

Coacervation in cationic soap solutions is a function of micellar charge and micellar size, expressed in terms of micellar length or axial ratios. Within limits, not as yet clearly defined, the lower the micellar charge density, the shorter is the critical length of the micelles necessary for coacervation in cationic soap systems.

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The Extraction and Constitution of Peat Wax. Chromatographic Fractionation of Wax

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Abstract

The extraction and properties of crude peat wax were investigated. The composition of this wax obtained with various solvents was assessed in terms of the components: resin, asphalt, and wax. It was concluded that extraction processes using benzene were likely to be of greatest value. Crude peat wax was further fractionated by chromatography to yield nine major fractions. These were distinguished by chromatographic behavior, fluorescence and spectral characteristics, and analytical composition. Preliminary work toward identification of individual components showed the presence of compounds of various types including hydrocarbons (e.g. perylene and many compounds containing one or more carboxyl, ester, carbonyl, or hydroxyl groups).

EXTRACTION of peat with organic solvents yields a dark colored wax of 5 to 15% yield, the amount depending on the source of the peat, its pretreatment, and on the solvent used. Chemical interest in this wax arises from the information it might yield as a possible precursor of coal, and as material of potential industrial value with applications similar to montan wax.

The term "crude peat wax" designates the residue after evaporation of the organic solvent. Earlier work has been reported (1,2,3,4). Most workers have regarded crude peat wax as consisting of three main fractions: resin, wax, and asphalt (5), though several methods have been adopted to separate the three sub-fractions. The resin is composed of polar compounds usually extractable with cold alcohol. The wax fraction is soluble in light petroleum, while the asphalt fraction is insoluble in this solvent. The relative proportions of these fractions obtained from any given wax specimen depend on the solvent used for the original extraction. This aspect will be considered further but the usual range is: resin 9-40%, wax 45-90%, and asphalt 1-18%.

Separation of individual constituents of peat wax has been reported by the authors (4). Since then

Wiedenhoff (6) has surveyed four plant waxes, using separations on alumina columns, followed by X-ray analysis of the fractions. Some hydrocarbons and alcohol components were detected. Cole and Brown (7) have examined ouricuri wax on alumina columns and consider that there is *a priori* evidence that the constituents of natural plant waxes are not simply mixtures of esters. In the case of sugar cane wax Lambertson and Redcliffe (8) have suggested that long-chain aldehydes in polymeric form are a major constituent. Although hydrocarbons and free alcohols and acids were also present there was no conclusive evidence for the presence of esters.

Published work indicates that most workers have preferred to use saponification and other severe reactions as a preliminary fractionation step. These methods are likely to degrade or destroy the natural constituents, and complicate any subsequent assembly of the results. In the present work the chromatographic fractionation of peat wax has been carried out directly. Nine major fractions were obtained and the further separation of these is under study. Results on extraction yields, and general chemical and physical properties of the wax are also presented.

Experimental

Materials. For this work peat was obtained from a bog situated east of Londonderry and referred to as "Altnahullion" in the Northern Ireland Peat Bog Survey (9). The material received was irregularly shaped pieces the size of bricks. Moisture content by the Dean and Stark method was 25 to 30%. Before extraction the peat was broken down in a jaw crusher and reduced in a hammer mill. A fraction passing a 3.2 mm but retained on a 2 mm sieve was used.

Wax. A crude wax extracted with benzene was used for the entire investigation. This was selected after preliminary separations had demonstrated that each of the three main components: resin, wax, and asphalt were present in benzene extracts. A further consideration was that benzene extraction appeared most feasible for commercial scale production.

Reference Compounds. As a guide in the selection of suitable adsorbent and solvent systems for chro-

matography, a synthetic mixture was made of pure substances representative of the various functional groups thought to be present in the crude wax. The compounds selected were similar to those used by Cole and Brown (7) namely: (a) dotriacontane, (b) octadecyl stearate, (c) n-octadecyl alcohol, (d) stearone, (e) 12-hydroxystearic acid, and (f) stearic acid. These reference compounds were from proprietary sources excepting octadecyl stearate, which was prepared by the method of Hilditch and Paul (10) from n-octadecyl alcohol and stearic acid. The purity of this and the other reference compounds was checked by melting point determination.

Adsorbents and Solvents. Published work on column chromatography indicated that alumina and silica gel would probably separate complex mixtures of the type present in the wax. Preliminary experiments with these showed that silica gel was not suitable for the initial fractionation as no combination of solvents could be found to produce satisfactory band separations.

Findley (11) and others have reported varying degrees of degradation of waxes separated on alkaline alumina. This may be overcome by pretreating the alumina with acid. This, however, is a tedious process and leads to variations in the adsorption characteristics of a particular sample of alumina. For the final series of separations alumina from M. Woelm of Eschwege was selected. This is available in an acid form with pH 4.0, and had been found satisfactory for the resolution of ouricuri wax (7).

A modified version of the Brockman and Schodder method of grading alumina suggested by Cole and Brown (7) was used. The most suitable activity was found to be between grades I and II, and p-methoxyazobenzene and sudan yellow were used for standardization. The alumina was of suitable activity when these two dyes were just separated on the column. It was necessary to add 2% by weight of water to the alumina as supplied, to obtain the desired activity. This was shaken vigorously in a stoppered vessel and allowed to stand for 2 hr.

The low solubility of crude peat wax in many solvents made the selection of a suitable solvent schedule difficult. It would have been simple to use a crude wax extracted with a petroleum fraction, but for the reasons given it was desired to use benzene extracted material. The asphaltic materials present ruled out the use of such petroleum fractions as heptane for the early stages of elution. The solvent schedule of Fuchs and de Jong (12) for beeswax was also unsuitable, as it commenced with carbon tetrachloride in which the benzene-extracted peat wax was not completely soluble.

After several experiments, using an alumina column, the following solvent schedule was chosen: (a) benzene, (b) benzene + 3% chloroform, (c) benzene/chloroform 4:1, (d) benzene/chloroform 2:1, (e) benzene/chloroform 1:1, (f) benzene/chloroform 1:1 + 5% methyl alcohol, (g) benzene chloroform/methyl alcohol 1:1:1, (h) benzene/chloroform/methyl alcohol 1:1:1 + 1% acetic acid.

After using this sequence of eluants the column was dismantled and the alumina, which was a pale buff color, was placed in a Soxhlet thimble and extracted with benzene/methyl alcohol, 2:1.

Methods

Effect of Solvent on Wax Yield. Peat was extracted with organic solvents in all glass Soxhlet extractors. Extractions were regarded as complete when

the effluent from the syphon had become colorless and remained so over several cycles, usually about 5 hr. The partially evaporated extract was transferred to a tared evaporating dish and dried at 105°C. The yield of wax was recorded as percentage of the dry weight of the peat.

To obtain sufficient wax for later separation processes an extractor was made on the Soxhlet principle, which held about 7 kg of peat. Several kg of crude wax were prepared.

Separation of Resin, Wax, and Asphalt. Several methods for separating components were studied. The method of Boyd-Barrett *et al.* (5) was found the most satisfactory of the published methods; but this also had disadvantages, namely: the amount of crude wax used was fairly large; the number of filtrations and transfers of material was excessive; the solubility of the resin component is temperature-dependent, and the process described is not well adapted for the accurate temperature control required for consistent results. A modified separation method follows:

(a) **Resin.** A glass Soxhlet extractor was adapted for extraction with methyl alcohol at constant temperature (18°C). The extract was reduced by distillation and then transferred to a tared dish, dried at 105°C, cooled, and weighed.

(b) **Wax.** This was determined in essentially the manner of Boyd-Barrett (5) except that the deresinified wax from (a) was used without removing it from the extraction thimble. It was, however, thoroughly dried of all traces of methyl alcohol. This was necessary to avoid the formation of azeotropes between the petroleum to be used and any residual alcohol. Azeotropes lead to extraction of some asphalt. The material obtained by complete extraction of the resin-free crude wax with 60/80°C petroleum ether gave the wax content of the crude wax.

(c) **Asphalt.** The content was determined by re-extracting the contents of the same extraction thimble with benzene. It was not necessary to remove the residual petroleum ether as the asphalt is insoluble in this solvent. The weight of extract obtained was recorded as the asphalt content of the crude wax.

Functional Group Analysis. To determine the extent to which any chromatographic technique was separating the constituents of the crude wax each fraction was analyzed for the functional groups present. The methods were based on those of previous workers: Findley (11), Findley and Brown (13), Sundgren and Rauhala (14), and Cole and Brown (7). Certain modifications had to be introduced to overcome such difficulties as the small amount of each fraction available for analysis, the low solubility of certain fractions, and the dark color of the crude wax and several of the major fractions separated on the chromatographic columns.

Hydroxyl Value. The sample (0.3 g) was weighed into a 100 ml wide necked stoppered flask and 3 ml of acetylating reagent (acetic anhydride, pyridine, 1:3, freshly prepared) was added. The stopper was moistened with pyridine to ensure a better seal. The flask was heated on a steam bath for 1 hr, 6 ml of water was added, and the flask heated for 2 min more. Thirty-five ml of benzene was added and the flask reheated if necessary to dissolve the sample. One ml of phenolphthalein indicator was added and the solution titrated with N/2 alcoholic KOH using a pH meter to determine the end-point. A blank determination was run simultaneously. The titration was corrected where necessary for the amount of free acid

in the sample. The end point by the pH meter was found to coincide with the change from colorless to pink of the indicator; it was therefore possible to determine the end point of the lighter colored fractions by either method. In all titrations the solution was stirred continuously by a magnetic stirrer. The method was checked against octadecanol, threo-9,10-dihydroxystearic acid, and 12-hydroxystearic acid.

Carbonyl Value. Ca. 0.3 g of sample was weighed into a wide necked stoppered flask and dissolved in 10 ml of benzene. Ten ml of freshly prepared hydroxylamine hydrochloride solution was added, and the mixture was refluxed for 4 hr on a steam bath. The liberated HCl was titrated with $N/2$ KOH solution to a pH of 3.8 using a pH meter. The flask was then returned to the steam bath and once refluxing had recommenced the heat was cut off and the flask remained on the bath over-night. The contents were again titrated to pH 3.8. This procedure was found to be necessary as the peat wax fractions reacted much more slowly than the reference compounds where the initial reaction went to completion and the second titration was negligible. In the case of some of the wax fractions, the second titration was sufficiently large to have a significant effect on final result. The flask was then reheated for a further 2 hr to ensure complete reaction. Blank determinations were run simultaneously. Stearone was used to check accuracy.

Acid Value. Ca. 0.3 g of the sample was weighed into a stoppered flask and dissolved in 10 ml benzene. Seven ml of 94% ethanol was added. The flask was heated to dissolve the sample, cooled, and titrated with $N/2$ KOH from a microburette using the pH meter. Again, with the lighter colored fractions, this was found to coincide with color change of phenolphthalein. Benzoic acid was used to check accuracy.

Saponification Value. This was determined on the sample previously used for the acid value determination. Ten ml $N/2$ KOH was run in and the mixture refluxed for 90 min. The condenser was washed down with 5 ml of 94% ethanol and 10 ml of benzene was added. The contents of the flask were acidified to pH 3. Using the pH meter, the sample was then titrated with $N/2$ KOH. The titration curve has two inflection points, the first representing the end point due to the strong acid, and the second that due to the liberated high molecular weight organic acids. The saponification value is calculated from the amount of $N/2$ KOH used between the two inflection points. With the lighter colored fractions it was possible to check the results obtained with the above method by the standard back titration procedure using indicators. Octadecyl stearate was used to confirm the accuracy.

Molecular Weights. The Rast method for the determination of molecular weight by the depression of the melting point of camphor was used. With molecular weights of the order of 500, depressions of 3–4C were observed. It was necessary to read temperatures to the nearest 0.05C, and to ensure that the rate of increase of temperature around the melting point was not greater than 0.25C per min. With care the method was found accurate to within 5% of the theoretical value for the reference substances used. The error of this method tends to increase with increasing molecular weight. However, with molecular weights likely to be found in peat wax and its constituents an error of 5% could be accepted.

Chromatography. Chromatographic separations were made in a glass column 4.0 cm diam and 40 cm

long. The column was packed with a benzene slurry of a standard amount (250 g) of the Woelm alumina adjusted to Grade I–II. After packing, the column stood overnight and usually packed down about 2 cm, the final length being about 30 cm. The upper end of the column was fitted with a reservoir of 5 l capacity. Fractions of 50 ml were collected in 6 x 1 inch glass tubes with a syphon operated fraction collector. The wax sample was prepared by weighing 15 g of the benzene-extracted crude wax and dissolving in 200 ml of hot benzene. The solution was cooled and filtered. After standing overnight the solution was again filtered. The two filtrations were necessary to remove particles of undissolved solids which tended to block the column. One hundred ml of solution prepared in this manner contained about 6.5 g of wax.

Before applying the solution to the column the reservoir was removed and a disc of filter paper placed on the surface of the alumina. The benzene was run out until a layer about 2 cm deep remained covering the alumina. The wax solution (150 ml) was then poured carefully down the interior wall of the column to avoid disturbing the surface of the alumina. When the level of the wax solution fell to about 2 cm above the alumina a small quantity of benzene was added. The sample was washed into the column with repeated small additions of benzene. The column was colored dark brown for a depth of about 10 cm from the top. Immediately below this was a slate grey band about 3 cm. The column was then filled with benzene, the reservoir replaced and the rate of flow adjusted to about 120 drops/min and 50 ml fractions were collected. A number of fluorescent bands soon became visible in ultraviolet light and the appearance of the column during the course of its development was recorded. Elution was continued with the selected solvent until the eluate became colorless, or the movement of the fluorescent bands down the column ceased. The next solvent in the series was then applied. The contents of each tube were transferred to porcelain dishes, evaporated, and the weight recorded. Usually about 150 fractions were collected in this manner by which time the column had become a pale lilac color in UV light, and a pale buff in ordinary light. At this point further development was stopped, the alumina removed from column, transferred to Soxhlet apparatus, and extracted with benzene and methyl alcohol (2:1). The extract was thereafter treated as one of the fractions, evaporated, and weighed.

Results

Effect of Different Solvents on the Yield of Crude Wax and on the Proportions of Resin, Wax, and Asphalt. A range of solvents was selected to show the effect of typical members of various classes of solvents in common use in industrial extraction processes. The properties of benzene-extracted crude wax are shown in Table I and the yield figures with various solvents are given in Table II. The various crude waxes thus obtained were split into resin, wax, and asphalt components by the solvent fractionation method described.

TABLE I
Properties of Crude Peat Wax (Benzene Extract)

Melting point °C.....	75
Molecular weight (Rast).....	686
Acid value.....	56
Ester value.....	60
Hydroxyl value.....	86
Carbonyl value.....	45
Resin content %.....	21
Wax content %.....	64
Asphalt content %.....	15

TABLE II
Yields of Crude Wax, Resin, Wax, and Asphalt
Obtained with Various Solvents

Solvent	Yield crude wax (% dry peat)	Resin % of crude peat wax	Wax % of crude peat wax	Asphalt % of crude peat wax
Benzene.....	10.6	21	64	15
Ethyl alcohol.....	10.4	32	56	12
Dichlorethane.....	11.0	20	70	10
Esso solvent 20 ^a	7.0	10	88	2
Esso solvent 50 ^a	7.8	12	87	1
Hexane.....	6.7	9	90	1
Methyl ethyl ketone.....	12.2	23	63	14
Benzene/ethyl alcohol (9/1).....	14.1	39	48	13
Benzene/ethyl alcohol (7/3).....	14.6	33	49	18

^a Petroleum fractions.

TABLE III
Chromatographic Fractionation of Crude Peat Wax and Characteristics of Fractions

Fraction	Tube numbers (see Fig. 1)	% Wt of crude wax	Texture	Color	Fluorescence
1.....	4, 5	0.81	Buttery	Pale yellow	Pale lilac
2.....	6-9	5.83	Soft, waxy	Yellow	Brilliant blue
3.....	10-48	7.26	Waxy	Light brown	Yellowish-green
4.....	49-53	5.54	Cheesy	Light brown	Yellowish-green
5.....	54	3.57	Soft-sticky	Olive green	Dull blue
6.....	55-71	13.31	Soft-sticky	Green	Dull blue
7.....	72-106	12.06	Brittle, shiny	Brown
8.....	107-134	17.10	Brittle, dull	Brown
9.....	CaH ₂ MeOH extract of alumina	19.71	Brittle, dull	Brown

TABLE IV
Analytical Data for the Major Chromatographic Fractions of Crude Peat Wax

Fraction	Acid value	Ester value	Carbonyl value	Hydroxyl value (mg KOH/g)	Molecular weight	Melting point °C	C %	H %	O %
1.....
2.....	0	59	29	10	604	67	81.02	12.7	6.28
3.....	3	70	49	12	508	68-71	81.55	11.48	6.97
4.....	21	150	38	47	612	60	74.93	11.25	13.82
5.....	17	49 ^a	89	586	57-58	78.96	11.68	9.36
6.....	18	63	32	89	634	57-59	79.97	11.90	8.13
7.....	15	74	34	108	681	62	78.02	11.28	10.70
8.....	88	50	28	82	742	80	75.31	11.21	13.48
9.....	87	68	16	91	850	Softens	74.18	10.79	15.03
Crude peat wax.....	56	60	45	86	686	75	77.26	12.34	10.40

^a Insufficient material to determine carbonyl value.

These figures are also included in Table II. The yields are given as percentages of crude wax, and dry weight of peat.

Chromatographic Fractionation of Crude Peat Wax. The crude wax used in all subsequent fractionation work was prepared by benzene extraction. The wax was separated into 4 major fractions (Fig. 1) and these were further subdivided on the basis of color and fluorescence differences to give nine main fractions. A recovery of 86% of the crude wax placed on the column was obtained, and the procedure was repeated until sufficient material had been produced for determination of functional groups and other properties of each fraction.

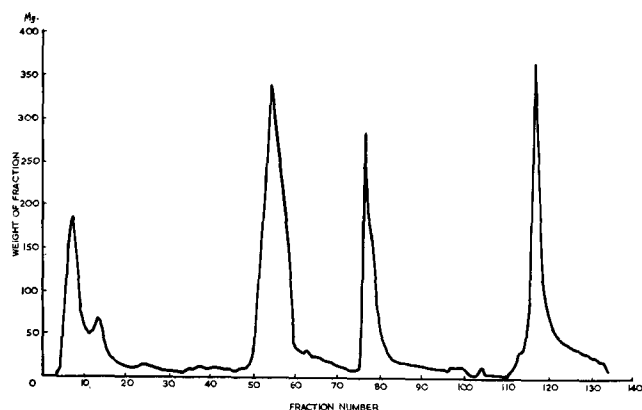


Fig. 1. Resolution of crude peat wax or Woelm acid alumina Grade II.

The weight distribution of the crude wax among the various fractions is shown in Table III, and the analytical data obtained for each fraction, together with similar data for the crude wax, is shown in Table IV.

In addition to the physical and chemical properties reported there was also a marked difference in texture and physical appearance of some of the fractions (Table V). These were helpful in separating the fractions. A striking feature of some of the earlier fractions was their brilliant fluorescence in UV light.

Further Separation and Characterization of Major Chromatographic Fractions. After the separation of

crude wax into its major chromatographic fractions attempts were made to establish the purity of each fraction before proceeding to consider methods for characterization.

At this stage none of the fractions exhibited any tendency to crystallize. From fraction 2, however, a white noncrystalline precipitate separated when a solution in hot cyclohexane was allowed to cool to room temperature. This material was designated subfraction 2-A, and had composition: % carbon, 81.43; % hydrogen, 13.16; molecular weight, 760. It melted at 81.5°C and had a slight fluorescence. There was insufficient material for functional group analysis and further investigation was limited to the determination of the UV and IR absorption characteristics. The IR spectrum indicated the presence of a carbonyl group. The empirical formula derived from microanalysis was C₂₀H_{38.6}O, but it has not been possible to find a suitable molecular formula; obviously further purification of the material is necessary, followed by a redetermination of the chemical and physical properties. It is of interest, however, that Pschorr and Pfaff (15) have reported the presence of cerotone (C₂₅H₅₁)₂CO, and montanone (C₂₇H₅₅)₂CO in montan wax, and Findley (11) the presence of a ketone or

TABLE V
Properties of Subfractions of Fraction 2

Subfraction	Texture	Color	Fluorescence
2-B.....	Waxy	White	Intense blue
2-C.....	Waxy	Lemon yellow	Turquoise
2-D.....	Semicyrystalline	Pale yellow	Slight green
2-E.....	Waxy	Orange yellow	Slight blue

group of ketones with a chain length of 57 to 65 carbon atoms in sugar cane wax.

The cyclohexane soluble portion of fraction 2 was further separated into components soluble and insoluble in ethyl alcohol, and from the soluble material a polycyclic hydrocarbon characterized as perylene has been isolated (16).

Failure to obtain separation by fractional crystallization suggested that the fractions were still of a heterogeneous nature, and further chromatography offered the most suitable method for the separation of additional constituents. Fractions 1, 2, and 6 were rechromatographed on alumina, using the solvents which seemed most appropriate after solubility experiments. The results were:

Fraction 1. This was readily soluble in benzene and was chromatographed on a column of Woëlm acid alumina, Grade II. The main fraction, designated subfraction 1-A, was a yellow waxy solid, mp 59.5C, with an intense lilac fluorescence. Associated with it was a small quantity of a white solid which was immiscible with the main fraction, on solidification, and in the molten state. This fraction, designated subfraction 1-B, was separated by allowing the subfraction 1-A to solidify, then collecting the still molten subfraction 1-B in a melting point tube by capillary attraction. By this means a very small quantity of a soft white waxy solid was obtained. This had a mp of 33.8C and intense lilac fluorescence. The extremely small amount of both materials available limited further investigation of characteristics to the determination of UV absorption spectra.

Fraction 2. This fraction was dissolved in cyclohexane and, after removal of subfraction 2-A, was chromatographed on Woëlm acid alumina, Grade II. Four main fractions were obtained with the properties shown in Table V.

Again, further investigation of the subfractions was limited by the small amounts available. Subfractions 2-B and 2-C were in sufficient quantity to determine melting points and spectra. MP were 56.3C and 65C, respectively.

Fraction 6. This fraction was readily soluble in cyclohexane, and after dissolving it was chromatographed on Woëlm acid alumina, Grade II, and separated into 3 main fractions. See Table VI. In the final stages of the separation it was necessary to use first benzene and then benzene/chloroform 4:1 to elute the subfractions.

Only subfraction 6-A was recovered in sufficient quantity for a melting point determination: 57C. Absorption spectra were also determined.

At this stage further work on rechromatographing the remaining fractions was abandoned as it became apparent from the number of subfractions being obtained that the main fractions were still relatively complex mixtures, and that further separations to isolate pure compounds for characterization would necessitate the preparation of much larger quantities of the nine main fractions. Although no well defined crystalline subfractions were obtained, there was sufficient indication of an increased tendency to crystallize to encourage the view that the method, if done on a large enough scale, would be successful in the separation of pure components of the original wax.

Discussion

This study of the solvent extraction of peat determined the effect of various solvents on the yield of wax. A considerable variation in yield was found; the highest yields were obtained with mixed solvents such as benzene/alcohol. In view of the economic importance of maximum yields an explanation was sought. The increased yields with mixed solvents could be explained by an increase in the resin and asphalt components of the crude wax, particularly the former (Table II). The wax content of the extracted crude material did not seem dependent on the solvent used. This is important as, for most commercial requirements, resin and asphalt are undesirable components and their elimination from waxes can be costly. The results obtained make it possible to select an extraction solvent based on the desired properties of the final product. In the course of the work an improved method for the separation of the resin component was developed.

The data obtained suggest that, for the extraction of peat wax on a large scale, the process should be based on a single solvent. The selection of this solvent will depend on the proportions of resin and asphalt required in the final product. If a high content of the wax component is desired, then a petroleum fraction will be the most suitable solvent. However, if a proportion of asphalt and resin can be accepted, benzene or methyl ethyl ketone are suitable. As part of a development program of the Department of Industrial and Forensic Science of Northern Ireland, this extraction work has been extended to pilot plant scale. Results obtained in extraction tests on 15 cwt batches of peat were substantially the same as those obtained in the laboratory. Yields were generally slightly lower, but the same relative compositions were observed.

The second part of this investigation, the development of methods for the isolation of original constituents of crude peat wax, is a difficult problem. Until recently the chemistry of waxes has been based largely on the examination of the degradation products of such destructive procedures as saponification. To avoid severe treatments, adsorption chromatography offered most promise in that it would be essentially nondestructive, sensitive, and versatile.

A suitable combination of adsorbent and solvent was first selected to achieve the maximum degree of separation of the wax components. The system chosen yielded nine main fractions. These were examined in detail by functional group analysis and, although it was evident that no clear-cut separations had been achieved, there was definite gradation of properties varying from nonacidic components in the first fractions to acids and hydroxy acids removed in the later fraction. An unexpected feature was the very general distribution of compounds in which the carbonyl group is present. The presence of carbonyl compounds throughout such a series of fractions does not appear to have been reported elsewhere in the literature and is of interest in view of recent similar findings by Lambertson and Redcliffe (8) on sugar cane wax.

A more detailed examination of fraction 2 led to the isolation of two interesting components. One was not completely purified, but appeared to consist predominantly of a carbonyl compound in the range C₂₀ to C₃₀. Secondly, and perhaps most striking, was the isolation of the polycyclic hydrocarbon, perylene. It is not possible at this stage to suggest what the biological precursor of this compound would be, but it seems that in the humification process there must be cycliza-

TABLE VI

Subfraction	Texture	Color	Fluorescence
6-A.....	Waxy	Orange brown	Slight bluish
6-B.....	Waxy dull	Green	Turquoise
6-C.....	Waxy shiny	Green	Turquoise

tion and dehydrogenation reactions taking place. Further study should reveal other related hydrocarbons, some more complex, some simpler, if this is the case.

Further work is proceeding on the resolution of fraction 2, and of other fractions. After considerable subfractionation, yields become small and it is apparent that characterization of components will have to depend mainly on spectroscopic identification.

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Preparation and Purity of Linoleic Acid from Commercial Corn, Cottonseed, and Safflower Oils¹

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Abstract

Linoleic acid from commercial corn, cottonseed, and safflower oils was prepared by low temperature crystallization using acetone and petroleum ether as solvents; temperatures ranged between -70 and 50°C. This method has the advantages of simple equipment and of flexibility in preparatory capacity. The crystalline fraction obtained at -55°C was shown to be "pure" linoleic acid.

Isomerization with potassium tertiary butoxide, oxidative cleavage by periodate-permanganate, and analysis by liquid-liquid and gas-liquid partition chromatography were used to ascertain the purity and the presence of isomers in the final product. This fraction was found to contain 90 to 95%, 9,12 dienoic acid; approximately 5% of dienes with the first double bond at the C₈ position and the second bond either at the C₁₂ or C₁₃ positions; and small amounts of nonconjugatable 9,15 *cis,cis* dienes. Linoleic acid from these oils was similar in composition, except that from corn oil showed the presence of diene with the first double bond at the C₁₁ position.

(6) employed urea complex precipitation procedures for preparation of large quantities of linoleic acid concentrate of about 95% purity as shown by alkali isomerization. Beal and Brekke (7) used liquid-liquid partition technique for separation of linoleic acid and gas-liquid chromatography (GLPC) for analysis. Ozonolysis, followed by liquid-liquid partition chromatography (LLPC) of dibasic acids, was used by Allen and Kiess (8), and by Cousins *et al.* (9) to judge the purity of their linoleic acid preparations from safflower oil. While Allen and Kiess reported small amounts of an isomer with a double bond at the 8 position, Cousins and his coworkers attributed the presence of such small amounts of isomers, with double bonds in positions other than 9,12-, to the shift of these bonds caused by alkali during the saponification of the oil for preparing the fatty acids.

Scholfield, Nowakowska, and Dutton (10) describe a countercurrent distribution procedure to prepare methyl linoleate from soybean and safflower oil methyl esters, and by using GLPC they indicate the possible presence in their preparations of palmitoleate, which has a similar partition coefficient to linoleate.

Of the procedures mentioned, only adsorption chromatography on silicic acid columns (5) and countercurrent distribution (10) are reported to yield methyl linoleate of greater than 99.0% purity. However, the investigators who used the two methods assumed that their preparations were the 9,12-octadecadienoate and did not investigate the possible presence of other isomers. Besides, adsorption chromatography yields only small amounts of products, and countercurrent distribution needs special apparatus.

In the present investigation low temperature crystallization technique was chosen not only because of its easy adaptability when solid carbon dioxide is available, but also because its flexible preparatory capacity is limited only by the size of cooling baths and crystallization cylinders. Since GLPC is the most versatile of the available analytical techniques in revealing the presence and identity of individual compounds in mixtures, all samples were analyzed by this method. Isomerization with potassium tertiary butoxide (11) was used to estimate the amounts of linoleic and trienoate acids in some fractions, mainly to confirm the quantitative aspects of GLPC. The

LINEOLEIC ACID is the most widely distributed and abundantly occurring dienoic acid of nature. Among its best known sources are corn, cottonseed, and safflower oils. It has been shown to have the structure, *cis,cis*-9,12-octadecadienoic acid (1). Isolation of linoleic acid in its native form by low temperature crystallization from corn, cottonseed, grape seed, poppy seed, and sesame oils was first described by Frankel, Stoneburner, and Brown (2). Their preparations were about 93-97% pure, using the tetrabromide number as the criterion of purity. Swift, Rose, and Jamieson (3) prepared methyl linoleate by adsorption chromatography on alumina columns and used iodine value to estimate purity.

After introduction of the alkali isomerization technique (4) it was used to determine the purity of methyl linoleate obtained by adsorption chromatography on silicic acid columns (5). Swern and Parker

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