Distribution study of radioactivity in rats after oral administration of the lipido/sterolic extract of *Serenoa repens* (Permixon®) supplemented with \(1^{14}\text{C}\)-lauric acid, \(1^{14}\text{C}\)-oleic acid or \(4^{14}\text{C}\)-\(\beta\)-sitosterol

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SUMMARY

The study carried out on rats given orally the \(n\)-hexane lipido/sterolic extract of *Serenoa repens* (LSESR), supplemented with \(1^{14}\text{C}\)-labelled oleic or lauric acids or \(\beta\)-sitosterol, demonstrated that radioactivity uptake in prostatic tissues shows the highest level in the case of administration of LSESR supplemented with \(1^{14}\text{C}\)-labelled oleic acid. This was clearly demonstrated on a rat with an induced fibro-muscular hyperplasia of the prostate and by quantitative measurements of radioactivity. Ratios of radioactivity in tissues compared to plasma show an uptake of radioactivity greater in prostate as compared to other genital organs, i.e. the seminal vesicles or to other organs such as liver.

INTRODUCTION

The saw palmetto *Serenoa repens* is a scrub dwarf palm very common in the open areas through much of the South-Eastern part of the US (South Carolina, Florida and Alabama) (1,2).

\(^4\) The drupe-like fruits, green becoming yellowish and blue-black when ripe, are eaten by wild animals and were highly appreciated by the natives as a food, for giving an 'increase of fat, flesh and strength'. The anti-irritating properties of the fruits of *S. repens* were confirmed in the mid-XIXth century; the extracts demonstrated activity on mucosae and appeared to be efficient against catarrhal affections and chronic bronchitis.

The present major medicinal use of *S. repens* started in 1894 with a description of the beneficial and

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Footnote: Permixon® is marketed by Pierre Fabre Médicament (Castres, France) whose other trademarks include Capistan, Libeprosta and Sereprostat.
more specific action of the extracts on prostatic dysfunctions (3). These efficacious properties are described in several American books of materia medica (4–7). Since then, S. repens has entered different national pharmacopeia (8,9).

Now, the saw palmetto extracts have regained an important place in urological therapy thanks to their lenitive action on the functional symptoms of benign prostatic hyperplasia, associated with an absence of side effects. Numerous clinical confirmations were made in countries such as France (10–13), Germany (14,15), Italy (16–19) and Spain (20). The beneficial effects of the extracts (21) may be related not only to their anti-inflammatory effects (22,23), but also to their interference with hormonal metabolism, either at the level of androgen (24) and prolactin (25) receptors, or on the biosynthesis of 5α-dihydrotestosterone reductase (26).

The drupes of S. repens have been extracted with various solvents, such as ethanol, acetone, hypercritical carbon dioxide or hexane. The hexane extract of the entire drupe (pulp and seed), the basis of the French pharmaceutic speciality Permixon® appears as a complex mixture of essentially free fatty acids and their esters, small quantities of phytosterols (β-sitosterol, campesterol, stigmasterol), cycloartenol, aliphatic alcohols (C26, C28, C30) and several polyprenic compounds. The chief more potent fatty acids, determined on 11 batches from two autumnal crops, are oleic (36 ± 3%), lauric (27.5 ± 3%) and myristic (12 ± 1%) acids (27).

In order to understand the mechanism of the pharmacological effects of the hexane extract of S. repens, the tissue distribution of its constituents must first be systematically investigated. In the experiments reported here, rats were administered orally the lipido/sterolic extract of S. repens (LSESR), to which was added [1-14C]-labelled oleic, or lauric acid, or β-sitosterol.

MATERIALS AND METHODS

Chemicals

[1-14C]-Lauric acid, [1-14C]-oleic acid and β-[4-14C]-sitosterol were purchased through Amersham International. [1-14C]-Lauric acid was available as a n-hexane solution and sealed under nitrogen in a borosilicate vial. Its specific activity was 2.18 GBq/mmol (59.5 mCi/mmol). The radiochemical purity was checked using radio-thin layer chromatography on silica gel plates (Merck F256) in ether:hexane:formic acid (25:75:2). It was found to be higher than 98.5%.

[1-14C]-Oleic acid was available as a dry deaerated toluene solution sealed under nitrogen in a borosilicate vial. Its specific activity was 2.04 GBq/mmol (55 mCi/mmol). The radiochemical purity was checked using radio-thin layer chromatography on silica gel plates (Merck F256) in ether:hexane:formic acid (25:75:2). It was found to be higher than 98%.

[4-14C]-β-Sitosterol was available as a solution in toluene:ethanol (9:1). Its specific activity was 2.07 GBq/mmol (56 mCi/mmol). The radiochemical purity was checked using radio-thin layer chromatography on silica gel plates (Merck F256) in cyclohexane:chloroform (50:50). It was found to be higher than 97%.

The n-hexane lipido/sterolic extract of S. repens (LSESR) was supplied by Pierre Fabre Médicament, Castres, France (batch no. 646).

Preparations

Each vial containing 9.25 MBq (250 μCi) of either [1-14C]-lauric acid, [1-14C]-oleic acid or [4-14C]-β-sitosterol was evaporated under a gentle nitrogen flow. The vial was then filled with 2.5 ml of LSESR, so that the reactive volume was 100 μCi/ml. The vial was stirred for 30 min at room temperature in the darkness. Then, an aliquot was weighed in a vial which was filled with 10 ml of Instagel® (Packard, France). Radioactivity counting was carried out 5 times in a liquid scintillation counter (Packard 2200 CA) for 1 min.

Animals

The study was performed on male Long Evans rats, weighing 150 g upon arrival in the animal unit. They were purchased through Elevages Janvier (Le Genest-Saint-Isle, France). They were maintained in the animal unit for 15 days before being included in the protocol. They were housed, one rat per cage, under a 12 h light-12 h dark lighting regime. They received a controlled diet for rats (Vilemoisson-sur-Orge, France) and water ad libitum.

The rats were randomized into 3 groups:

Group A
18 animals were included in the protocol of the distribution study carried out by whole-body autoradiography. They were divided into 3 groups of 6 animals.

Group B
14 animals were included in the protocol of the quantitative study. They were divided into 2 groups of 7 animals.