Constitution of Mamey Wax

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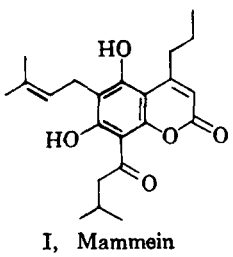
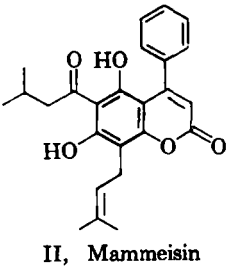
Mamey wax, m.p. 79.5°, isolated from the seed oil of *Mammea americana* L. is shown to be a mixture of saturated, long straight-chain fatty esters with carbon contents ranging from C_{38} to C_{54}. The major constituent is the symmetrical C_{48} homolog, tetracosanyl tetracosanoate. After hydrolysis, in addition to even carbon-numbered species, small amounts of odd carbon-numbered acids were detected.

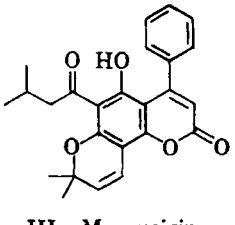
SCIENTIFIC INTEREST in various parts of the mamey tree (*Mammea americana* L., family *Guttiferae*) stems, at least in part, from the century old report of de Grosourdy (1) on its insecticidal activity. Several studies of this property of mamey seed extracts (2–5) led eventually to the isolation (6) of a crystalline active principle. Degradative and spectroscopic (7, 8) as well as synthetic evidence (9) was adduced (8) in support of I for this substance which was named mammein (7). Subsequently, a yellow toxic (10) compound was isolated from the fruit peelings and shown (11) to possess II. [Compound II was later named mammeisin (12).] Recently, a new coumarin with yet unknown physiological properties, mammeigin, was isolated from the seed oil and reported (13) to have the closely related structure III.



I, Mammein



II, Mammeisin



III, Mammeigin

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As part of a continuing study of the constituents of mamey seed oil, we have characterized the wax obtained by acetone precipitation from this oil. The present paper is concerned with these experiments.

RESULTS and DISCUSSION

In connection with their original isolation of mammein, Morris and Pagán (6) reported that a "nontoxic white, powdery solid, melting sharply at 78°," was obtained as a precipitate from an acetone solution of mamey oil. Although Morris and Pagán provide no additional details concerning this substance, there is no doubt that this material is identical to that which we have obtained by a similar procedure and named mamey wax. The melting point of the crude wax is 74–76° but can be raised by repeated recrystallization from dioxane, isopropanol, ethyl acetate, or chloroform to 79.5°.

The presence in the molecule of a saturated ester grouping was indicated by the bands in the infrared at 1733 (carbon-oxygen double bond stretching) and 1179 cm.$^{-1}$ (carbon-oxygen single bond stretching) (14). In addition to these bands and the strong absorption in the 2900 cm.$^{-1}$ region corresponding to carbon-hydrogen stretching modes, the spectrum showed relatively few more bands. A series of small evenly spaced peaks between 1340 and 1190 cm.$^{-1}$ indicative of band progression in long-chain fatty acid esters, suggested a chain length of at least 22 carbons (15). A pair of bands at 730 and 724 cm.$^{-1}$ associated with skeletal vibrations of methylene chains was present (16) as was a band at 1377 cm.$^{-1}$ arising from the carbon-hydrogen deformation frequency for a terminal methyl group. This latter band, however, was of significantly low intensity when compared to the band at 1471 cm.$^{-1}$ arising from the analogous vibrations of methylene groups. Finally, it was noted that absorption due to hydroxyl or carbon-carbon double bonded groupings was absent.

The conclusion that the wax was indeed saturated was supported by a negative tetranitromethane test (17) and by the absence of vinyl hydrogen absorption in the proton magnetic resonance spectrum. (These observations were also consistent with the complete absence of absorption in the ultraviolet region.) Although some difficulty was encountered in the determination of the NMR spectrum (low solubility), it did serve to confirm the features already indicated by the infrared measurements. The terminal methyl absorption at 9.13 τ was quite weak compared to the intense signal at 8.75 τ caused by the methylene groups. In addition, the unique pair of methylene groups which flank the ester carboxyl function gave rise to a pair of weak

TABLE I.—PARENT MASS PEAKS IN MAMEY WAX

Mass	Molecular Formula C	H	O	Peak Ht., Chart Divisions
564	38	76	2	4
592	40	80	2	11
620	42	84	2	18
648	44	88	2	28
676	46	92	2	47
704	48	96	2	69
732	50	100	2	30
760	52	104	2	12
788	54	108	2	4

TABLE II.—MASS SPECTRAL ANALYSIS OF MAMEY WAX SAPONIFICATION PRODUCTS

Carbon No.	Alcohols Wt., %	Acids (as Methyl Esters) Wt., %
18	...	0.2
19	...	...
20	5	1.5
21	...	...
22	6	11.1
23	...	0.7
24	62	67.8
25	Less than 1	1.2
26	15	15.1
27	...	0.2
28	8	1.8
29	...	0.1
30	4	0.3
31	...	0.02
32	...	0.01

triplets (J = 6.5 cps.) situated appropriately (18) at 5.95 τ (—$CH_2\underline{CH_2}O$—) and 7.72 τ (—$CH_2\underline{CH_2}$-CO_2—). With the presence of at least two oxygen atoms established spectroscopically, the microanalytical results led to the formulation of mamey wax as $C_{48}H_{96}O_2$. The Kuhn-Roth determination indicated the presence of two C—CH_3 groups. Although the microanalytical data might be accommodated by nearby homologs of the C_{48} formula when taken in combination with the data obtained from the wax hydrolysis products, the formula indicated represents the best fit.

Saponification of the wax could be effected by reaction with boiling ethanolic potassium hydroxide solution or, more conveniently, by reaction with sodium hydroxide in boiling aqueous dioxane solution. Both the alcohol and the acid moieties were isolated in high yield and identified as lignoceryl alcohol, $CH_3(CH_2)_{22}CH_2OH$, and lignoceric acid, $CH_3(CH_2)_{22}CO_2H$. These structural assignments were based on the microanalytical results, including Kuhn-Roth determinations, and on comparisons (melting points and infrared spectra) with authentic materials.[1] Furthermore, the acid was converted to its methyl ester and this in turn compared to an authentic specimen.[2] The combined chemical, microanalytical, and spectroscopic evidence then requires that mamey wax be represented as lignoceryl lignocerate, $CH_3(CH_2)_{22}CO_2(CH_2)_{23}CH_3$. The central location of the ester function was verified by lithium aluminum hydride reduction which provided lignoceryl alcohol in accordance with this formulation.

It is generally recognized that natural waxes usually consist of mixtures of homologous components whether they be esters, acids, alcohols, ketones, or hydrocarbons (19, 20). It seemed likely that mamey wax was similarly constituted; we therefore made use of mass spectrometric measurements to extend and complete the chemical definition of the wax. The wax itself produced a spectrum with parent ion peaks ranging from mass 564 ($C_{38}H_{76}O_2$) to mass 788 ($C_{54}H_{108}O_2$). The relative peak heights are shown in Table I, where it can be seen that the most abundant species is the one at mass 704 corresponding to $C_{48}H_{96}O_2$. The baselines show small bulges at the positions of the parent masses of the odd-carbon esters, with those at C_{47} and C_{49} being the most pronounced. Although these were too small (the order of one chart division) to measure unambiguously, their magnitude is entirely sufficient to account for the presence of the odd-carbon acids discussed below. No information could be derived from the fragment region of the spectrum with respect to the location of the ester group in the chain. Examination of the saponification products, however, did provide verification of this point. The acid resulting from hydrolysis of the wax was analyzed as its methyl ester. It can be seen in Table II that this acid consists of all the saturated even numbered homologs from C_{18} to C_{32}. By far the most abundant species is the C_{24} member, lignoceric acid, which is present to the extent of nearly 68%. The fragmentation pattern was virtually identical to that observed for authentic methyl lignocerate.[2]

A most interesting feature of this analysis was the detection of the odd-numbered acids from C_{23} to C_{31}. The older view that natural fats and waxes consisted exclusively of even-numbered homologs[3] has been eroded gradually by the application of the sensitive analytical techniques of gas chromatography and mass spectrometry. The "unnatural" homologs since first being detected in certain animal fats (21–24), have been observed to occur in such diverse materials as human hair fat (25), soil extracts (26), human skin wax (27), petroleum reservoir waters (28), seawater (29), bituminous (montan) wax (30–32), wool wax (33), and beeswax (34). The first report of the occurrence of the odd-numbered (unnatural)[3] species in a plant wax (sugar cane cuticle wax) appeared in 1960 (35); it seems that their widespread occurrence will depend only on the sensitivity of the analytical method (36, 37).

Also listed in Table II is the distribution of alcohols obtained by saponification of mamey wax, which clearly shows the major constituent (62%) to be lignoceryl alcohol. The analysis of the alcohol, however, is subject to uncertainties greater than that of the methyl esters. In the first place, parent ion peaks do not appear for long-chain alcohols (38). Peaks of high sensitivity do appear at masses 18

[1] Lignoceric acid was obtained from the Applied Science Laboratories, Inc., State College, Pa. The corresponding alcohol was prepared from this material by lithium aluminum hydride reduction. The acid was stated to have a purity of 99+%.

[2] Methyl lignocerate was also obtained from the Applied Science Laboratories (see *Footnote 1*) and was stated to have a purity of 99.8+%. Our mass spectral analysis indicated a purity of 99.2% (by weight); the contaminants were the methyl esters of the homologous C_{25} (0.6%) and C_{26} (0.2%) acids.

[3] Hydrocarbon waxes, of course, consist predominately of odd-numbered homologs, presumably arising by decarboxylation of the even-numbered acids. Recent years have brought the detection of the rare even-numbered hydrocarbons, however.

units (loss of H_2O) and 20 units (additional loss of two hydrogens) below the parent mass number. In addition, a fraction of the long-chain alcohol molecules simultaneously lose one or more methylene groups, and therefore significant contributions appear at mass numbers corresponding to the principal peaks of other members of a mixture of homologous alcohols—the contribution is especially large for the loss of two methylene groups (38). The value for the alcohol at C_{22} is subject to the most uncertainty due to the large contribution resulting from the loss of two methylene groups from the main constituent at C_{24}. The spectra of two pure compounds, lignoceryl alcohol[1] and behenyl alcohol [$CH_3(CH_2)_{20}CH_2OH$], were used to establish contribution factors for the analysis of the mixture. Within the accuracy of these factors, the observed peaks corresponding to the odd-carbon alcohols (except the C_{25} member) were accounted for by contributions from even-carbon alcohols of higher molecular weight. The most abundant odd-carbon alcohol is apparently the C_{25} homolog which, incidentally, corresponds in chain length to the most abundant odd-carbon acid. Table III shows the distribution of alcohols obtained by lithium aluminum hydride reduction of mamey wax. This distribution, corrected for contributions, reflects the superposition of the alcohol and acid distributions shown in Table II. None of the specimens examined showed evidence for branching in the hydrocarbon chains.

In 1922, Brigl and Fuchs reported the isolation of lignoceryl lignocerate from beech tar wax and proved its structure by a combination of degradative and synthetic means (39). The chemical and physical properties which we have observed for mamey wax and its hydrolysis products accord well with those reported by Brigl and Fuchs for their ester. More recently, Bell and Harvey have shown that lignoceryl lignocerate is an important constituent of the wax from the heartwood of *Phyllocladus trichomanoides* (40).

EXPERIMENTAL[4,5]

Isolation of Mamey Wax.—Thirty-four kilograms of dried and ground seeds of *M. americana* L. were extracted with a total of 12 gal. of *n*-hexane. The residue remaining after removal of the solvent by distillation was treated with 1 gal. of technical grade acetone. A yellow semisolid material precipitated and was collected on a filter. A single recrystallization from isopropanol or dioxane provided 20 Gm. of slightly yellow solid, m.p. 74–76°, (0.06% yield based on the amount of dried seed). Treatment with charcoal, followed by several recrystallizations from dioxane, isopropanol, or ethyl acetate provided pure white material melting sharply at 79.5°. The literature melting point for lignoceryl lignocerate is 79° (39).

Anal.—Calcd. for $C_{48}H_{96}O_2$: C, 81.74; H, 13.72; O, 4.54; 2 C—CH_3, 4.26. Found: C, 82.27, 81.78, 82.10, 81.98; H, 13.44, 13.40, 13.43, 13.46; O, 4.09, 4.48; C—CH_3, 2.25, 2.58, 3.09.

Table III.—Mass Spectral Analysis of Alcohols Derived from Mamey Wax by Reduction

Carbon No.	Alcohol from Reduction, Peak Ht., Divisions
18	7
20	27
22	183
24	252
26	97
28	35
30	28
32	13

The infrared spectrum (KBr) of mamey wax showed strong bands at 2933, 2841, 1733, 1471 (doublet), 1179, 730, and 724 cm.$^{-1}$. The material was transparent in the ultraviolet (20.3 mg. in 10 ml. $CHCl_3$) and gave a negative tetranitromethane test for unsaturation (17). The NMR spectrum was determined in deuterochloroform solution using tetramethylsilane as an internal standard. Signals were observed at 5.95 τ (triplet, J = 6.5 cps.), 7.72 τ (triplet, J = 6.5 cps.), 8.75 τ (singlet), and 9.13 τ. Mass spectra were determined on two samples, m.p. 77.5–78.5° and m.p. 79.5°, with essentially identical results.

Saponification of the Wax.—One gram of ester, m.p. 79.5°, was heated at reflux in dioxane solution (50 ml.) with 20 ml. of aqueous sodium hydroxide (20%). After 48 hours, an additional 50 ml. of dioxane was added, and the mixture again heated at reflux for 1 hour. The hot mixture was then filtered and the white salt washed three times with 25 ml. of hot dioxane. The combined dioxane solutions were concentrated to about 100 ml. and cooled in an ice bath. The alcohol crystallized from solution and was collected on a filter, 380 mg., m.p. 75°. Two additional recrystallizations from chloroform provided 345 mg. of product, m.p. 76°. The literature melting point of lignoceryl alcohol is 76° (39). Microanalyses were performed on samples with m.p. 74–75° and 76°.

Anal.—Calcd. for $C_{24}H_{50}O$: C, 81.28; H, 14.21; O, 4.51; mol. wt., 354.6; C—CH_3, 4.23. Found: C, 81.28, 81.41; H, 14.08, 13.81; mol. wt., 385; C—CH_3, 3.04.

The infrared spectrum (KBr) showed prominent bands at 3584, 3106, 3040, 1484, 1064, 728, and 718 cm.$^{-1}$ and was similar to that of an authentic sample of tetracosanol, m.p. 76.5–77°; literature[6] m.p. 77.5° (39). No absorption was observed in the NMR spectrum at lower field than the triplet (J = 6 cps.) at 6.36 τ indicating the absence of vinyl protons. The mass spectral measurements were carried out on two samples, one with m.p. 75–75.5° and another (less extensively purified) with m.p. 69–73°.

The salt obtained from the saponification mixture described above was heated on a steam bath with 5 ml. of 2 *N* hydrochloric acid. After 30 minutes, the mixture was diluted with 15 ml. of water, and the acid was collected on a filter, washed with water until the washings were neutral, and dried in a

[4] The present work was initiated by E. J. Eisenbraun while in the Department of Chemistry, Wayne State University, and continued by R. A. Finnegan while in the Department of Chemistry, The Ohio State University, Columbus.

[5] Melting points were determined on a Fisher-Johns block and are uncorrected. Microanalyses were performed by Dr. A. Bernhardt, Mulheim, Germany. The infrared spectra were recorded on Baird model B and Perkin-Elmer model 137 Infracord instruments. The ultraviolet measurement was made on a Perkin-Elmer Spectracord, and the NMR spectra were determined with the Varian Associates A 60 spectrometer.

[6] The melting points of the synthetic materials are invariably somewhat higher than the corresponding naturally derived materials. This is known to be caused by the presence of homologs in the latter.

vacuum oven. This material weighed 428 mg., m.p. 74–76°. A single treatment with charcoal and three recrystallizations from ethyl acetate raised the melting point to 78.5°. Lignoceric acid is reported to melt at 79° (39). Microanalyses were carried out on samples with m.p. 77.5–79.5° and 78.5°.

Anal.—Calcd. for $C_{24}H_{48}O_2$: C, 78.19; H, 13.13; O, 8.68; C—CH_3, 4.08. Found: C, 78.37, 77.91, 78.30; H, 12.97, 12.88, 12.95; O, 8.22; C—CH_3, 0.68.

The infrared spectrum (KBr) showed bands at 3100, 2900, 2850, 2600, 1700, 1455, 925, 725, and 717 cm.$^{-1}$. The spectrum of an authentic specimen of tetracosanoic acid differed from that of the mamey wax acid only in the number of low intensity bands which were better resolved.

The saponification was also carried out in a more usual fashion by heating a solution of the wax in ethanolic potassium hydroxide at reflux for 24 hours. The properties of the products obtained in this way were the same as for the products described above.

Methylation of the Wax Acid.—A solution in anhydrous methanol (50 ml.) of 231 mg. of the acid, m.p. 73–76°, and a few drops of concentrated sulfuric acid was heated at reflux temperature for 14 hours. The white precipitate obtained when the reaction mixture was poured onto ice, was dissolved in ether, and washed several times with aqueous potassium bicarbonate solution. After being dried, the solvent was removed. The residual methyl ester weighed 200 mg. and had m.p. 54–56°. The infrared spectrum of this sample was similar to that of an authentic sample[2] of methyl tetracosanoate, m.p. 57–58°, differing only in the degree of resolution of the less intense bands. The sample was used for mass spectral measurements without further purification.

Reaction of the Wax with Lithium Aluminum Hydride.—A 3.50-Gm. sample of mamey wax, m.p. 76–78°, was added to a Soxhlet extraction apparatus mounted on a 2000-ml., three-necked flask containing 1000 ml. of anhydrous ether. The ether was stirred vigorously with a mechanical stirrer, and 4 Gm. of lithium aluminum hydride was added in portions. The suspension was stirred and heated at reflux temperature for two 8-hour periods until all of the wax had been dissolved and carried to the reaction vessel. Refluxing was continued for an additional 2 to 3 hours, the heating mantle was removed, the reaction allowed to cool, and water was added dropwise until the stirred suspension turned from gray to completely colorless.

The suspension was filtered through a layer of magnesium sulfate in a sintered-glass funnel. The ether was removed from the filtrate by evaporation to give 2.5 Gm. of a colorless solid. Sodium hydroxide (10%) was added to the salts, and the resulting suspension was extracted successively with ether and chloroform. The ether and chloroform extracts were combined, dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated to give 1.05 Gm. of colorless solid which was combined with the earlier fraction. The crude product melted at 67–68.5° with some softening at 60°. A fraction (500 mg.) of this material was recrystallized twice from ethyl acetate to give 360 mg. of the alcohol, m.p. 73.5–74°. Additional recrystallizations from the same solvent did not raise the melting point. The infrared and NMR spectra of this material were virtually identical to those of the alcohol obtained by saponification.

Mass Spectrometric Determinations.—The instrument used is a Consolidated Electrodynamics Corp. model 21-103 (modified) mass spectrometer. The operating conditions were as follows: ionizing voltage, 70 v.; ionizing temperature, 270°; inlet temperatures, 250–275° for the alcohols and methyl esters, 340° for mamey wax.

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