Isolation and characterization of a mixture of higher primary aliphatic alcohols of high molecular weight from henequen (*Agave furcroydes L.*) wax

Ernesto Méndez*, Mayra Blanco*, Abilio Laguna* [+] and Ezequiel García**

* Centro de Productos Naturales, C.N.I.C., Ave 25 #15202, Reparto Cubanancán, Playa, Habana, Cuba, e-mail: dalmer@etecsa.ip.cu
** Dirección de Producción, C.N.I.C., Ave 25 #15202, Reparto Cubanancán, Playa, Habana, Cuba
[+] Correspondence address

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RESUMEN: Partiendo de una colecta de epidermis de hojas de henequén, específicamente de la especie *Agave furcroydes* L. y empleando un disolvente orgánico, fue extraída la cera de dicha planta. La cera después de someterla a una reacción de hidrólisis básica o saponificación y empleando nuevamente un disolvente orgánico le fue extraída una mezcla de alcoholes alifáticos, lineales y de alto peso molecular. Dicha mezcla fue estudiada mediante las técnicas de Espectrometría Infrarroja y Cromatografía Gaseosa acoplada a Espectrometría de Masas, lo que permitió una caracterización química de la misma. Finalmente fueron identificados y cuantificados en la mezcla los once alcoholes siguientes: 1-hexacosanol, 1-heptacosanol, 1-octacosanol, 1-nonacosanol, 1-triacontanol, 1-hentriacontanol, 1-dotriacontanol, 1-tritriacontanol, 1-tetratriacontanol, 1-pentatriacontanol y 1-hexatriacontanol. Los alcoholes más abundantes en la mezcla son 1-octacosanol y 1-triacontanol. El proceso de obtención de este producto, compuesto por la mezcla de once alcoholes, muestra una composición reproducible lote a lote que resulta muy estable y definida.

ABSTRACT: Colleted epidermises from henequen (Agave furcroydes L) leaves were submitted to a process of extraction, using an organic solvent, to obtain the wax of this specie. This wax, after submitting to an alkaline hydrolysis reaction, was submitted in another process of extraction, this time, to obtain a mixture of eleven, aliphatic, straight and high molecular weight alcohols. This mixture was characterized chemically by means of the Infrared Spectrometric and the Gas Chromatography coupled to Mass Spectrometric techniques; Finally, the identified and quantified alcohols were: 1-hexacosanol, 1-heptacosanol, 1-octacosanol, 1-nonacosanol, 1-triacontanol, 1-hentriacontanol, 1-dotriacontanol, 1-tritriacontanol, 1-tetratriacontanol, 1-pentatriacontanol and 1-hexatriacontanol, being 1-octacosanol and 1-triacontanol the main components. This product, composed for the mixture of eleven alcohols, shows in its process of obtainment a very stable, well-defined and reproducible composition from batch to batch.

INTRODUCTION

Other authors have previously, described biological effects of higher primary fatty alcohols. Thus, 1-triacontanol was reported as a plant growth stimulator[1] also showing moderate anti-inflammatory and antiviral effects[2,3]. Hexacosanol has been referred as stimulant of the neural cell growth in tissue culture and experiments[4-6], also, showing inmunological properties[7]; octacosanol has been described as an ergogenic compound[8] and related to the lipid metabolism in rats[9]. Also, in 1984, Sho and coworkers[10] described that partially purified Okinawan sugarcane wax lowered levels of cholesterol on serum and liver, while triglycerides and phospholipids remained unchanged, in rats with induced hypercholesterolaemia, but concluded that fatty alcohols did not induce such effects. Nevertheless, Shimura and coworkers[11] studying the effects of octacosanol on motor endurance in mice found that these animals fed with a supplement extracted from sugarcane wax had a significant reduction in cholesterol and triglycerides in the liver.

Other mixture of these type of alcohols, named policosanol, was obtained from sugarcane (*Saccharum officinarum* L.) wax,[12] showing cholesterol-lowering effects, demonstrated in different experimental models.[13,14] It is a cholesterol-lowering drug indicated for patients with type II hypercholesterolaemia and dyslipi-demia associated to an insulin dependent diabetes mellitus, which significantly raises moderately high-density lipoproteins cholesterol (HDL-C).[15,16] Data obtained from preclinical[17,18] and clinical studies[19-27] have proven that policosanol is very safe and well tolerated and no drug-related adverse effect has been demonstrated up to date. Also, a mixture of these alcohols, obtained from beeswax, shown activity against gastric and duodenal ulcers as well as anti-inflammatory.[28-30] This mixture, also, shows antioxidant activity.[31-33] Data obtained from preclinical studies[34-36] have proven that this mixture is very safe.

Henequen wax have always been a matter of interest, because of its possible industrial application, [37] considering the large extensions of the plant that are cultivated for the production of natural fibres. The amount of wax in the leaves of henequen ranges between 0.1 to 0.3 %, depending on its age, soil, climatic conditions, etc. This wax is made up of esters, aldehydes, ketones, hydrocarbons, fatty acids and free alcohols, the amount of each one depends on the origin of the henequen plant and the technology used to obtain the wax. Different methods for the isolation of this type of alcohol have been described.[38, 39]

Present work reports the isolation and purification of the mixture of alcohols from henequen (*Agave furcroydes L.*) wax for the development of further pharmacological studies.

MATERIALS AND METHODS

All the reagents used on the extraction procedure were of commercial grade (Merck, Darmstadt, Germany). The reagents used for the quantification of the mixture of fatty alcohols are of chromatographic quality are 1-eicosanol (used as internal standard, 98 % GC), 1-tetracosanol (99.0 % GC), 1-hexacosanol (98.0 % GC) and 1-octacosanol (99.0 % GC, Janssen Chimica, Beerse, Belgium), 1-heptacosanol (98.0 % GC) and 1-triacontanol (99.0 % GC) (Sigma St. Louis, U.S.A). Methyl N-trimethylsilyl-trifluoracetamide (MSTFA) (97.0% GC, Fluka, Buchs, Switzerland). Chloroform, analytical grade (99.8% GC, E Merck, Darmstadt, Germany).

Henequen wax (3000 g) was obtained in laboratory by solvent extraction of the epidermis of henequen leaves. Previously, the leaves were collected in the factory of Mariel (Havana, Cuba) during the harvest of 1998-1999 from 8-years old plants.

The henequen wax was taken to melt at 100 ^{0}C, adding 80 g of KOH dissolved in 100 mL of ethanol: water (1:1). After the hydroxide solution is completely added, the reaction continues for three hours with continuos stirring, then the saponified henequen wax is cooled at room temperature.

The mixture of fatty alcohols was extracted in the following manner: Saponified henequen wax (500 g) is extracted in a 2 L Söxhlet apparatus using 1000 mL of dichloroethane, during 24 hours. Solvent was evaporated to a third of its volume under reduced pressure and the extract was cooled at room temperature overnight. Whereby, the mixture of fatty alcohols was dissolved in chloroform (2 L) and let to stand at 4 ^{0}C overnight. The crystallization process was repeated once more and after that time, the solution was filtered and the crystals were dried on a vacuum oven at 45 ^{0}C, overnight.

Chromatographic analysis, for identification and quantification of the mixture of fatty alcohols, were performed as described for the identification and quantification of policosanol from sugarcane[40,41] using a GC-14B (Shimadzu-Kyoto, Japan) gas chromatograph with flame ionisation detector (FID), coupled to a C-R4A (Shimadzu-Kyoto, Japan) computerised data processor. A 3 % OV-101 on Chromosorb HP 80-100 mesh (Supelco, Bellofonte, U.S.A) glass column 3 m length x 3 mm i.d. was used. The GC analysis was adjusted to the following conditions: injector and detector temperature: 320 ^{0}C, oven temperature: from 200 to 320 ^{0}C (10 ^{0}C/min) and held for 10 min, carrier gas (argon) flow: 30 mL/min, hydrogen and air flows for FID were adjusted to 40 and 400 mL/min respectively and the injection volume was 1 µL.

The quantitative determination of the mixture of fatty alcohols was done using the internal standard method[42,43], in which 1-eicosanol was used as internal standard. In this case, a known quantity of the 1-eicosanol solution is added to the sample of the mixture of fatty alcohols. The mixture was derivatized using MSTFA as sylanizing agent, in the following manner: 10 mg of the mixture of fatty alcohols were weighed into a 3 mL vial with screw cap and 100 µL of MSTFA were added to it, heating the solution at 60 ^{0}C for 15 min on a dry thermostat.

IR spectra were recorded on a PU 4990 spectrophotometer. Mass spectra of the individual alcohols, present in the mixture of aliphatic alcohols, were obtained in a GC/MS MD 800 (Fisons, Instruments, England) equipment coupled with a Lab-Base (VG Mass Lab, England) software using a SE-54 (25 m length, 0.32 mm id.) capillary column (Supelco, Bellofonte, U.S.A.) with the following chromatographic conditions: temperature of detector and the injector 320 ^{0}C, of the ionisation chamber 250 ^{0}C and that of the interface 200 ^{0}C at 40 ^{0}C/min, from 200 to 320 ^{0}C at 8 ^{0}C/min, carrier gas (He) flow 1.0 mL/min and the energy of ionisation was of 70 eV. Sample need, firstly, to be derivatized using MSTFA as sylanizing agent in the same manner as was previously described. One µL of this solution was injected to the GC-MS system, the chromatogram is recorded and the mass spectra analysed further.

RESULTS AND DISCUSSION

The quantitative composition of the mixture of aliphatic alcohols is the following: 1-octacosanol (24.60 %), and 1-triacontanol (24.39 %) are the main components of the mixture, the other alcohols in the mixture are 1-hexacosanol (0.69 %), 1-heptacosanol (0.41 %), 1-nonacosanol (2.45 %), 1-hentriacontanol (9.25 %), 1-dotriacontanol (20.82 %), 1-tritriacontanol (4,85 %), 1- tetratriacontanol (8.90 %), 1-pentatriacontanol (2.10 %) and 1-hexatriacontanol (1.56 %), this quantification is obtained from the gas-chromatogram of the mixture of alcohols (Figure 1).

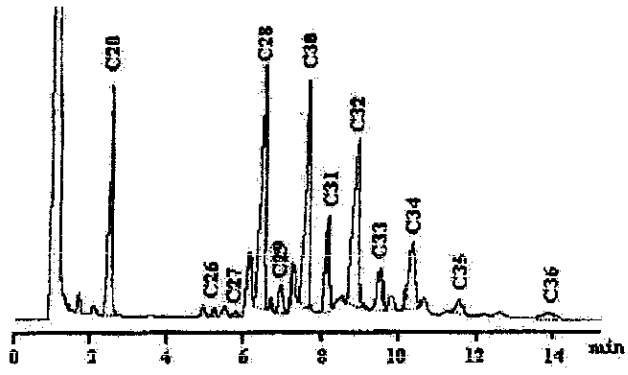

Figure 1: Gas-chromatogram of the mixture of alcohols obtained from *Agave furcroydes* L. wax

IR bands of the mixture of fatty alcohols (KBr discs) were 3236.5, 2912.8, 1460.2, 1609.0 and 791.1 cm^{-1}. This spectrum shows a strong similarity, including the fingerprint region, with respect to those of the commercial samples of the individual alcohols present on it, which were, also, measured.

The mass spectra of the sylanized fatty alcohols, present in the mixture of fatty alcohols (after resolution by GC) are: 1-hexacosanol: 439 (100 %), 423, 125, 103, 83, 75 (41 %), 57 and 43 d; 1-heptacosanol: 453 (100 %), 437, 125, 103, 83, 75 (40 %), 57 and 43 d; 1-octacosanol: 467 (100 %), 465, 125, 103, 83, 75 (70 %), 57 and 43 d; 1-nonacosanol: 481 (100 %), 479, 125, 103, 83, 75 (60 %), 57 and 43 d; 1-triacontanol: 495 (100 %), 479, 125, 103, 83, 75 (45 %), 57 and 43 d; 1-hentriacontanol: 509 (100 %), 507, 125, 103, 83, 75 (70 %), 57 and 43 d; 1-dotriacontanol: 523 (100 %), 507, 125, 103, 83, 75 (52 %), 57 and 43 d; 1-tritriacontanol: 537 (100 %), 535, 125, 103, 83, 75 (65 %), 57 and 43 d; 1-tetratriacontanol: 551 (100 %), 535, 125, 103, 83, 75 (28 %), 57 and 43 d; 1-pentatriacontanol: 565 (100 %), 563, 125, 103, 83, 75 (45 %), 57 and 43 d and 1-hexatriacontanol: 579 (100 %), 577, 125, 103, 83, 75 (55 %), 57 and 43 d.

The sylanization of the alcohols 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 analysed 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 these 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.

CONCLUSIONS

It was possible to isolate, purify and characterize, studying its chromatographic and spectroscopic properties, a mixture of eleven fatty alcohols of high molecular weight from henequen (*Agave furcroydes* L.) wax. The following alcohols compose this mixture: 1-hexacosanol, 1-heptacosanol, 1-octacosanol, 1-nonacosanol, 1-triacontanol, 1-hentriacontanol, 1-dotriacontanol, 1-tritriacontanol, 1-tetratriacontanol, 1-pentatriacontanol and 1-hexatriacontanol being 1-octacosanol and 1-triacontanol the main components of it.

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