. He used 95% ethanol to extract the lipids from pulverized ergot.

Around 1910, Power and his co-workers (3,4,5,6,7,8,9)

extracted the oil of numerous plants by percolation with hot ethanol.

In 1932, Zeichmeister and Cholnoky (10) extracted intermittently the oil of the dry blossoms of the plant, Calendula officinalis, with 96% ethanol.

The oil of the algae, Fucus Vesiculosus, was extracted with ethanol in the cold by Phipers and Wright in 1934 (11).

Simpson in 1937 (12) worked on dried Sanega root with boiling ethanol and extracted the oil.

Dicholesteryl ether was obtained from the spinal cord of oxen by Silbermann (13) in 1945 by continuous extraction of the dried cord with hot ethanol.

The second solvent used was petroleum ether. In 1878, Hesse (14) in Germany extracted the lipid material from powdered Calabar beans with this solvent.

More recently, Clenshaw and Smedley Mac Lean (15) extracted green leaves with light petroleum ether (BP: 40^{o}-60^{o}).

Another solvent which has been used was ethyl ether to extract the oil and, subsequently, the sterols.

Chibnall and Channon (16) in 1927 obtained the oil from cabbage leaf cytoplasm with ethyl ether.

Anderson (17) also extracted wheat and corn germ with ethyl ether.

Cholesterol was extracted from blood by drying the serum over sodium sulfate and then extracting it with chloroform (22)(Myers-Wordell method, 1918).

In 1932, Hart and Heyl (18) extracted dried spinach leaves with acetone.

The total steroidal content of steers' urine was determined by Marker in 1939 (19) after a treatment with hydrochloric acid and an extraction with butanol.

Fernholz and Moore (20) in 1939 extracted dehydrated alfalfa meal with hexane.

In 1944, the oil of ground dried seeds of the plant, Calycanthus floridus, was obtained with benzene by Cook and Page (21).

Schoenheimer and Sperry (22) used acetone-ethanol to precipitate the proteins and extract the cholesterol from whole blood.

B. Isolation of the Sterols after Saponification.

The foregoing extractions were carried out on various biological tissues involving the isolation of total lipids along with the sterols.

The most used procedure for the extraction of the sterols involved a preliminary saponification by ethanolic or methanolic potassium or sodium hydroxide, either in the cold or at a warm temperature. Water was added to the unsaponifiable material and it was then extracted by a solvent, usually ethyl ether. The sterol was extracted from the ether phase either by formation of a derivative, such as, oxalate, acetate, digitonide, or by a fractionated crystallization in ethanol. However, there are a few exceptions to this general rule, for sterols can be extracted from a biological source without carrying out a saponification. These cases will be listed at the end of this section.

Beneke (1) used a lead salt, PbO, to precipitate the impurities. He dried the precipitate and extracted it with ethanol. He recovered the sterols after saponification with barium hydroxide and then made subsequent extractions of the unsaponifiable material with ethanol.

Schulze (23) working with wool fat, Hesse (14), Tanret (2), Power (3,4,5,6,7,8,9), Clenshaw and Smedley Mac Lean (15), Simpson (12), all previously mentioned, used the usual procedure mentioned above for the extraction of sterols.

Chibnall and Channon in 1927 (16) treated the ethyl ether extract with acetone to remove the phosphorus containing lipids. A double saponification, first with sodium hydroxide and then with sodium ethylate, was followed by the usual procedure.

Fernholz and Moore (20) used the usual procedure but evaporated the ethyl ether extract to dryness and dissolved it in acetone. The acetone soluble material, evaporated to dryness, was dissolved in hexane which was extracted with methanol. The methanol extraction yielded crystals which were purified by recrystallization in ethanol.

Zeichmeister and Cholnoky (10) after addition of water transferred the sterols to an ethyl ether - petroleum ether phase, dried the epiphasic layer over sodium sulfate and then applied the usual procedure.

Hardt and Heyl (18) and Phipers and Wright (11) eliminated the chlorophyll by petroleum ether before proceeding in the ordinary way.

Anderson (17) extracted the unsaponifiable material fraction with ethyl ether, dried it in vacuum at 100°C., washed it with ice cold pentane and obtained practically colorless crystals. Then an additional saponification, extraction with ethyl ether and recrystallization from ethanol yielded crystals of high purity.

Fernholz (24) holds a patent in which he claimed to obtain the sterols from soybean oil by saponification; the resulting soapstock was decomposed by hydrochloric acid and the fatty acid, steam distilled in vacuo. The sterols were recovered from the residue by ethyl ether.

In another patent, sterols having one reactive double bond in the hydrophenanthrene nucleus were isolated by Yoder in 1944 (25). The separation consisted of saponification, solution of the unsaponifiable material in an anhydrous solvent, such as, ethylene dichloride or acetone, and treatment of this solution with anhydrous oxalic acid. The sterols which were precipitated as oxalates, were liberated by hydrolysis.

Mitchell (26) in a patent extracted the dried saponified product with liquid sulfur dioxide in order to obtain sterols.

As mentioned above, methods have been reported which have eliminated the saponification phase of the sterol preparation.

In 1942, Oliver and Palmer (27) passed "talloel", extracted by a solvent such as petroleum naphta, through an adsorbent clay in order to recover the sterols.

Kruse and his co-workers (28) claimed to have neutralized the fatty acids and precipitated the

phosphatides of soy-bean oil by addition of a base, with insufficient alkali to saponify the oil itself. The solid portion was then extracted with an organic solvent such as acetone.

Kraybill, Brewer and Thornton (29) reported in a patent a process for preparing an adsorbent suitable for removing phosphatides, mucilages, sterols and pigments from vegetable oils. The adorbent was prepared by mixing a water solution of sodium silicate with a water solution of an acid reacting aluminum salt.

Asquew (30), Almquist (31), Drummond (32), Embree (33), Fawcett (34), Hickman (35), and some others worked on the molecular distillation of fats and oils. In such a method, phospholipids and mucilaginous matters were eliminated by a preliminary treatment. During the distillation, the fatty acids passed off first, then the hydrocarbons and then the sterols, which came off just before the triglycerides.

EXPERIMENTAL

The material used was dehydrated alfalfa leaf meal, the so-called Leafy Irish Brand. The meal used had the following analysis according to the report of its manufacturers, Ireland Alfalfa Mills Inc.

Crude Protein, not less than 17%

Crude Fat, not less than 1.75%

Crude Fibre, not more than 25.0%

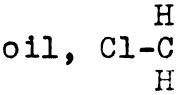
Carbohydrates nitrogen free extract not less than 60%

For comparison, the alfalfa leaf oil was extracted by two different methods: the first preparation by the use of ethanol and ethyl ether whereas, in the second preparation, ethylene dichloride was used. Both extractions were performed at room temperature in order to lessen the effect of heat.

The solvents used were:

1. 95% Ethanol from Commercial Solvents, Inc.
2. Ethyl Ether, USP 10, from Merck Co. Inc.
3. Ethylene Dichloride from the Dow Chemical Co.

Ethylene dichloride is a colorless liquid the boiling point of which is 83.7°. It is insoluble in water, soluble in alcohol and ether and has a specific gravity of 1.257. This 1-2 dichloroethane, or Dutch oil,



is prepared industrially by direct chlorination of ethylene in the presence of a catalyst, such as a ferric ion or a peroxide.

Extraction No. 1.

1 kg. of alfalfa leaf meal was weighed and put into a cloth bag. The bag was placed in a 12 liter cylinder. Five liters of 95% ethanol were poured into the cylinder, the latter then covered with a glass top. After twenty-four hours of standing, the liquid was siphoned off and the process repeated until the extracting solvent remained colorless. This condition was obtained after ten extractions.

The accumulated solvent containing the oil was concentrated on the steam bath under reduced pressure. The receiving flask was packed in ice. This is shown in Figure 1.

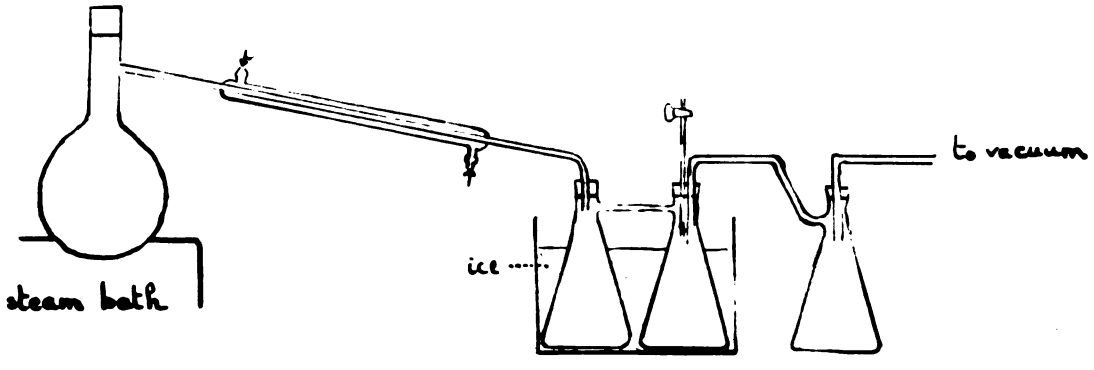


Fig. 1

Despite all these precautions a large part of the ethanol did not condense but was lost through the water pump.

The concentration was stopped when the liquid began to boil too violently. At this stage, about 1.5 l. remained in the flask.

The residue, after the ethanol extractions, was treated with ethyl ether, USP. The ethyl ether was poured into the cylinder containing the alfalfa leaf meal; and the same procedure as described for the ethanol extractions was used. The ethyl ether extract was condensed to about 1 l. in the same manner as described for the ethanol extractions and then combined with the ethanol extract. The preparation was now ready for saponification.

Extraction No. 2.

As in the case of the ethanol and ethyl ether extractions, 1 kg. of alfalfa leaf meal was put in a bag and extracted with ethylene dichloride until the solvent remained colorless. For this purpose 18 extractions were necessary.

The ethylene dichloride extract was evaporated to dryness under reduced pressure and then 600 ml. of ethanol were added. Since ethylene dichloride is soluble in ethyl ether, trouble would have occurred later in the separation

of the unsaponifiable portion from the bulk of material if the ethylene dichloride had not been removed.

Saponification and Obtention of the Unsaponifiable Material.

From now on, the instructions given by Fernholz and Moore (20) were followed. Since they started with 2.8 kg. of dehydrated alfalfa meal while, in these preparations, 1 kg. only of dehydrated material was used, proportional amounts of the reagents were employed. The same method was applied to both extractions.

The saponifications were done directly on the concentrated extracts. For that purpose 18 g. of potassium hydroxide dissolved in 360 ml. of 95% ethanol were heated with the extracts on the steam bath with a reflux condenser for a period of two hours. After cooling at room temperature, 2 l. of water were added. About 1 l. of the solution was placed in a 2 l. separatory funnel and 200 ml. of ethyl ether were added. A gentle rotation permitted the upper layer to absorb the unsaponifiable matter and thus to become colored. The same operation was repeated about seven times for each liter of the solution. Some persistent partial emulsions were encountered at this point but were ignored for in later concentration, as described below, the two

phases separated completely. The combined ether extracts were concentrated to 1.5 l. and washed thoroughly with water using phenolphtalein to denote the removing of alkali present. The three last washings were made with a solution of water containing 10% of ethanol in order to remove the water dissolved in the ether.

PURIFICATION OF THE STEROLS

The ether extract containing the unsaponifiable matter was evaporated to dryness. In order to prevent the oxidation of the product, the distillation was done under reduced pressure and an atmosphere of dry carbon dioxide. A cylinder furnished the carbon dioxide which was dried by passing through concentrated sulphuric acid. The liquid to be evaporated was contained in 100 cc. beaker which in turn was placed on a ground glass plate. A truncated suction flask with the bottom cut off was placed on the ground glass plate. Silicone grease was put on the low rim of the flask to effect a seal. When the pressure equilibrium was obtained, the current of carbon dioxide was kept at about one bubble every second through the sulfuric acid.

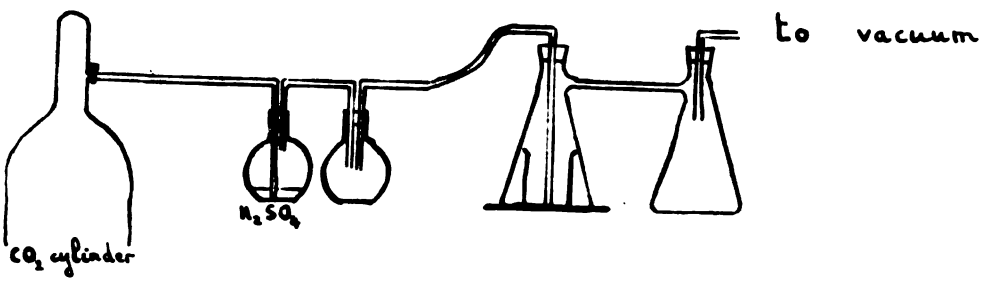


Fig. 2

Between evaporations, the carbon dioxide cylinder was disconnected in order to prevent the sulphuric acid from backing up in the rubber tubing. The apparatus described above is shown in Figure 2.

The evaporation of ethyl ether to complete dryness required about 20 hours. A dark brown waxylike material remained at the bottom of the flask. This residue was dissolved in a proportional amount (71 ml.) of hot acetone, and the material coming out on cooling was removed by filtration and set aside. This was probably phosphatides but a crystalline substance, not further considered, was present.

The acetone filtrate was evaporated to dryness with the same apparatus as described above. This evaporation required about the same period of time as the ether evaporation.

At this step of the method, a modification was made from that followed by Fernholz and Moore (20) because of the lack of hexane. Instead of hexane, pentane was used. The residue was dissolved in a proportional amount (71 ml.) of pentane and the solution was placed in a 500 ml. separatory funnel. This dark solution was washed five times in succession with 71 ml. each time of 95% methanol saturated with pentane. The methanol layer was hypophasic. The first washing was brown; subsequent washings change progressively from yellow to about colorless.

After concentration on the steam bath, the combined methanol extracts were evaporated under reduced pressure and atmosphere of carbon dioxide with the apparatus described above. The resulting product consisted of a brownish waxylike material.

The results of the different extractions both with alcohol-ethyl ether and with ethylene dichloride as the starting materials are shown in Tables I and II.

TABLE I

Amount of Material Obtained
by
Alcohol - Ethyl Ether Extractions

No. of Preparation	Crude Material (after ethyl ether evaporation)	Partially Refined Material (after acetone evaporation)	Methanol Extracted Material (after treatment with pentane)
1st	5.1 g	2.1 g	.630 g
2nd	11.15	6.6	1.3
3rd	32.70	7.0	1.6

TABLE II

Amount of Material Obtained
by
Ethylene Dichloride Extractions

No. of Preparation	Crude Material (after ethyl ether evaporation)	Partially Refined Material (after acetone evaporation)	Methanol Extracted Material (after treatment with pentane)
1st	4.6 g	1.60 g	.189 g
2nd	9.1	4.2	.417
3rd	16.1	5.21	1.18

ACETYLATION AND IDENTIFICATION

This waxylike material obtained from either the ethanol-ethyl ether extraction or the ethylene dichloride extraction was dissolved in acetic anhydride. (30 ml. per gram) and the solution heated on the steam bath for 1 hour. The solution was let stand overnight and then filtered.

An attempt was made to recover from the mother liquors some of the acetylated product dissolved in the excess of acetic anhydride. For that purpose the solution was concentrated and water added, but only a negligible quantity of steryl acetate appeared.

The solid acetate, crystallized after acetylation, was separated from the acetic anhydride by filtration and saponified with an excess of 5% alcoholic potassium hydroxide during two hours on the steam bath with a reflux condenser. The alkalinity of the solution was checked in order to be sure to obtain a complete saponification. Then water was added to the solution and the solution was extracted with ethyl ether seven times. The ethyl ether solution was washed with water until free from base and finally washed with 10% ethanol solution to remove the water. Then the ethyl ether solution was evaporated to dryness on the steam bath and the crude sterols obtained were dissolved in just sufficient boiling 85% ethanol to effect a complete solution. This solution was let stand overnight and the material which crystallized out of it was separated. The crystals were then

redissolved in the minimum amount of 85% ethanol and let stand over night. This procedure was repeated eight times. For the last time the solvent was methanol instead of ethanol.

The sterols obtained with the ethanol - ethyl ether extraction melted at 156° after the first crystallization from ethanol and 168° after the last crystallization. The sterols obtained with the ethylene dichloride extraction melted at 160-162° after the first crystallization from ethanol and 168-168.5° after the last crystallization. The identity of the sterols obtained with these two different solvents was furthermore proved by the melting point of their acetate. The acetates were prepared by the usual method. The melting points were respectively 179-181° for the sterols obtained from the ethanol-ethyl ether extraction after one recrystallization from ethanol, and 180-182° for the sterols obtained from the ethylene dichloride extraction after one recrystallization from ethanol.

DETERMINATION OF HYDROXYL GROUPS

In order to see whether there was some other substances containing an hydroxyl group in the mother liquors of the crystalline material, a determination of the hydroxyl group was attempted.

P. J. Elving and B. Warshowsky (36) used the phthallization method with phthalic anhydride in hot pyridine solution for the determination of the alcoholic hydroxyl content of complex mixtures.

An attempt to apply this method for the determination of the hydroxyl containing substances was tried.

A preliminary experiment applying this method was performed on cholesterol (m.p. = 137-138°) from Eastman Kodak Company. A quantity of cholesterol (1.714' g) was placed in a dry volumetric flask and dissolved in 50 ml. of dry redistilled pyridine (b.p. 114-115°). The phthallization mixture was prepared by dissolving 20 g. of phthallic anhydride in 200 ml. of redistilled pyridine. Water must be avoided, otherwise low values may be obtained, because of the hydrolysis of phthallic anhydride. Twenty-five ml. of the phthallic anhydride solutions were pipetted into a 24/40 standard taper flask. To this were added 10 ml. of the solution containing the sample. A condenser was attached to the flask and the later was heated in a boiling water bath for one hour. A blank determination was made in the same manner on the reagents employed. In each case

50 ml. of distilled water were then added and the solution was cooled and titrated immediately with 0.2027 N. sodium hydroxide. The difference in amount of alkali consumed between the blank and the sample represented the amount of esterifiable hydroxyl present in the solution.

$$\% \text{ hydroxyl} = \frac{V \times N \times .017 \times 100}{W}$$

W = weight in grams of the sample in the aliquot taken.

V = difference in volumes required by the blank and sample titrations.

N = normality of the standard sodium hydroxide.

.017 = the milliequivalent weight of the hydroxyl group.

The solution containing the sample was yellow after phthallization. When the water was added, a white cloudiness appeared due to the presence of phthallic anhydride, very insoluble in water. It persisted almost until the end of the titration; about 5 ml. before the end point the solution became transparent but still yellowish.

Two different solutions of cholesterol in pyridine were used. The percentage of hydroxyl group are given in Table III.

In order to verify further this phthallization method, and the purity of the cholesterol used of which neither the origin, nor the properties appeared on the label, the following experiment was performed. A saponification of the cholesteryl

TABLE III

Weight of Cholesterol		Ml. 0.2027 N. NaOH		% Hydroxyl
		Blank	Sample	
1st Exp.	0.343 g	154.91	150.84	4.01
2nd Exp.	0.343	154.60	150.15	4.15
3rd Exp.	0.209	153.08	151.44	4.07

acetate prepared from the sample of cholesterol used above was carried out in order to know the number of milligrams of sodium hydroxyde necessary for the hydrolysis of the acetate and consequently the OH content. A saponification with ,2027 N. sodium hydroxyde was performed and the solution back titrated with .1015 N. hydrochloric acid. A similar experiment was performed using stigmasterol acetate, which has been recrystallized 14 times.

The results of both saponifications are given in Table IV.

To check the presence of water in cholesterol, an attempt to dehydrate the material at 100° under atmospheric pressure failed because of decomposition before a sensible loss of water.

The phthallization method however was applied to characterize the hydroxyl containing group in the mother liquors of crystallization after purification of the sterols.

The mother liquors were evaporated to eliminate the ethanol and methanol. The respective weights of dry material were .021 g. for the ethanol-ethyl ether extractions and .016 g. for the ethylene dichloride extractions (only the third extractions were considered in both cases).

The results are given in Table V.

TABLE IV

Sterol Used	Weight of Steryl Acetate	%OH in Acetate calculated	%OH in Acetate found	%OH in Sterol calculated	%OH in Sterol found
Cholesterol	.456 g	3.97	4.21	4.40	4.63
	.093		4.18		4.60
	.235		4.36		4.80
Stigmasterol	.105	3.74	4.06	4.10	4.47
	.113		4.23		4.65

TABLE V

Ethanol-Ethyl Ether Extractions		Ethylene Dichloride Extractions	
Wgt. of Material in g.	% OH	Wgt. of Material in g.	% OH
.004	8.6	.0032	8.4
.008	9.6	.0064	9.48

DISCUSSION

The appearance of the two different extracts is not materially different until the last phase of the purification. Whereas, the extract coming from the ethanol-ethyl ether extraction is dark brown, that coming from the ethylene dichloride extraction is light brown, almost orange in color.

The acetylation products are also different. The dark brown material from the ethanol-ethyl ether extraction gives crystals which are small, flat, and seem to have a tendency to be agglomerated one with the other. The light brown material on the contrary gives large, flat, transparent crystals.

If the best preparations in each of the methods used in this study are compared with the method used by Fernholz and Moore (20), the yields are still low. In Table VI is given a comparison of the results published by Fernholz and Moore with those obtained in the present study showing the yields at the different steps of the purification, all on the basis of g. per kg. of alfalfa leaf meal.

The extraction of crude material by diffusion, used in the present work, gave a better yield then the extraction with the percolation method as described by Fernholz and Moore (20). At the subsequent step the results are about the same although those of the present work are a little

TABLE VI

(g. per kg. of alfalfa leaf meal)

	Fernholz and Moore Hexane Method	Present Work Ethanol-Ether Method	Present Work Ethylene Dichloride Method
Crude Material (after ethyl ether evaporation)	10	32.70	16.1
Partially refined Material (after acetone evaporation)	7.14	7.0	5.21
Methanol extracted Material (after treatment with pentane)	3.92	1.6	1.18

lower, particularly for the ethylene dichloride extraction. At the last phase of this purification, the yield of the Fernholz and Moore's percolation method is far ahead of the yields obtained in this study. From the comparison of these results, we can conclude that the extraction in the stationary diffusion method isolates something else than the sterols in an amount different from that obtained by percolation. Beside the phosphatides which are extracted in all the cases, it might consist of pigments, probably not quite destroyed by the saponification. This might be ameliorated by a second saponification recommended by different authors such as, Chibnall and Channon (16), Anderson (17) and others, after the extraction of the unsaponifiable matter with ethyl ether. In fact, in the ethyl ether extract, there was some greenish material standing at the interphase and overlapping therefore into the ethyl ether phase.

The final yields in this work are effectively much smaller than the one obtained by Fernholz and Moore (20), but there is to be noted that the cited authors used an extraction by percolation and do not mention the duration of their extraction, or the amount of solvent used. In this study, the renewing of solvents was stopped when an extraction remained colorless after twenty-four hours; but nothing proves that the extraction of sterols stops when

the extraction of pigments ceases. Besides, if the solvent is left long enough, for example seventy-two hours, it still becomes colored.

The solvents act by diffusion. This method does not attain 100% extraction but rather approaches complete extraction asymptotically due to the fact that there is always an equilibrium of solute between the solvents and the plant tissue immersed in the solvent.

If a comparison is made with the work of King (37), it can be seen that the sterols obtained in this work are similar to the one obtained by King from alfalfa seed. The melting points of the purified sterols are about the same and also the melting point of the acetates. The amount of material obtained in the present work was too small to attempt a separation of the different homologues of spinasterol as performed by King.

For the titration of the hydroxyl group, the phthallization method is not too satisfactory. A comparison between the percentage of hydroxyl group of a known sterol shows a fairly wide discrepancy as shown in Table IV.

This error can be explained partly by the small size of the sample. In the method Ewing and Warshowsky (36) a sample of 1 g. of ethanol was advised (MW = 46 g.) to obtain reliable results. For cholesterol the same accuracy would be obtained with 8.4 g. $(\frac{386}{46})$ in proportion to the MW. Consequently the results obtained for the hydroxyl content of the mother

liquors is not definite, due to extremely low weight of the sample. However, the result may indicate the presence of some OH containing substances, probably of lower molecular weight.

The phthallization method was checked by a method consisting of the saponification of the sterol acetate, but here also the titration is not very accurate. In this method the acetate is dissolved in ethanol, a volume of standard sodium hydroxyde is added, the flask refluxed on the steam bath for two hours. The back titration, with standard hydrochloric acid and always gives a higher result than the theoretical because of an insufficiency of acid. The phenolphthalein in ethanol has its PK raised, and besides that the equilibrium seems to be very slowly obtained.

These methods of titration of the hydroxyl content can give an idea but are not very reliable, especially when the samples are small.

The results given in Table V show however that some other OH containing substances of lower molecular weight were present in the mother liquors from the crystals of spinasterol.

SUMMARY AND CONCLUSION

A comparison of the yield of extraction of ethanol-ethyl ether and of ethylene dichloride has been determined. The pair, ethanol-ethyl ether, extract more unsaponifiable product than ethylene dichloride.

A qualitative comparison between the unsaponifiable portion has been carried out. The ethanol-ethyl ether extract does not yield as large an amount of sterols as expected from the weight of the unsaponifiable matter. This pair of solvents extract a slightly larger amount of sterols than does ethylene dichloride.

A derivative has been prepared from each of the solid fractions separated.

The main crystalline substances in each of these fractions has been identified as α spinasterol.

There is an indication of some uncrystallized material containing hydroxyl groups.

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