CHEMICAL STUDY OF THE CARNAUBA (*Copernicia cerifera Martius*) WAX

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Key words: *Copernicia cerifera Martius*, wax, fatty alcohols, fatty acids, hydrocarbons.

RESUMEN : Se realizó el estudio de la composición química de la cera de Carnauba (*Copernicia cerifera Martius*) una palma que crece en Argentina, Brasil y Paraguay. Cuando se realiza previamente su hidrólisis básica, se aislan e identifican fracciones que contienen diferentes grupos de compuestos, entre los que mencionamos: alcoholes grasos de alto peso molecular (C_{24}-C_{36}) en donde el 1-dotriacontanol es el alcohol principal; ácidos grasos de mediano peso molecular (C_{12}-C_{20}) en donde el ácido hexadecanoico es el mas abundante y de alto peso molecular (C_{24}-C_{36}) donde el ácido octacosanoico es el mas abundante. Así como hidrocarburos de la serie homóloga a dichos alcoholes entrte las que se destaca el heptacosano. Además, fueron aislados 4 hidroxi-ácidos: ácido 16-hidroxihexadecanoico, ácido 18-hidroxioctadecanoico así como ácido 26-hidroxihexacosanoico y ácido 28-hidroiyoctacosanoico. Durante el desarrollo del trabajo no se observó la presencia de ácidos hidroxiácidos insaturados. Estos compuestos fueron caracterizados mediante el estudio de las propiedades cromatográficas y espectroscópicas de cada uno de estos grupos de compuestos.

ABSTRACT: The chemical composition of Carnauba (*Copernicia cerifera Martius*) wax (a palm tree that grows in Argentina, Brazil and Paraguay) was studied. When a previous saponification of the wax was done, fractions that contain different groups of compounds were isolated and identified. Among them, could be mentioned: fatty alcohols of high molecular weight (C_{24}-C_{36}) in which 1-dotriacontanol in the main one; fatty acids of medium (C_{12}-C_{20}) where hexadecanoic acid is the main one and those of high molecular weight (C_{24}-C_{36}), being octacosanoic acid the main one. A group of paraffins was, also, isolated, being heptacosane the main one corresponding to the homologue series of that of the alcohols. Also, were isolated 4 hydroxy acids: 16-hydroxyhexadecanoic, 18-hydroxyoctadecanoic acid as well as 26-hydroxyhexacosanoic and 28-hydroxyoctacosanoic acid. No unsaturated hydroxy acids were observed in our study. These compounds were characterized by means of the chromatographic and spectroscopic properties of each one of these groups of compounds.

INTRODUCTION

The study of natural waxes has increased its interest since it was obtained policosanol[1] a cholesterol-lowering drug indicated for patients with type II hypercholesterolaemia and dyslipidemia associated to an insulin dependent diabetes mellitus, which significantly raises moderately high-density lipoproteins cholesterol (HDL-C), from sugarcane wax.[2-5] Another mixture of this type of alcohols, obtained from beeswax shown activity against gastric and duodenal ulcers as well as anti-inflammatory properties.[6-8] This mixture, also, shows anti-oxidant activity.[9] Also, a mixture of fatty acids of high molecular weight isolated from sugarcane wax[10] has demonstrated good hypocholes-terolaemic[11] as well as anti-platelet and anti-thrombotic[12] effects in experimental models.

Carnauba wax is the most extended wax in the world and there have been a number of authors that studied its components. Sttrocke[13] and Koonce and Brown[14] studied the composition of the primary alcohols of high molecular weight of even number of carbon atoms (C_{24}-C_{34}), being the majors those of 30 and 34 carbon atoms. Murray and Schoenfeld.[15, 16] studied the composition of the unsaponificable fraction of the wax using molecular distillation obtaining 1-tetracosanol, 1-octacosanol, 1-triacontanol, 1-dotriacontanol and 1-tetratriacontanol. They also, isolated, others 3 alcohols, that represents less than 2.0 % of the mixture, showing a higher melting point of that of the primary alcohols. These authors propose to be compounds with chemical structure similar to the alcohols, such as diols, being identified as those of 22, 24 and 26

carbon atoms. Leys[17] identified the diol of 28 carbon atoms in this wax. In 1953, Findley and Brown[18] reported the presence of fatty acids, with a melting point of 83.5-84.5 ^{0}C, that represents a 50.0 % of the total composition of the wax, against a 43.0 % reported by Koonce and Brown. [19] Murray and Schoenfeld[16] demonstrated that the fatty acids present in this wax have between 18 and 30 carbon atoms, they also isolated seven hydroxy acids: 18-hydroxyoctadecanoic, 20-hydroxyeicosanoic, 22-hydroxydocosanoic, 24-hydroxytetracosa-noic, 26-hydroxyhexacosanoic, 28-hydroxyoctacosanoic and 30-hydroxytriacontanoic. The hydroxy acids present in this wax are saturated and unsaturated, the saturated are ω-hydroxy acids while the unsaturated are able to form resinous complexes.

Some authors[20, 21] reported that paraffins represent 1.0 % of the total content of the wax. Gottfried and Ulzer[20] identify the main hydrocarbon as heptacosane, while Farcy[21] report between 1.0 to 1.62 % of paraffins in this wax, identifying those of 27, 29 and 31 carbon atoms where that of 27 is the major one.

MATERIALS AND METHODS

A commercial sample of medium clear class Carnauba wax (melting point 84.0-85.5 ^{0}C, ≤ 5 % of water) was obtained from Brazil in 1992.

Reagents used in the extraction process were of commercial grade and the ones used in the separation process were pure for analysis (p.a.). Potassium hydroxide, 1-eicosanol, chloroform and 1,2- dichloroethane (Fluka, Buchs, Switzerland), methanol (Reactivul, Hungary), Hydrochloric acid and n-hexane (Merck, Darmstadt, Germany), N-methyl N-trimethylsililtrifluoracctamide, MSTFA (Pierce, USA), Silicagel G (70-230 mesh ASTM) and Silicagel G 60 (Merck, Darmstadt, Germany).

The following equipments were used in this research: 2 L Söxhlet extractor (Quickfit, England), analytical balance, precision 0.1 mg (Sartorius, Germany), dry thermostat (CNIC, Cuba), water bath (Unipan, Poland), oven with air circulation (WEB, Germany), centrifuge (WEB, Germany), as well as:

- Gas chromatograph PU 4550 (Pye Unicam, England) with the following chromatographic conditions: glass column 3 m and 4 mm i.d., filled with Chromosorb W-AW-DMCS with OV-101 at 3.0 %; carrier gas (Argon) flow: 30 mL/min; temperature of the detector and the injector: 320 ^{0}C, temperature of the oven 200-320 ^{0}C (10 ^{0}C/min) and 320 ^{0}C isothermal for 15 min; air flow: 400mL/min; hydrogen flow: 40 mL/min. and attenuance: 100 x 32.
- Gas chromatograph with mass detector MD-800 (Fisons Instruments, Manchester, England) coupled with a Lab-Base (VG Mass Lab, England) used with capillar column SE-54 (30 m and 0,25 mm i.d.) (Supelco, Bellofonte, USA). The following chromatographic conditions were used: temperature of detector and injector: 320 ^{0}C, of the ionization chamber: 250 ^{0}C and the interface: 200 ^{0}C. Temperature of the oven: 100-200 ^{0}C at 40 ^{0}C/min, from 200-320 ^{0}C at 8 ^{0}C/min; carrier gas flow (He): 1.0 mL/min. and ionization energy: 70 eV.

EXPERIMENTAL PART

Carnauba wax was saponified as follows: Melts the wax at 100 ^{0}C and after that add, drop by drop, a concentrate solution (10 % w/w) of KOH/EtOH. The reaction was developed for 3 hours and the saponified wax was cooled up to room temperature and milled in order to obtain a homogeneous powder.

Saponified wax (300 g) were placed in a 2 L Söxhlet extractor, with 1000 mL of n-hexane extracting for 24 h. After that, the system was cooled and the cerous residue retained in the thimble extractor (180 g) was dried on an air flow in order to eliminate the solvent. The solution was cooled at 4 ^{0}C for 2 h and centrifuged. The residual solvent was evaporated to dryness in order to obtain a crude extract (100 g) of non-polar compounds. This crude extract was dissolved in n-hexane, cooled at 4 ^{0}C for 2 h, centrifuged, decanted and the solution was evaporated to dryness, obtaining a crude paraffin extract (15 g) that was dried and weighed. Paraffins were identified using gas chromatography with mass detector as well as the comparison with reference samples of these hydrocarbons.

The remaining residue (180 g) was extracted in the Söxhlet extractor with 1 000 mL of 1,2 dichloroethane for 24 h. After that, the system was cooled and the cerous material retained in the thimble (69 g) was dried on airflow, in order to eliminate the solvent. The organic solution was centrifuged and the fatty alcohols fraction was crystallized from 1,2 dichloroethane using the same process, of dissolving and crystallizing, described previously. In this manner was obtained a mixture of fatty alcohols (43 g) that was identified using gas chromatography with mass detector as well as the comparison with reference samples of some of these alcohols. In order to quantify the alcohols was used gas chromatography, comparing the mass of an internal standard with known concentration of the alcohols under study.[22]

The cerous residue of the previous process (69 g) was extracted again in the Söxhlet extractor using firstly 1 000 mL of n-hexane, discarding this solution. Secondly, was extracted with 1 000 mL of acetone for 24 h, the organic solution was evaporated to dryness and purified with ethanol using the same process of dissolving and crystallizing described previously. After purification, the regeneration of fatty acids (23 g) was done with 1.0 moll/L HCl, heated up to 95 ^{0}C for one hour.

The cerous residue that was present in the Söxhlet extractor was dried and treated with 1.0 moll/L HCl at 95 ^{0}C for 1 h. The aqueous solution was filtered and the residue was washed several times with desionized water up to neutral pH, obtaining another mixture of fatty acids (25 g).

Both mixtures of fatty acids were methylated and then, characterized using gas chromatography with

mass detector. Also, the comparison with reference samples of some of these fatty acids was done.

Chromatographic analysis of the fractions

The paraffin extract (10,0 mg) was weighed, and 25 μL of 1,2-dichloroethane were added, heating in order to help the dissolution. The gas chromatograph with mass detector was adjusted to the conditions previously described and, firstly, injected 1 μL of 1,2-dichloroethane running the chromatogram for 20 min. in order to check the stability of the baseline. Then were injected 3 μL of the sample. Then, each one of the paraffins present in the wax was identified according to its mass spectrum.

The crude extract of alcohols (10mg) was weighed and 150 μL of MSTFA added as well as a solution of 1-eicosanol that is used as internal standard. Heat the solution at 60 ^{0}C for 20 min. The equipment was adjusted to the conditions previously described and was injected a 1 μL of 1,2-dichloroethane running the chromatogram for 20 min in order to check the stability of the baseline. After that 3 μL of the sample were injected. Then, each alcohol present in the wax was identified according to its mass spectrum.

Fatty acids fractions were methylate treating them with a mixture of methanol and hydrochloric acid reacting for 60 min. After methylation 2 μL of each fraction were injected to the chromatograph with mass detector that has been adjusted to the chromatographic conditions previously described. Then, each fatty acid present in the wax was identified according to its mass spectrum.

RESULTS AND DISCUSSION

The paraffin fraction obtained from Carnauba wax was studied and it was observed that much more paraffins are present in this batch of wax that those reported. It was possible to identify and quantify the hydrocarbons reported in Table 1. As can be observed, there were identified all the linear hydrocarbons between 23 and 31 carbon atoms, being the major ones those from C_{25} to C_{28} carbon atoms, especially C_{27}, this result correspond with those previously reported.[21]

Table 1. Paraffins characterized from Carnauba wax.

Paraffins	Formula	Content in the mixture (%)
Tricosane	$C_{23}H_{48}$	2.6
Tetracosane	$C_{24}H_{50}$	7.4
Pentacosane	$C_{25}H_{52}$	15.8
Hexacosane	$C_{26}H_{54}$	17.2
Heptacosane	$C_{27}H_{56}$	28.6
Octacosane	$C_{28}H_{58}$	13.9
Nonacosane	$C_{29}H_{60}$	8.5
Hentriacontane	$C_{31}H_{64}$	4.9

The alcohols of high molecular weight that were identified were reported in Table 2, being 1-dotriacontanol, together with 1-triacontanol and 1-tetratriacontanol the major components in the mixture.

Table 2. Fatty alcohols isolated from Carnauba wax.

Alcohol	Formula	Content in the mixture (%)
1-tetracosanol	$C_{24}H_{49}OH$	0.1
1-hexacosanol	$C_{26}H_{53}OH$	0.1
1-heptacosanol	$C_{27}H_{55}OH$	0.1
1-octacosanol	$C_{28}H_{57}OH$	0.6
1-triacontanol	$C_{30}H_{61}OH$	8.1
1-dotriacontanol	$C_{32}H_{65}OH$	64.3
1-tritriacontanol	$C_{33}H_{67}OH$	1.1
1-tetratriacontanol	$C_{34}H_{69}OH$	17.6
1-hexatriacontanol	$C_{36}H_{73}OH$	0.6

In this research was found a percent of 1-dotriacontanol higher than that reported by other authors[15, 16], as well as a lower concentration of 1-tetracosanol and 1-hexacosanol that those reported by these authors.

The mass spectra of the sylanized alcohols were the followings: 1-tetracosanol: 411 (100 %), 395, 125, 103, 83, 75 (41 %), 57, 43 d; 1-hexacosanol 439 (100 %), 423, 125, 103, 83, 75 (41%), 57, 43 d; 1-heptacosanol 453 (100 %), 437, 125, 103, 83, 75 (40 %), 57, 43 d; 1-octacosanol 467 (100 %), 451, 125, 103, 83, 75 (42 %), 57, 43 d; 1-nonacosanol 491 (100 %), 465, 125, 103, 83, 75 (70 %), 57, 43 d; 1-triacontanol 495 (100 %), 479, 125, 103, 83, 75 (45 %), 57, 43 d; 1-dotriacontanol 523 (100 %), 507, 125, 103, 83, 75 (52 %), 57, 43 d; 1-tetratriacontanol 551 (100 %), 535, 125, 103, 83, 75 (28 %), 57, 43 d and 1-hexatriacontanol 579 (100 %), 125, 103, 83, 75 (52%), 57, 43 d, that corresponds with those spectra reported in literature for these alcohols. The sylanization of the alcohol's produce an increase of 57 d in the molecular weight of each of them, and the base peak in this spectrum corresponds to the lost of 15 d of this molecular ion (M^+ - 15).

The lost of the analyzed fragment with a simultaneous rearrangement of hydrogen gave place to the 75 d fragment $[OH\text{-}Si(CH_3)_2]^+$ an intense peak that is common for the 8 alcohols. Another characteristic peak of this type of alcohols is that of 103 d which structure corresponds to $[CH{=}O\text{-}Si(CH_3)_3]^+$, characteristic of the sylanized terminal hydroxyl groups. The fragments at 43 ($C_3H_7^+$) and 57 d ($C_4H_9^+$) are characteristic of compounds showing a hydrocarbon chain.

When both fractions that contain fatty acids from Carnauba wax were analyzed (Table 3) it could be observed the presence of the essential fatty acids with a high yield of them than that reported by other authors.[18, 19] As can be seen from the Table, there were present fatty acids of high molecular weight with even number of carbon atoms, but in lower concentration than that reported[18, 19] specially those of 24, 26 and 30 carbon atoms. Those fatty acids of 32, 33, 34 and 36 carbon atoms were not reported previously.[16,18,19] Also, were isolated four hydroxy acids: 16-hydroxyhexadecanoic, 18-hydroxyoctadecanoic acid as well as 26-hydroxyhexacosanoic and 28-hydroxyoctacosanoic acid. These results were different to those reported by Murray and Schoenfeld[16] which did not report the presence of these hydroxy acids but reported the presence of those of 20, 22, 24 and 30 carbon atoms. No unsaturated hydroxy acids were observed in our study.

Table 3. Fatty acids from Carnauba wax.

Fatty acid	Formula	Content in the mixture (%)
Dodecanoic	$C_{12}H_{24}O_2$	0.25
Tridecanoic	$C_{13}H_{26}O_2$	< 0.1
Tetradecanoic	$C_{14}H_{28}O_2$	0.50
Pentadecanoic	$C_{15}H_{30}O_2$	< 0.1
Hexadecenoic	$C_{16}H_{30}O_2$	0.43
Hexadecanoic	$C_{16}H_{32}O_2$	54.62
Heptadecanoic	$C_{17}H_{34}O_2$	0.39
Octadecenoic	$C_{18}H_{34}O_2$	25.05
Octadecanoic	$C_{18}H_{36}O_2$	2.47
Eicosenoic	$C_{20}H_{38}O_2$	1.83
Eicosanoic	$C_{20}H_{40}O_2$	1.04
16-Hydroxyhexadecanoic	$C_{16}H_{32}O_3$	0.29
18-Hydroxyoctadecanoic	$C_{18}H_{36}O_3$	0.34
Tetracosanoic	$C_{24}H_{48}O_2$	3.05
Hexacosanoic	$C_{26}H_{52}O_2$	2.06
26-Hydroxyhexacosanoic	$C_{26}H_{52}O_3$	0.25
Octacosanoic	$C_{28}H_{56}O_2$	3.89
28-Hydroxyoctacosanoic	$C_{28}H_{56}O_3$	0.19
Triacontanoic	$C_{30}H_{60}O_2$	<0.1
Dotriacontanoic	$C_{32}H_{64}O_2$	0.27
Tritriacontanoic	$C_{33}H_{66}O_2$	< 0.1
Tetratriacontanoic	$C_{34}H_{68}O_2$	1.17
Hexatriacontanoic	$C_{36}H_{72}O_2$	0.88

CONCLUSIONS

It was studied Carnauba (*Copernicia cerifera Martius*) wax and were isolated and characterized, using chromatographic and spectroscopic properties, a mixture of paraffins, being heptacosane the main one, a mixture of fatty alcohols in which 1-dotriacontanol in the main one, as well as two mixtures of fatty acids, in one of them were included those of medium molecular weight (C_{12}-C_{20}) where hexadecanoic acid was the main one and another one in which are included those of high molecular weight (C_{24}-C_{36}) being octacosanoic acid the main one. Also, were isolated 4 hydroxy acids: 16-hydroxyhexadecanoic, 18-hydroxyoctadecanoic acid as well as 26-hydroxyhexacosanoic and 28-hydroxyoctacosanoic acid. No unsaturated hydroxy acids were observed in our study.

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