Discussion

The storage of aceituno seed presents no special problems except that one must take care against insect infestation. The seed may be attacked by the larvae of the Indian meal moth, *Plodia interpunctella* (Hbn.), and the almond moth, *Ephestia cautella* (Wlkr.).

The residual cake is rich in proteins (50%), but it contains a bitter principle that is related to quassine and is probably similar to or identical with simarubin, isolated from *Simarouba amara* Aubl. The meal is toxic to livestock and is therefore now being used only as a fertilizer. The cake contains 8.12% nitrogen, 1.90% phosphoric acid as P_2O_5, and 1.17% potash (K_2O).

Work is under way to develop an economical method of detoxication of the cake in order to permit its use as feed for livestock.

Summary

The seed of the tree *Simarouba glauca*, which is known as aceituno, aceituno silvestre, and aceitillo in Central America, contains about 65% of a solid or plastic fat. The crude fat is greenish in color and has a slightly bitter taste, but after refining it yields a snow-white, odorless, and practically tasteless product having the same uses as commercial shortenings. Refining is carried out in a manner similar to that used for coconut oil.

The fat is produced commercially in El Salvador, where it is used for practically all purposes in which vegetable shortening would be used. The residual press cake is toxic to livestock and can be used at present only for fertilizer.

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The Hydrocarbons of Ouricuri Wax

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THE ouricuri tree is one of Brazil's most important palm trees. Its seeds yield a fatty oil, and from its leaves while they are still green, there is recovered a hard wax by scraping them with glass or metal. It was not until some 15 years ago, following the adoption of improved purification techniques, that ouricuri entered the list of the several waxes exported from this country. To the United States at that time were sent a mere 3.075 kg. Three years later exports of this product to the States alone had increased over 100-fold (2).

Ouricuri wax finds use as a substitute for carnauba wax in floor waxes, shoe creams, other polishes, and inks used in producing typewriter carbon paper. Like carnauba it can also be used as a "melting point booster" for paraffin waxes. It is reported that ouricuri wax has been used in the finishing of bombing and fighter planes because of the high polished surfaces that can be produced with it so that they are resistant to air friction and to wetting or condensation (5).

The wide technological utilization of this wax stands in marked contrast to the paucity of available literature on chemical investigations pertinent to its constitution. The only intensive study of any significant merit appears to be that reported by Luedecke (1), who has claimed the presence herein, among other substances, of hydrocarbons and esters of myristic and cerotic acids.

In this communication we report the results of a study undertaken not so much to substantiate or to contradict existing views on the chemical composition of ouricuri wax as to explore the possibilities of using an approach other than the conventional saponification-fractional crystallization techniques to the analysis of plant, and perhaps insect, waxes. The immediate objective however was the characterization of the hydrocarbon components of this wax. The following account will show that solvent-fractionation, molecular distillation, and column chromatography were successfully used to bring about not only the resolution of ouricuri wax into groups of its components but also the isolation of the individuals comprising the mixtures in question.

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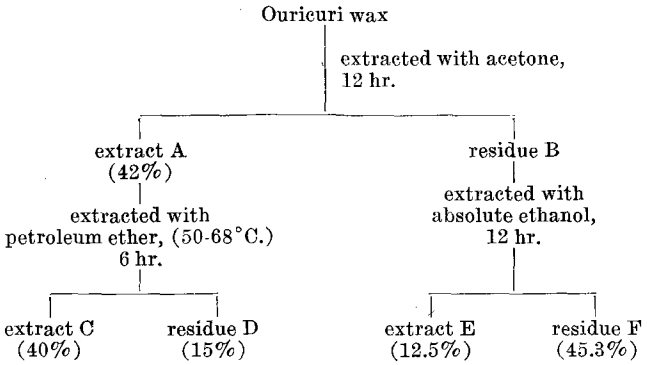
FIGURE 1
Solvent-Fractionation Flow Sheet



Experimental

Solvent-Fractionation. The regular article of commerce, previously ground to a coarse powder in the laboratory, was extracted with acetone, petroleum

ether, and ethanol under conditions described in the flow sheet of operations (Figure 1). Two of the resulting fractions were then critically studied.

Analysis of Fraction F. The acetone- and ethanol-insoluble fraction (m.p. 90-92.5°C.) did not yield satisfactory chemical constants because of its poor solubility and dark brown color. However on repeated attempts sufficient evidence was obtained to contraindicate the presence of free acids, alcohols, and unsaturated components in this fraction.

A part of this fraction was heated under reflux for eight hours with equal volumes of alcoholic potassium hydroxide solution and benzene. After saponification the solution, while still hot, was decanted from the small residue of unchanged material still in the reaction mixture. The solvent was then removed from it by evaporation after which the residue of potassium soaps was converted to insoluble barium soaps by the addition of barium chloride with vigorous and continued stirring. The barium soaps, thoroughly dried after recovery from the reaction mixture, were then treated with petroleum ether (b.p. 60-68°C.) in a Soxhlet extractor. The extract had a slightly yellow color. This was subsequently removed by treatment with activated charcoal. The final product, soluble in acetone, ethanol, and other organic solvents, yielded, on further purification and repeated crystallization, a crystalline material melting at 86-87°C. (melissyl alcohol, m.p. 85.8-87°C.). As a final precaution to ensure complete freedom from unsaponified material the barium soaps were extracted once more, this time with benzene. These, when decomposed with hydrochloric acid, yielded a dark product which however became colorless on several treatments with charcoal. One recrystallization of the purified acid gave a product of m.p. 79-80°C. and neutralization equivalent 140 (hexacosanoic acid 141). These findings along with the other facts strongly indicate that fraction F is composed mostly of the wax ester, melissyl cerotate. An ester of closely similar composition has been reported as a component of the ouricuri wax by others (1).

Molecular Distillation of Fraction C. A 400-g. portion of that fraction which had been recovered from the original wax by extraction, in turn, with acetone and petroleum ether was subjected to distillation in a type CMS-5 centrifugal molecular still (Distillation Products Inc.). Six fractions were collected (Table I). The apparatus was maintained at a pressure of 15 microns throughout the entire process.

Preliminary tests based on chromatographic analysis (4) revealed the presence of paraffin hydrocarbons in the first four fractions.

Isolation of Hydrocarbons. The first molecular distillate was almost entirely paraffin in composition while 75% of the second distillate was accounted for by the hydrocarbons. The third and fourth distillates yielded about 30 and 15%, respectively, as their hydrocarbon components. The procedure adopted for isolation in the case of each distillate individually was as follows. A 20-g. portion was dissolved in a minimum quantity of petroleum ether and introduced into a 1.75″ x 10″ column of activated alumina (Alcoa, Grade F-20). Percolates, in suitable portions, were collected consecutively and evaporated on a steam bath in order to follow the progressive elution of the hydrocarbon component. After the first appearance of the hydrocarbon in the percolate, the solid contents

TABLE I

Molecular Distillation of Acetone- and Petroleum Ether-Soluble Fraction of Ouricuri Wax

Distillate	Weight	Distillation temperature
	g.	*°C.*
1	45	130
2	46	150
3	84	180
4	50	200
5	12	230
6	17	250

of the following few percolates accounted for all the hydrocarbon present. Anhydrous petroleum ether (60-68°C.), purified by means of sulfuric acid treatment, was used throughout the entire process.

Purification of the Hydrocarbon. The hydrocarbon was heated with constant stirring at 70-80°C. with about 20 times its volume of sulfuric acid for one hour. The mixture was cooled, and the spent acid was removed from under the solid cake of hydrocarbon. The whole process was repeated until there was no discoloration upon heating the hydrocarbon with fresh acid. The product was washed with cold water and then with hot water until completely free of acid. The material was then thoroughly dried and dissolved in a minimum volume of purified, anhydrous petroleum ether, after which the solution was passed through a short column of activated alumina. A 2 x 20 cm. column of alumina was found suitable for a one-gram quantity of hydrocarbon.

Chromatographic Separation of Hydrocarbons. Each hydrocarbon fraction, isolated from the above mentioned molecular distillates and purified as described in preceding paragraphs, was separately treated in the following manner. One to two grams of the material under examination, dissolved in a minimum quantity of purified anhydrous petroleum ether, was put through a 2 x 96 cm. column of activated alumina wet before use with the same solvent which was also used for subsequent development of the entire chromatographic process. Immediately following the appearance of the first traces of hydrocarbon, the percolates were collected in 5-10 ml. portions until all the material placed on the column had been brought down. About 40 fractions, after the removal of the solvent by evaporation, were thus obtained. Three or more suitable fractions, covering the entire range of and equally spaced over the complete set of these fractions, were chosen and, after recrystallization from a mixture of ethanol and petroleum ether, were examined for melting point and long crystal, or A, spacings (3). The latter were determined with the aid of a Model XRD-3 X-ray Diffraction Unit (General Electric Corporation, Milwaukee, Wis.).

The following facts were noted by the application of the foregoing process of resolution with the aid of column chromatography:

1. No separation was noticeable in the case of the hydrocarbon obtained from the molecular distillate No. 1. All the fractions examined individually gave a melting point of 51.1-51.3°C. and a long crystal spacing of 32.6°Å.
2. Molecular distillate No. 2 provided a mixture of hydrocarbons. The melting points covering the entire range of chromatographic fractions progressively increase from 56.8-57°C. to 58.9-59°C. Long crystal spacings showed a variation of the order of 2.6 Å. However X-ray analysis in this case failed to yield satisfactory individual values.
3. The hydrocarbon obtained from the molecular distillate No. 3 yielded fractions exactly identical in physical properties, m.p. 65.6-65.8°C. long crystal spacings, 40.0 Å.
4. The hydrocarbon obtained from molecular distillate No. 4 was resolved into fractions, showing the greatest spread in the

melting points. A group of three fractions, chosen in the manner identical to the one indicated earlier, gave 69-69.2°C., 72.7-72.9°C., and 76-76.2°C., respectively, as their melting points.

Discussion

Chromatography over alumina was found not only to be a remarkably effective tool in bringing about resolution of hydrocarbon mixtures but also a very dependable criterion for judging the purity and homogeneity of a paraffin sample. In the light of this observation it follows beyond any reasonable doubt that the hydrocarbons obtained from the molecular distillates Nos. 1 and 3 are single hydrocarbons. Their physical constants would identify them as C_{24} and C_{30} *n*-paraffins, respectively. Molecular distillate No. 2 however yielded a mixture of hydrocarbons, which on the basis of chromatographic behavior and observed physical properties can be presumed to be a mixture of C_{26} and C_{28} *n*-paraffins. Any prediction in the case of the hydrocarbon component isolated from the molecular distillate No. 4 is not so easy as in the above other cases. However the spread noted in the case of melting points of the widely separated chromatographic fractions makes it seem likely that this particular hydrocarbon fraction may very well be a mixture of C_{32}, C_{34}, and C_{36} *n*-paraffins. Although there may be some doubt as to the exact identity of some of the hydrocarbons that had appeared as mixtures, there seems to be little doubt however that the first four molecular distillates collectively included *n*-paraffin falling continuously within the range of C_{24} to C_{36}, and these, if presumed to be all of an even number carbon atom content, will be seven in number.

Summary

Solvent-fractionation used in conjunction with molecular distillation and chromatographic adsorption yielded wax fractions whose separation into their components was successfully and easily accomplished. Without benefit of either molecular distillation or chromatography however, but merely by a solvent-fractionation procedure there were isolated cerotic acid and melissyl alcohol under conditions which point to their occurrence in ouricuri wax in an ester combination.

Column chromatography was found to be a remarkably successful tool in resolving the mixture of hydrocarbons which this wax contains and in establishing the homogeneity of an individual hydrocarbon. Indicated beyond a reasonable doubt is the presence of C_{24} and C_{32} *n* paraffins; that of all the four even homologs between these limits is strongly indicated.

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Gossypol Material Balance, Denaturation of Protein, and Loss of Thiamine in Commercial Processing of Cottonseed

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INFORMATION on the material balance for gossypol, the denaturation of protein, and the destruction of thiamine in the commercial processing of cottonseed by hydraulic- and screw-pressing methods is considered basic to current consideration on the improvement of processing methods that may lead to the general production of meals of superior nutritive value and suitable for feeding extensively to swine and poultry (2, 7). The purpose of this report is to present data on this subject.

The yields and quality of oil have been given much attention in the development of the cottonseed processing industry. Cooking of the meats prior to pressing has become a general practice to facilitate oil extraction, to improve the quality of the oil, and to bind the gossypol and gossypol-like pigments (1), hereafter referred to as gossypol. The gossypol not bound during cooking and pressing and extractable with aqueous acetone is designated as free gossypol (13). The extent of gossypol binding, protein denaturation, and thiamine reduction is in each case a function of moisture content, temperature, and time of heating of the meats during cooking and pressing.

The utility of cottonseed meal in the rations of swine and poultry is influenced by the extent to which the free gossypol is reduced and by the availability of the protein. Processing conditions can be selected so that the free gossypol can be reduced to a level which does not interfere with the growth of swine and poultry (2, 9). Based on the evidence collected to date, the suggestion has been made that the free gossypol should be reduced to 0.03% or less in meals for feeding to swine and chickens (2).

Lacking specific analytical methods for laboratory evaluation of the change in nutritive or biological value of the protein induced by heat during processing, nitrogen solubility has been used as an approximate index (7, 12). Denaturation, measured by

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