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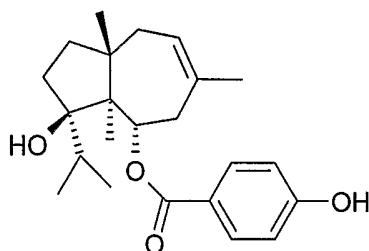
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(54) Title: A PROCESS FOR THE PREPARATION OF FERUTININE FROM *FERULA* GENUS PLANTS



(1a)

(57) Abstract: The invention relates to a process for the preparation of ferutinine (1a) from *Ferula spp* extracts comprising basic hydrolysis of the extracts and treatment with *p*-pivaloyloxybenzoic acid. The invention relates also to the use of the extracts and ferutinine in the cosmetic and dermatological field.

A PROCESS FOR THE PREPARATION OF FERUTININE FROM FERULA GENUS PLANTS

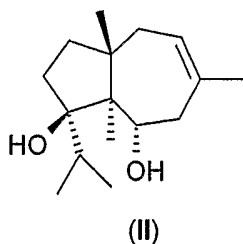
FIELD OF THE INVENTION

The present invention relates to vegetable extracts from *Ferula spp* and to a process for isolating ferutinine from said extracts.

BACKGROUND OF THE INVENTION

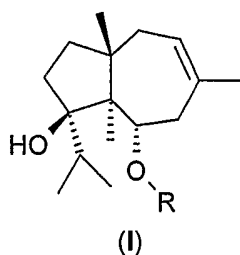
5 Numerous *Ferula* genus plants contain terpenes with estrogenic activity, known also as phytoestrogens, i.e. substances which regulate hormonal functions and are apparently a valid alternative to the use of synthetic hormones in the treatment of pre-menstrual syndrome and disorders related to menopause and aging. Extracts of some types of *Ferula* were used
10 in ancient times as contraceptives and in the treatment of impotence and menopausal disorders. Recently, alcoholic extracts from *Ferula asafoetida L.* have been disclosed (WO 0230438) as anticancer drugs.

The most abundant compounds in *Ferula* genus plants are derivatives of jaeschkenadiol (II):



15

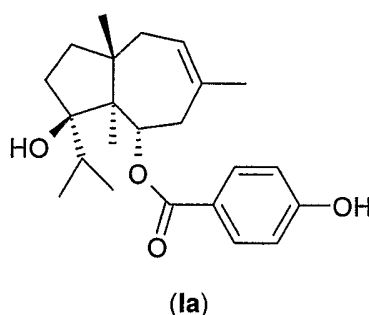
in particular daucane esters having the general formula (I)



Daucane esters are known compounds and are for example disclosed in
20 Phytochemistry, vol. 37, n° 3, pages 597 - 623, 1994. In the formula (I) R is a

straight or branched, saturated or unsaturated aliphatic acyl residue, or an optionally substituted aromatic acyl residue. Examples of R groups are iso-valeroyl, angeloyl, benzoyl, *p*-hydroxybenzyl, veratroyl or cinnammoyl.

Daucane esters from *Ferula spp* are estrogen modulators similar to
5 SERMs (selective estrogen receptor modulators); among them, ferutinine (**Ia**) shows marked estrogenic activity, whereas the others have a rather mild activity.



In particular, ferutinine is an estrogen receptor alpha agonist (ER α) and
10 an estrogen receptor beta agonist/antagonist (ER β). It has also been shown that ferutinine has higher binding to estrogen receptors than tamoxifen.

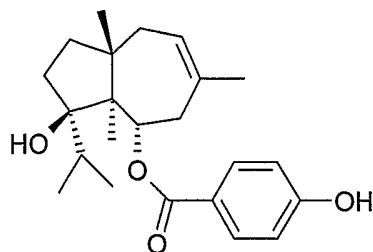
There is therefore the need to obtain ferutinine-enriched extracts or optimise the extraction of ferutinine in the pure form from plant materials containing its precursors.

15 A process comprising the hydrolysis of a daucane esters whole extract to give crude jaeschkenadiol and the subsequent re-esterification of jaeschkenadiol with suitably protected *p*-hydroxybenzoic acid, for example with *p*-acetoxybenzoic acid, is known from the literature (J. Org. Chem. USSR (Engl. Transl.); EN; 28; 10; 1992; 1666-1673). Nevertheless, this process gives rather
20 poor yields (about 45%), mainly due to competitive transesterification reactions.

DETAILED DISCLOSURE OF THE INVENTION

It has now been found that the use of *p*-pivaloxyloxybenzoic acid as esterifying agent allows to avoid competitive reactions responsible for low conversion yields.

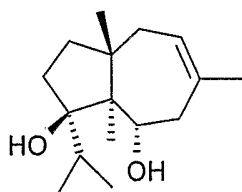
Object of the present invention is therefore a process for the preparation of ferutinine (**Ia**)



(Ia)

5 which comprises the following steps:

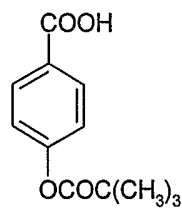
- a) extraction of daucane esters from *Ferula spp*;
- b) basic hydrolysis of daucane esters to give jaeschkenadiol (**II**)



(II)

;

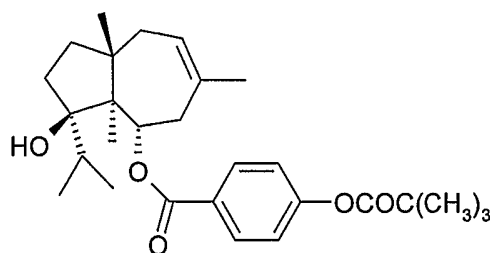
- c) esterification of jaeschkenadiol (**II**) with *p*-pivaloyloxybenzoic acid (**III**)



(III)

;

- to give *p*-pivaloylferutinine (**IV**)



(IV)

;

d) hydrolysis of *p*-pivaloylferutinine (IV) to ferutinine.

“Daucane esters” means compounds of the general formula (I) as defined above; said esters can be obtained by extraction of the rhizomes or aerial parts of *Ferula spp*, preferably *Ferula communis* and *Ferula hermonis*,
5 with conventional methods, for example by extraction with lower alcohols. Starting from *Ferula hermonis* rhizomes containing ferutinine and jaeschkenadiol benzoic ester (not easily separable by chromatography) in 1:1 ratio, pure ferutinine can be isolated by extracting the roots with methanol, treating the extract with 5% KOH and back-extracting the saponified extract
10 with aliphatic hydrocarbons, ethers, esters or chlorinated solvents.

Alternatively, daucane esters can be obtained by extraction with supercritical CO₂ at temperatures ranging from 35 to 65°C, preferably at 45°C, and pressures ranging from 200 to 260 bar, preferably at 245 bar. In the separator (or in the separators) the temperature ranges from 25 to 45°C and the
15 pressure is of about 50 bar. In these experimental conditions gummy materials, which make the recovery of the desired compounds difficult, are not extracted. The residue can be directly submitted to saponification according to what reported in the examples.

Jaeschkenadiol is esterified with *p*-pivaloylossibenzoic acid and treated,
20 in the same reaction solvent, with a base, preferably a primary amine, more preferably ethylenediamine, to give pure ferutinine. According to a preferred embodiment of the invention, steps c) and d) are conveniently carried out in sequence without recovery of intermediate *p*-pivaloylferutinine.

A further object of the present invention is the cosmetic and
25 dermatological use of ferutinine, *p*-pivaloylferutinine and extracts, of *Ferula spp*, preferably *Ferula communis* and *Ferula hermonis* extracts.

When applied on the skin, ferutinine and *Ferula spp* extracts surprisingly proved able to increase collagen biosynthesis and to exert a tonic,

trophic and moisturizing action, thus giving firmness and elasticity. Moreover, they reduce sebum secretion and play a remarkable role in the control of hirsutism and face virilization. Therefore, compositions containing ferutinine or *Ferula spp* extracts can be used in the cosmetic or dermatological field for the treatment of superficial or deep wrinkles or other unaesthetisms, as well as for the treatment of various acne and seborrhea forms.

Ferutinine and *Ferula spp* extracts can be formulated in the form of creams, gels and lotions in admixture with conventional excipients, for example those described in Remington's Pharmaceutical Sciences Handbook, XVII ed., Mack Pub., N.Y., U.S.A., preferably in the presence of soy lecithin or phospholipids, such as lauroylphosphatidylcholine and myristoylphosphatidylcholine, which can be incorporated in water/oil and oil/water emulsions or in transdermal plasters.

The following examples illustrate the invention in greater detail.

15 EXAMPLES

Example 1 - Isolation of jaeschkenadiol from *Ferula hermonis* roots

250 g of finely ground *Ferula hermonis* roots (particle size distribution: 2 mm) are extracted by percolation with 1 l MeOH. After maceration for two days and percolation of the solvent, the operation is repeated (4 x 1 l), to obtain 112.2 g of methanol extract (45%). Drug exhaustion is monitored by TLC (petroleum ether/EtOAc 8/2, ferutinine R_f : 0.14).

The methanol extract is refluxed with 513 ml of a 10% KOH methanol solution. After 1 h, TLC analysis (petroleum ether-EtOAc 8/2, ferutinine R_f : 0.14; R_f jaeschkenadiol: 0.31) shows that the reaction is complete. After cooling, the reaction mixture is diluted with water (500 ml) and extracted with petroleum ether (4 x 500 ml). The combined petroleum ether phases are washed with brine, dried and evaporated. The resulting semicrystalline residue is washed with cold petroleum ether (refrigerator temperature) to obtain 7.5 g

of crystalline jaeschkenadiol. The mother liquors are purified by chromatography (50 g silica gel, petroleum-ether-EtOAc 95:5), to obtain α -bisabolol (870 mg), a mixture of α -bisabolol and jaeschkenadiol, and pure jaeschkenadiol (3.4 g after crystallization). The mixture of jaeschkenadiol and α -bisabolol is pooled with the mother liquor and chromatographed (50 g silica gel, petroleum ether-EtOAc 95:5), to obtain 1.95 g of crystalline jaeschkenadiol (overall yield: 12.85 g, 5.1%).

The compound has the following chemical-physical and spectroscopic properties:

IR spectrum (KBr, cm^{-1}): 3339, 2965, 2905, 2875, 1470, 1375, 1047, 968, 858

Mass spectrum (C.I.)

$M^{+}+1 - \text{H}_2\text{O}=221$; $M^{+}+1 - 2\text{H}_2\text{O}=203$

^1H NMR spectrum (300 MHz, CDCl_3) δ : H9 5.43 m, H2 3.9 m, H14 1.78 s, H15 1.00 s, H12 0.95 d J 4.98, H13 0.91 d J 5.13.

Example 2 - Isolation of jaeschkenadiol from *Ferula communis* roots

250 g of finely ground *Ferula communis* roots (particle size distribution: 2 mm) are extracted by percolation using 1 l MeOH. After maceration for two days and percolation of the solvent, the operation is repeated (4 x 1 l); the methanol extracts are concentrated to a volume equal to the weight of the ground roots and the extract is added with 10% KOH (10 ml). The alkaline solution is refluxed for 2 hours, then cooled and back-extracted three times with 200 ml of *n*-hexane. Drug exhaustion is monitored by TLC (petroleum ether/EtOAc 8/2).

The combined hexane phases are washed with brine, dried and evaporated. The resulting semicrystalline residue is washed with cold petroleum ether (refrigerator temperature), to obtain 3.5 g of crystalline jaeschkenadiol. The mother liquor is purified according to example 1. Yield:

0.8 g of jaeschkenadiol having the same chemical-physical properties as that of example 1.

Example 3 - Isolation of jaeschkenadiol from *Ferula communis* aerial parts

5 1 kg of finely ground *Ferula communis* aerial parts are extracted with carbon dioxide at 45°C and 245 bar in an apparatus for extraction with supercritical gases. In the separator (or in the separators) temperature ranges from 25 to 45°C and pressure is of about 50 bar. In these conditions gummy materials that make it difficult to recover the desired compounds are not
10 extracted. The residue, which contains only lipophilic compounds and water, is taken up with methanol and treated with bases to hydrolyse jaeschkenadiol esters, as reported in examples 1 and 2. After purification, 5.1 g of pure compound having the same characteristics of the product of example 1 is obtained.

15 **Example 4 - Synthesis of *p*-pivaloyloxybenzoic acid**

 4-Hydroxybenzoic acid (114.5 g, 829 mmol) is dissolved under stirring in pyridine (1.15 l), cooling to $T < 5^{\circ}\text{C}$ on an ice bath. The resulting solution is added with 4-dimethylaminopyridine (DMAP, 0.3 equivalents, 248.8 mmol, 30.4 g) and pivaloyl chloride (3 equivalents, 2.487 mol, 300 g, 293.6 ml). The
20 solution is allowed to warm up to room temperature and left under stirring for 2 h, then added with water (2.29 l) (exothermic reaction: cool the solution in an ice bath) and left under stirring for further 3 h.

 The solution is poured into a separatory funnel and extracted with CH_2Cl_2 (3 x 750 ml). The combined methylene chloride phases are washed
25 with 2 M H_2SO_4 (4 x 750 ml) and a saturated NaCl solution (1 x 1150 ml), then dried over Na_2SO_4 (60 g).

 The solution is filtered through paper filter and the solvent is evaporated off under vacuum to obtain a residue which is triturated with

petroleum ether at 30°-50° (3 x 400 ml), filtered by suction and dried under vacuum in a static dryer at 45°C for 15 h. 130.5 g of product with the following spectroscopic characteristics is obtained.

IR spectrum (KBr, cm^{-1}): 3680, 2978, 2361, 1753, 1686, 1603, 1427,
5 1289, 1204, 1163, 1103.

Mass spectrum (C.I.): $M^{+}+1=223$

^1H NMR spectrum (300 MHz, D-DMSO) δ H3=7 8.00 d J=8.48,
H4=6 7.23 d J=8.55, CH3 1.34 s.

Example 5 - Synthesis of ferutinine from jaeschkenadiol

10 Jaeschkenadiol (100 g, 419.5 mmol) is dissolved under stirring at room temperature in CH_2Cl_2 (600 ml). The resulting solution is added with *p*-pivaloyloxybenzoic acid (1.4 equivalents, 587.3 mmol, 130.5 g) and DMAP (0.3 equivalents, 125.9 mmol, 15.4 g). The solution is left under stirring for 10 min. to complete reagents dissolution, then N,N'-dicyclohexylcarbodiimide
15 (DCC, 1.8 equivalents, 755.1 mmol, 155.8 g) is added. The reaction is complete after 2 h.

The solution is concentrated to 2 volumes (200 ml) and diluted with 5 volumes of CH_3CN (500 ml), thereafter the dicyclohexylurea precipitate is filtered off and washed with 5 more volumes of CH_3CN (2 x 250 ml). The
20 combined organic phases are poured into a separatory funnel, extracted with 10% w/v Na_2CO_3 (2 x 250 ml) and with a NaCl saturated solution (1 x 250 ml), then dried over Na_2SO_4 (100 g). Na_2SO_4 is filtered off and the solvent is evaporated under vacuum to give 360 g of compound (IV), having the following spectroscopic characteristics:

25 ^1H NMR (300 MHz, CDCl_3): δ 8.09 (d, J=9.0 Hz, H3'-H5'), 7.20 (d, J=8.7 Hz, H2'-H6'), 5.60 (brt, J=4.7 Hz, H9), 5.35 (td, J=10.4-2.9 Hz, H6), 2.58 (dd, J=13.1-10.9 Hz, H7b), 2.34 (dd, J=14.1-2.3 Hz, H7a), 2.07 (m, H10), 2.04 (d, J=2.7 Hz, H5), 1.97 (d, J=9.7 Hz, H2a), 1.89 (m, H11), 1.86 (s, H14),

1.63 (m, H2b), 1.57 (m, H3a), 1.41 (s, C(CH3)3), 1.30 (m, H3b), 1.14 (s, H15), 0.99 (d, J=6.9 Hz, H12), 0.89 (d, J=6.7 Hz, H13).

Compound (IV) is dissolved under stirring at room temperature in 2 l of CH₂Cl₂. The resulting solution is added with ethylenediamine (10 equivalents, 280 ml). After 3 h the reaction is complete. The solution is cooled to 0°C, poured into a separatory funnel, then washed with 3 M H₂SO₄ at 0°C (2 x 750 ml, exothermic reaction) and a saturated NaCl solution (1 x 500 ml). The organic phase is dried over Na₂SO₄ (100 g), filtered and evaporated to dryness. The residue (230 g) is loaded onto a silica gel column (2.5 kg) equilibrated with 5.8 l of a hexane:AcOEt=9:1 mixture and eluted with 70 l of the same mixture. The product-containing fractions are pooled, the solvent is evaporated off under vacuum and the product is dried in a static dryer at 45°C for 24 h.

139 g (92.4%) of product having the following spectroscopic characteristics is obtained:

IR spectrum (KBr, cm⁻¹): 3410, 1686, 1655, 1608, 1593, 1560, 1279, 1165, 1099, 771.

Mass spectrum (C.I.): M⁺+1 - H₂O=341

¹H NMR spectrum (200 MHz, CDCl₃): δ H3'=7' 7.94 d J=8, 4'=6' 6.88 d J=8, H9 5.56 m, H2 5.23 dt J=11, H14 1.80 brs, H15 1.10 s, H13 0.94 d J=6.5, H12 0.82 d J=6.5.

Example 6 - Preparation of a *Ferula hermonis* extract

1 kg of *Ferula hermonis* whole plant is extracted three times with 5 volumes of acetone. The combined acetone extracts are concentrated to 0.5 parts compared with the weight of the starting biomass and diluted with 2 parts of water. The aqueous solution is adjusted to pH 7.8 with diluted KOH, in the presence of hexane, under strong stirring. The hexane phase is discarded, the aqueous one is acidified to pH 5 and back-extracted with *n*-hexane. The hexane phase that contains ferutinine is concentrated to dryness

to give 52 g of extract containing about 35% of ferutinine.

Example 7 - Formulation containing ferutinine for the treatment of superficial wrinkles

5 Ferutinine is incorporated into a cream having the following composition:

	Ferutinine	0.20 g
	Carbomer 934 (Carbopol 934 P - Goodrich)	0.60 g
	Propylene glycol	3.00 g
	Imidazolinylurea	0.30 g
10	Kathon CG	0.05 g
	Disodium EDTA	0.10 g
	PEG-5 soy sterols (Generol 122 E5 - Henkel)	2.00 g
	Octyldodecanol (Eutanol G - Henkel)	4.00 g
	Wheat germ oil	4.00 g
15	Silicone oil 350 cps	0.50 g
	Glycerylstearate (Cutine GMS - Henkel)	7.00 g
	Polysorbate 60 (Tween 60 - ICI)	5.00 g
	Tocopherol	0.20 g
	Ascorbyl palmitate	0.10 g
20	10% NaOH solution	2.00 g
	Perfume (186909 - Dragoco)	0.20 g
	Purified water	up to 100.00 g

Example 8 - Formulation containing a *Ferula hermonis* pure extract with a ferutinine content of 30% and a jaeschkenadiol benzoic ester content of 20%

25	<i>Ferula hermonis</i> extract	0.5 g
	Carbomer 934 (Carbopol 934 P - Goodrich)	0.60 g
	Propylene glycol	3.00 g
	Imidazolinylurea	0.30 g

	Kathon CG	0.05 g
	Disodium EDTA	0.10 g
	PEG-5 soy sterols (Generol 122 E5 - Henkel)	2.00 g
	Octyldodecanol (Eutanol G - Henkel)	4.00 g
5	Wheat germ oil	4.00 g
	Silicone oil 350 cps	0.50 g
	Glycerylstearate (Cutine GMS - Henkel)	7.00 g
	Polysorbate 60 (Tween 60 - ICI)	5.00 g
	Tocopherol	0.20 g
10	Ascorbyl palmitate	0.10 g
	10% NaOH solution	2.00 g
	Perfume (186909 - Dragoco)	0.20 g
	Purified water	up to 100.00 g

Example 9 - Gel containing ferutinine

15	Ferutinine	0.30 g
	Imidazolinylurea	0.30 g
	Methylparaben	0.20 g
	Hydroxyethylcellulose (Natrosol 250 HHX - Aqualon)	2.00 g
	Purified water	up to 100 ml

20 Example 10 - Cosmetic formulation containing *Ferula spp* extract

	<i>Ferula hermonis</i> extract	0.5 g
	Imidazolinylurea	0.30 g
	Methylparaben	0.20 g
	Hydroxyethylcellulose (Natrosol 250 HHX - Aqualon)	2.00 g
25	Purified water	up to 100 ml

EXPERIMENTATION

Product effectiveness

The effectiveness of the cream of example 7 was determined in a double

blind study with 40 female volunteers, of age ranging from 39 to 56, evaluating the effects on skin elasticity and firmness and on rugometry. The study was preceded by a seven days conditioning period, wherein the subject had to refrain from the use of moisturizing products, sun-creams and liquid make-ups
5 and to avoid tanning treatments and excessive exposition to UV rays.

The subjects were allowed to use conventional eye and lip care products, face powders and non moisturizing soaps.

The subjects were randomly divided into two groups, one treated with a placebo cream and one treated with the cream of example 7. The creams were
10 applied on the face in standardized amount (0.5 g, i.e. 0.5 cm of cream coming out of the tube) twice a day, morning and night. Before and after five weeks of treatment the following measurements were carried out.

Before each measurement session all the subjects stayed for thirty minutes in a climatic chamber at 23°C and 50% of relative humidity. Each
15 session comprised three measurements with corneometer, three measurements with cutometer, and a silicon impression of the periorbital area, on the skin areas indicated in the following.

All the 40 subjects completed the study.

Cutometry

20 Cutometer is a commercially available device (Cutometer SEM 575, Courage & Khazaka, Germany) for measuring skin mechanical properties in a non-invasive way. In more detail, it measures the vertical deformation of the skin surface when subjected to a negative pressure of 500 mm Hg through a 2 mm opening of a probe. The length of skin penetration in the probe is
25 optically measured with 0.01 mm precision. The probe is connected with a computer that registers skin deformation over time. From the resulting curve, numerous variables can be extrapolated to evaluate skin elastic, viscoelastic and viscous behaviour.

The following parameters were recorded:

immediate distension (U_e), measured at 0.1 seconds;

delayed distension (U_v);

final distension (U_f), measured at 10 seconds; and

5 immediate retraction (U_r).

The test was carried out using the cutometer on both cheeks.

Significant variations were not observed in the placebo group. Delayed distention (U_v) in the treated group significantly decreased (16%, $p < 0.05$) after 5 weeks treatment. This parameter reflects skin viscoelastic properties and dermis behaviour. After 5 weeks, a significant change was also observed
10 (-12%, $p < 0.05$) in U_e , which is mainly influenced by hydration and mechanic properties of the corneum layer. The decrease in U_v and U_e , together with U_r stability, shows increased skin firmness.

Corneometry

15 Soft and smooth skin appearance mostly depends on the presence of an adequate amount of water in the corneous layer.

Corneometer is a commercially available device (Corneometer CM 825 Combi 3, Courage & Khazaka, Germany) which measures capacitance changes resulting from changes in skin hydration.

20 The test was carried out using the corneometer on both cheeks.

After 5 weeks, a significant change in the treated group was observed, in particular, skin hydration increased by 17.5%, while in the placebo group it decreased by 3%.

Rugometry

25 Silicon impressions were carried out on subjects in the seated position. The impressions (2 x 5 cm) were obtained at the beginning and after 5 weeks, using "Silflo silicon impression material" (available from Flexico, UK).

The impressions were then analysed with the Skin Image Analyzer

system using the Quantirides - Monaderm software, which distinguishes cutaneous microrelief from median wrinkles and deep wrinkles and calculate their number and depth; finally, the value of total wrinkle area is obtained.

After 5 weeks, significant changes were observed in the treated group.

- 5 In particular, a 21.3% ($p < 0.05$) decrease in the wrinkle area was observed, whereas in the placebo group the decrease was 0.4%.

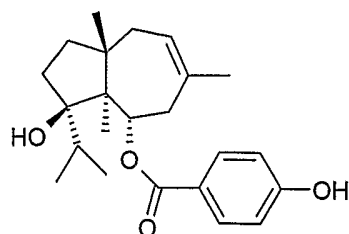
Statistically significant decrease has been observed mainly in the number and depth of median and deep wrinkles.

Conclusion

- 10 The study allowed to conclude that the cream of example 7 has good cosmetic activity in the treatment of skin with chrono- and photoaging signs, as it increases skin firmness and hydration and decreases mean wrinkled area, in particular deep micro- and macrorugosities. Skin visibly appeared firmer and smoother.

CLAIMS

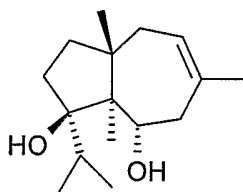
1. A process for the preparation of ferutinine (**Ia**)



(Ia)

which comprises the following steps:

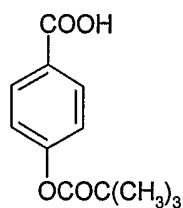
- a) extraction of daucane esters from *Ferula spp*;
- b) basic hydrolysis of daucane esters to give jaeschkenadiol (**II**)



(II)

;

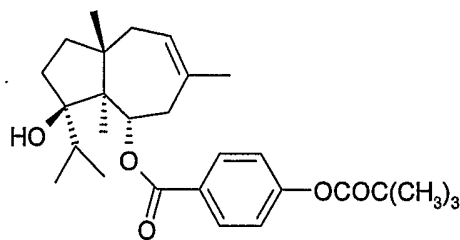
- c) esterification of jaeschkenadiol (**II**) with *p*-pivaloyloxybenzoic acid (**III**)



(III)

;

to give *p*-pivaloylferutinine (**IV**)



(IV)

;

d) hydrolysis of *p*-pivaloylferutinine (IV) to ferutinine.

2. Process according to claim 1 wherein daucane esters are extracted from *Ferula communis*.
3. Process according to claim 1 wherein daucane esters are extracted from
5 *Ferula hermonis*.
4. Process according to any one of claims 1-3 wherein daucane esters are extracted with supercritical carbon dioxide at temperatures ranging from 35 to 65°C and pressures ranging from 200 to 260 bar.
5. Process according to claim 4 wherein the temperature is 45°C.
- 10 6. Process according to claim 4 or 5 wherein the separation is carried out at temperatures ranging from 25 to 45°C and pressures ranging from 45 to 55 bar.
7. Process according to any one of claims 1-6 wherein steps c) and d) are carried out in sequence without recovering compound (IV).
8. Use of *Ferula spp* extracts for the preparation of cosmetic and/or
15 dermatological compositions.
9. Use of ferutinine for the preparation of cosmetic and/or dermatological compositions.
10. Use of *p*-pivaloyloxyferutinine for the preparation of cosmetic and/or dermatological compositions.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003055

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CH 672 251 A (AS UZB PLANT CHEM) 15 November 1989 (1989-11-15)	8,9
Y	page 9, line 6 - line 24; claim 1	10
X	TAMEMOTO KIMIKO ET AL: "Sesquiterpenoids from the fruits of Ferula kuhistanica and antibacterial activity of the constituents of F. kuhistanica" PHYTOCHEMISTRY (OXFORD), vol. 58, no. 5, November 2001 (2001-11), pages 763-767, XP004310053 ISSN: 0031-9422	8,9
Y	page 765, paragraph 5; table 3; compound 4	10
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

29 July 2004

Date of mailing of the international search report

12/08/2004

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003055

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>GALAL A M ET AL: "Daucane sesquiterpenes from <i>Ferula hermonis</i>." JOURNAL OF NATURAL PRODUCTS. MAR 2001, vol. 64, no. 3, March 2001 (2001-03), pages 399-400, XP002290428 ISSN: 0163-3864 abstract; compound 3</p> <p style="text-align: center;">-----</p>	8,9
X	<p>GB 1 604 225 A (SKOURIDES A A) 2 December 1981 (1981-12-02) claim 2; examples</p> <p style="text-align: center;">-----</p>	8
X	<p>PATENT ABSTRACTS OF JAPAN vol. 2000, no. 24, 11 May 2001 (2001-05-11) & JP 2001 206819 A (SANSHO SEIYAKU CO LTD), 31 July 2001 (2001-07-31) abstract</p> <p style="text-align: center;">-----</p>	8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/003055

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GB 1604225	A	02-12-1981	NONE	
JP 2001206819	A	31-07-2001	NONE	