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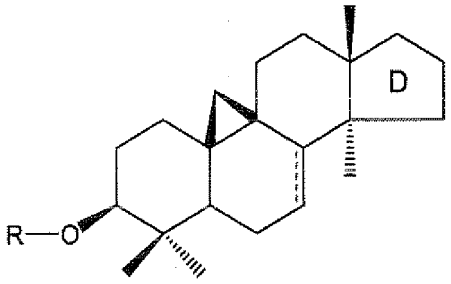
(54) VEGF PRODUCTION PROMOTER

(57) Provided are a VEGF production promoter, a hair quality improver, and an external preparation for skin, each of which has a VEGF production promoting activity and can be used as a pharmaceutical agent, a cosmetic, a food, or a material therefor.

The VEGF production promotor, the hair quality improver, and the external preparation for skin each comprises, as an active ingredient, a cycloartane-type glycoside represented by the following general formula (1) (where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f (where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group)).



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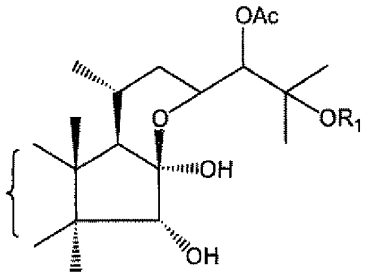

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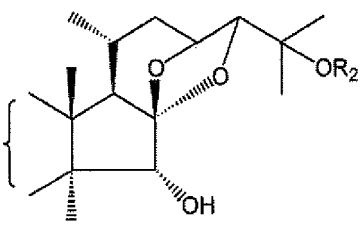

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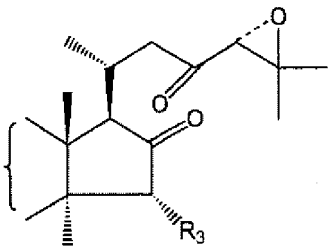

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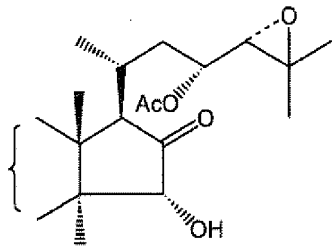

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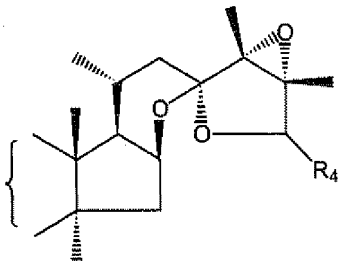

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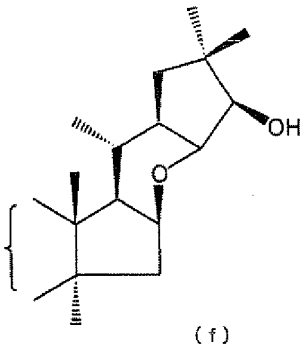


(f)

Description

Field of the Invention

[0001] The present invention relates to a VEGF production promoter, a hair quality improver, and an external preparation for skin, each of which can promote production of vascular endothelial growth factor (VEGF).

Background of the Invention

[0002] VEGF is known as vascular permeability factor, but recent studies report that VEGF is major angiogenesis factor in human skin. Therefore, VEGF is a molecule which attracts attention in fields of studies on healing of wound, improvement of skin color, hair growing/hair restoration, and the like.

[0003] In normal skin, VEGF is secreted from epidermal keratinocytes in a small amount and is bound to a specific receptor which is present on dermal microvascular endothelial cells. As a result, viability of the endothelial cells is kept to maintain upper blood vessels having reticular structures.

[0004] It has been reported that VEGF in the epidermis thickened during inflammation or healing of wound is very highly expressed, which leads to an increase in blood vessels and supply of nutrients (Non Patent Document 1). Further, it has been reported that, in the hair anagen phase, blood vessels around the hair follicle are drastically dilated and VEGF is expressed in cells of the hair follicle, while in the hair catagen and telogen phases, expression of VEGF is suppressed by regression of the blood vessels (Non Patent Document 2).
It has further been reported that VEGF promotes vascular formation, and hence promotes blood circulation to supply large amounts of nutrients to hair roots and is related to adhesion between hair cortex cells.
In particular, a recent study reports that a decrease in VEGF expression level is correlated with a decrease in hair firmness and elasticity, and application of a VEGF production promoter to a hair cosmetic has been a focus of attention (Non Patent Document 5).

[0005] Therefore, promotion of production of VEGF, which is involved in the increase in blood vessels and supply of nutrients in the dermis is very effective not only for recovery/healing of wound but also for improvement of skin color such as dullness or decreased transparency of skin caused by lowered skin metabolism or the like. In addition, promotion of production of VEGF, which is also involved in growth of hair/hair follicle is effective for prevention and improvement of symptoms such as hair loss/thin hair and decreased hair firmness and elasticity.

[0006] In view of the foregoing, various VEGF production promoters have been developed so far. For example, the following preparation and extracts have been reported to have VEGF production promoting activities: a preparation derived from soybean (Patent Document 1); an extract of each of *Boletinus cavipes, Suillus laricinus, Suillus grevillei, Boletinus asiaticus, Suillus bovinus, Suillus spectabilis, Acanthopanax senticosus Harms,* polygonatum rhizome, *Gentiana lutea,* Alexandrian Senna, *Eucommia ulmoides, rhubarb,* melilot, coix seed, Chinese wolfberry fruit, Japanese Angelica Root, rehmannia root, gardenia fruit, Glycyrrhiza, carrot, red ginseng, lithospermum root, and cymbidium (Patent Document 2) ; an extract of a plant belonging to the genus Pandanus (Pandanus L.f.) (Patent Document 3) ; and an extract of each of shii take mushroom, *Echinacea purpurea,* prune, bean sprouts, and jiaogulan (Patent Document 4). However, blending of each of the VEGF production promoters may be limited from the viewpoint of a side effect, and blending of each of the promoters in an effective amount may cause a problem such as coloring or unpleasant odor.

[0007] On the other hand, it has been reported that the cycloartane-type glycoside is isolated from a plant belonging to the genus *Cimicifuga* of the family *Ranunculaceae* such as *Cimicifuga simplex* and has pharmacological activities such as an thymidine intake inhibitory activity (Non Patent Document 3) and an antimalarial activity (Non Patent Document 4).

[0008] However, it was not known that the cycloartane-type glycoside has any VEGF production promoting activity.

Citation List

Patent Document

[0009]

[Patent Document 1] JP-A-11-286432
[Patent Document 2] JP-A-2000-212059
[Patent Document 3] JP-A-2004-43393
[Patent Document 4] JP-A-2004-35443

Non Patent Document

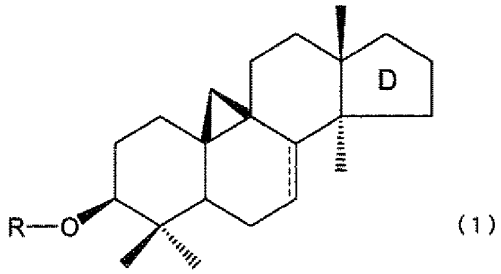
[0010]

[Non Patent Document 1] Detmar M. The role of VEGF and thrombospondins in skin angiogenesis, J Dermatol Sci. 24 (Suppl 1), S78-84, 2000
[Non Patent Document 2] Yano K, Brown LF, Detmar M. Control of hair growth and hair follicle size by VEGF-mediated angiogenesis, J Clin Invest. 107(4), 409-17, 2001
[Non Patent Document 3] Matsuhashi, Kusano and others, Proceedings of the 121st Annual Meeting of the Pharmaceutical Society of Japan, Vol. 4, p54
[Non Patent Document 4] Takahira M., Bio. Pharm. Bull., 21, 823-828 (1998)
[Non Patent Document 5] Moriwaki, Taguchi, Fragrance Journal, No. 12, 2007

Summary of the Invention

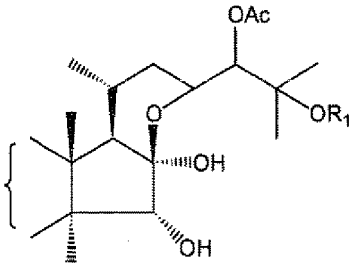
[0011] The present invention relates to the following items 1) to 9).

1) A VEGF production promoter, comprising, as an active ingredient, a cycloartane-type glycoside represented by the following general formula (1):



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



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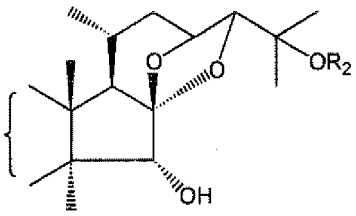

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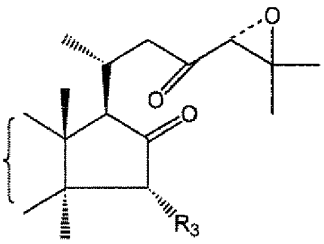

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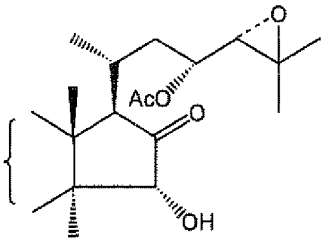

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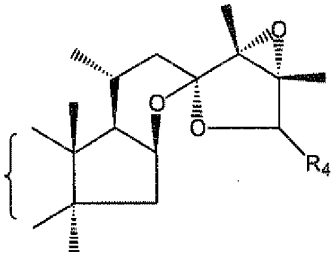

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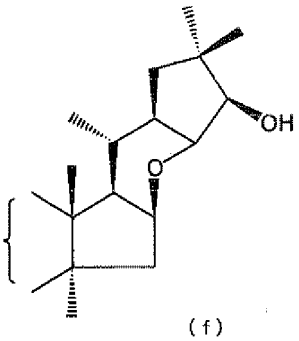
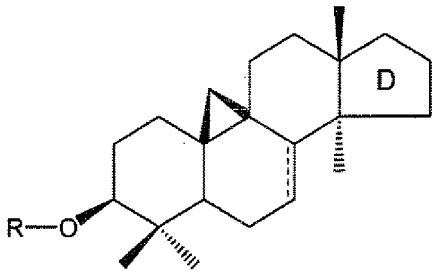


(f)

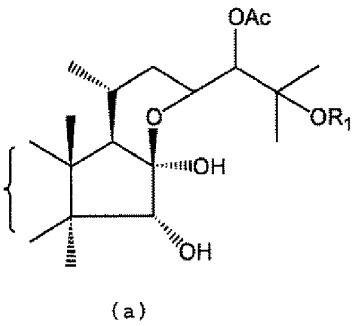
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

2) A hair quality improver, comprising, as an active ingredient, a cycloartane-type glycoside represented by the following general formula (1) :



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



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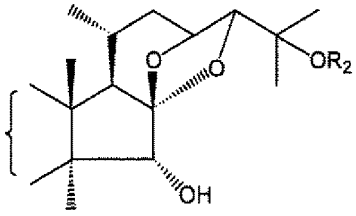
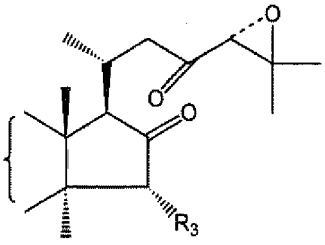


(b)



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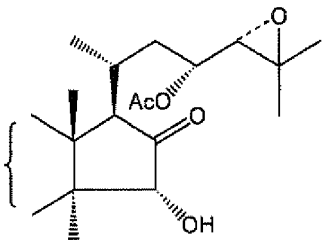

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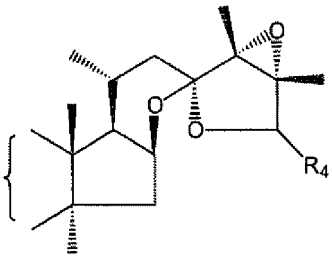

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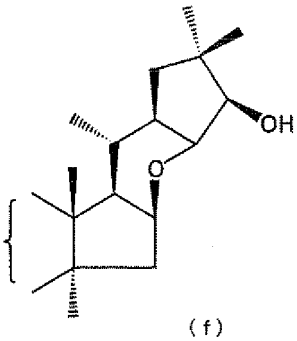
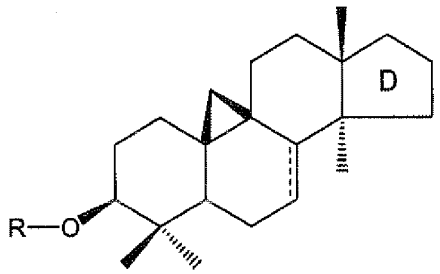


(f)

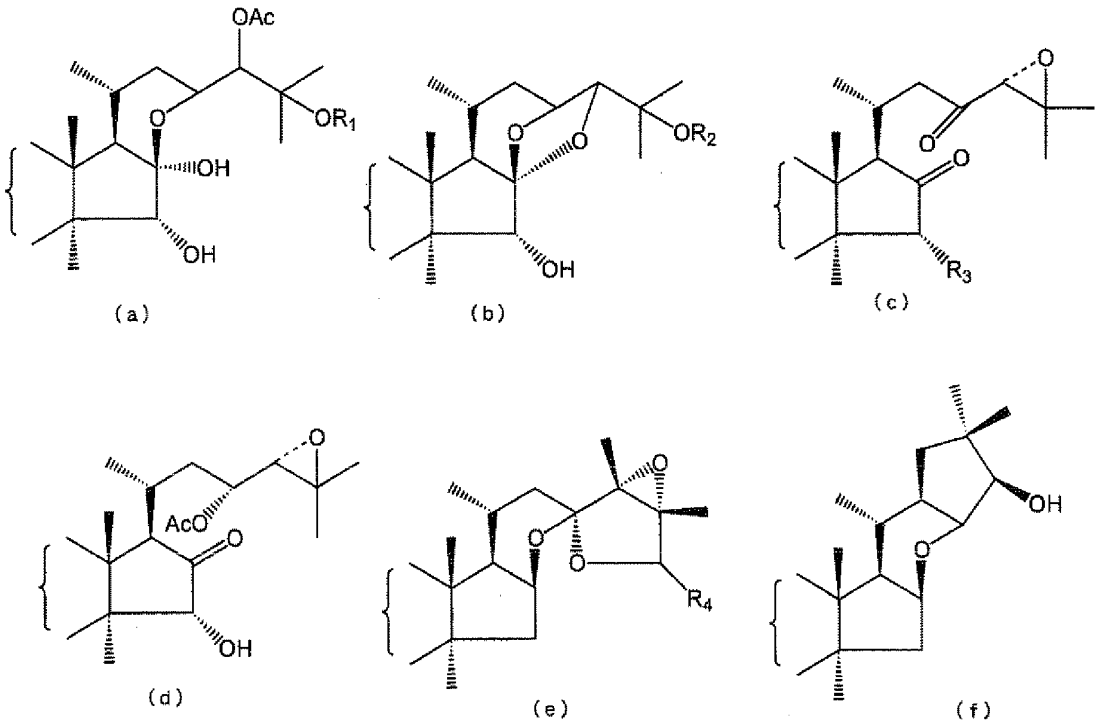
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

3) An external preparation for skin, comprising a cycloartane-type glycoside represented by the following general formula (1):



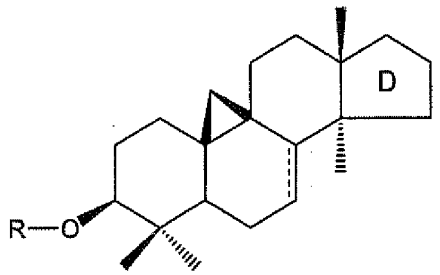
(1)

[0021] where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



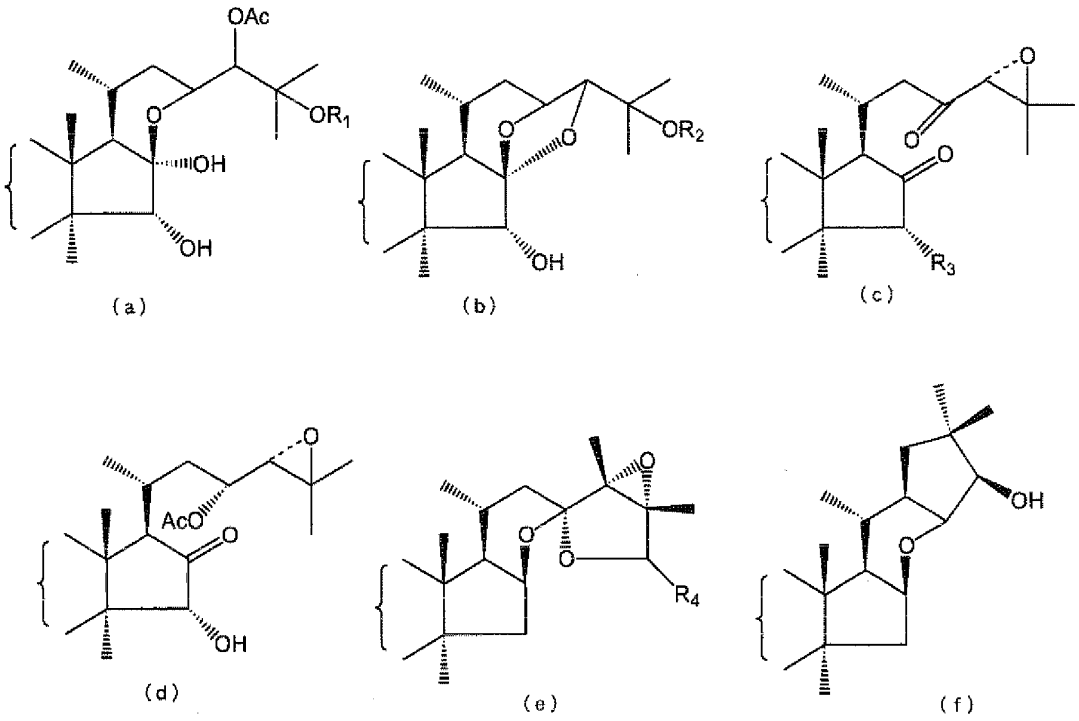
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

4) A method of promoting VEGF production, comprising administering or taking a cycloartane-type glycoside represented by the following general formula (1):



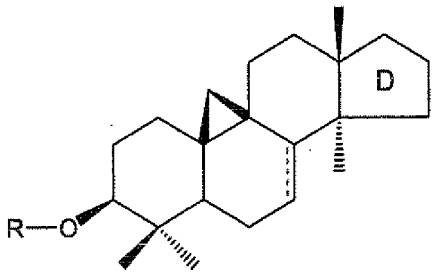
(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



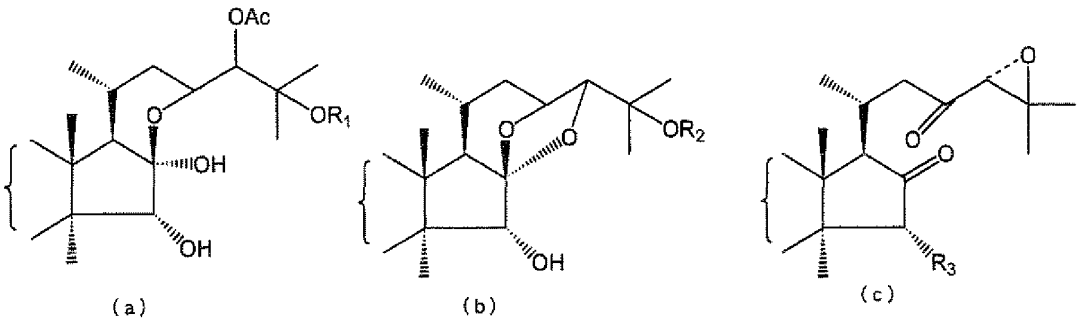
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

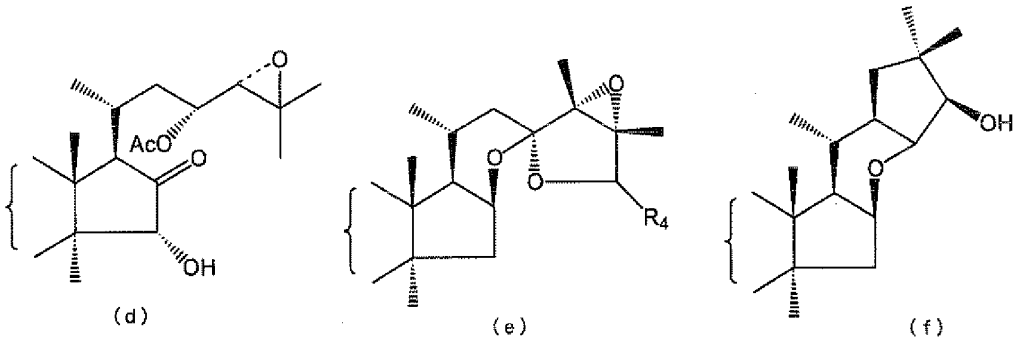
5) A method of improvinghairquality, comprising administering or taking a cycloartane-type glycoside represented by the following general formula (1):



(1)

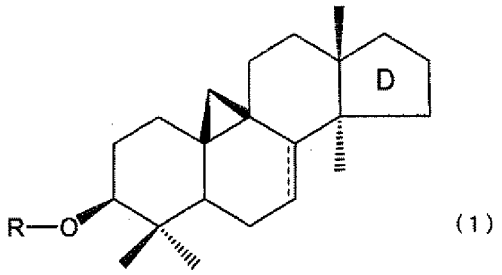
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



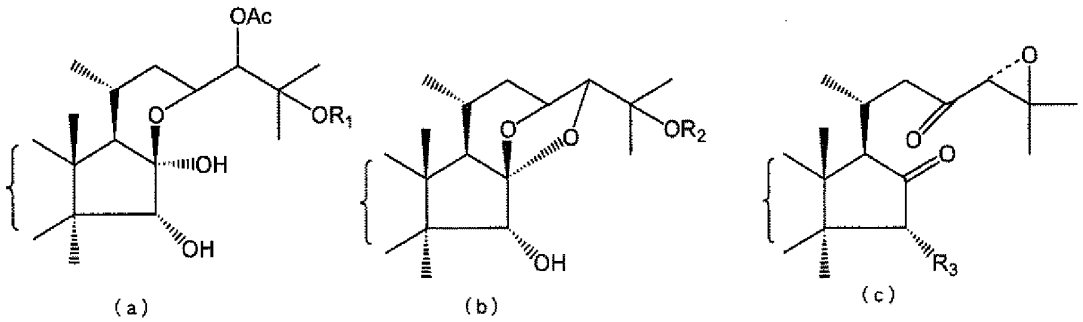


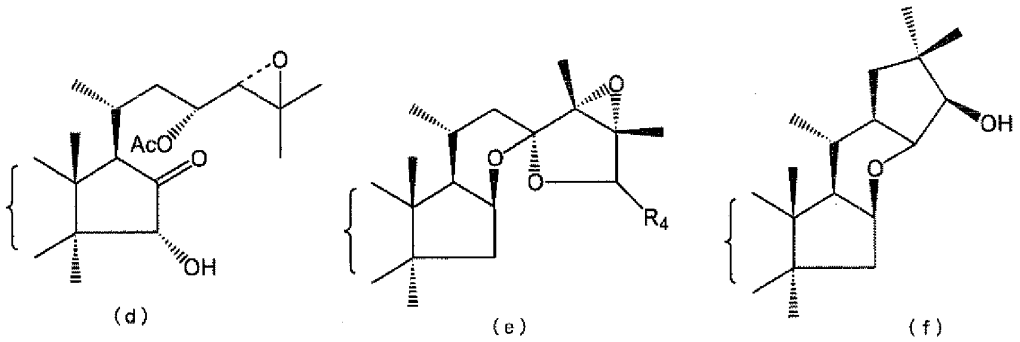
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

6) Use of a cycloartane-type glycoside represented by the following general formula (1) for manufacturing a VEGF production promoter:



where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:

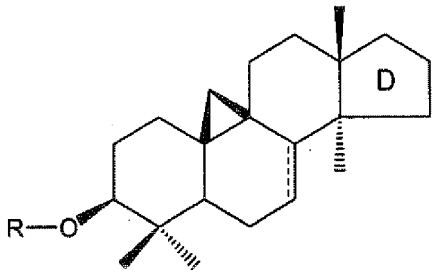




where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3

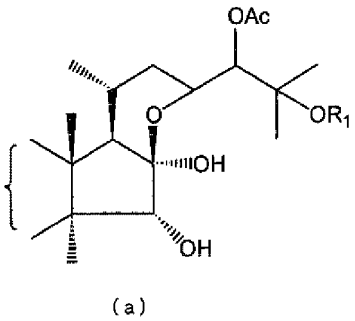
represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

7) Use of a cycloartane-type glycoside represented by the following general formula (1) for manufacturing a hair quality improver:



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



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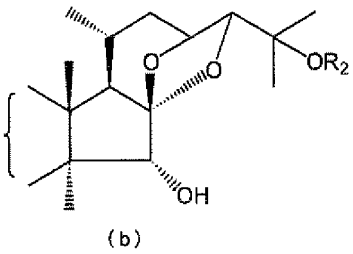

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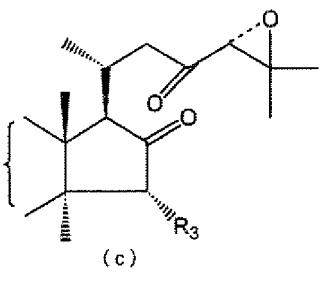

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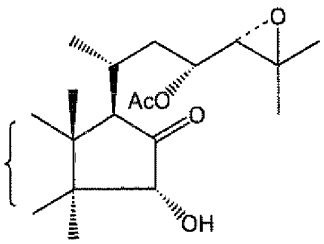

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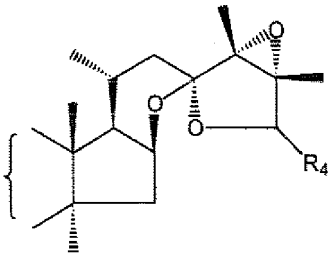

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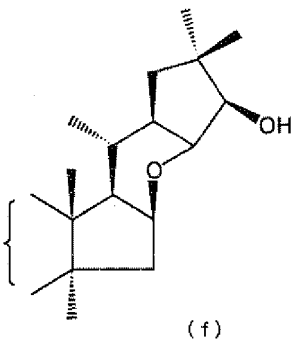
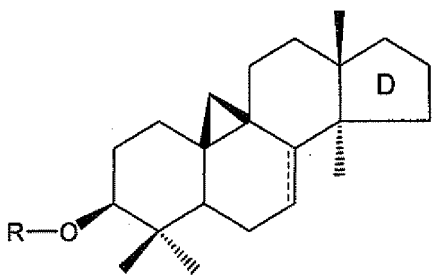


(f)

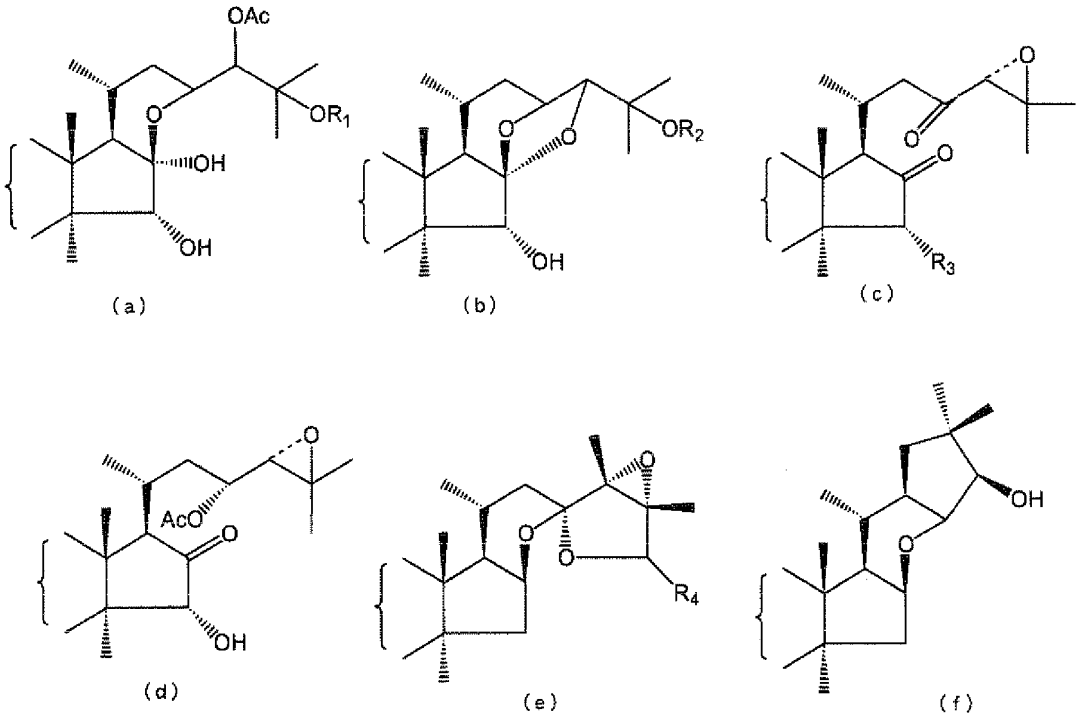
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

8) A cycloartane-type glycoside represented by the following general formula (1) for use in promoting VEGF production:



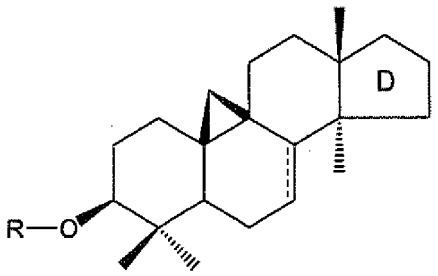
(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



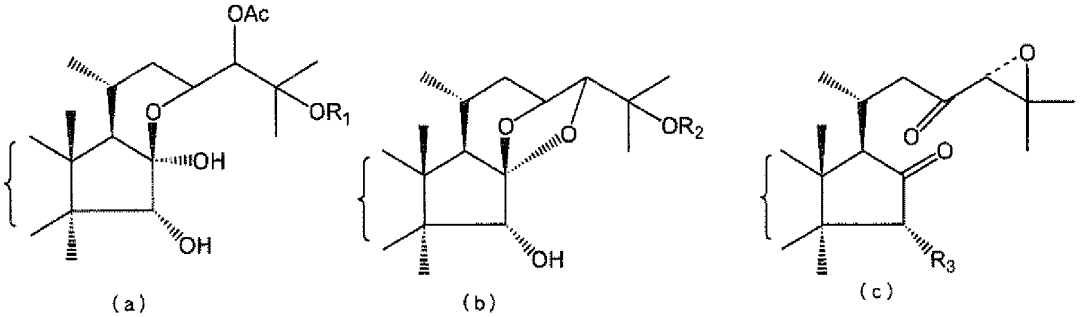
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

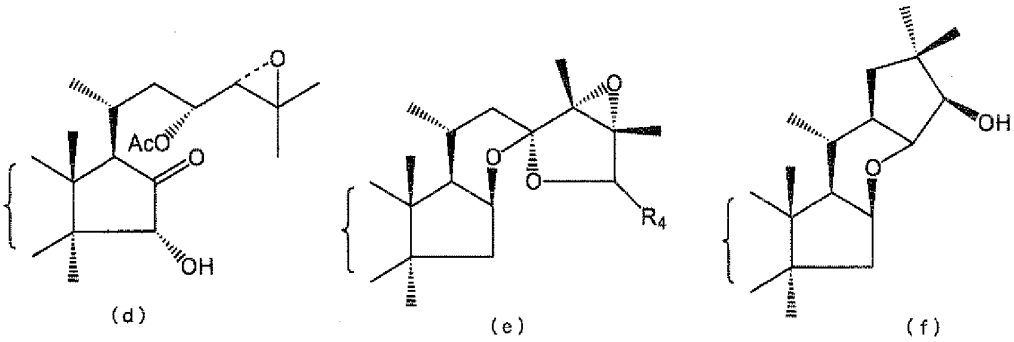
9) A cycloartane-type glycoside represented by the following general formula (1) for use in improving hair quality:



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:





where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

Detailed Description of the Invention

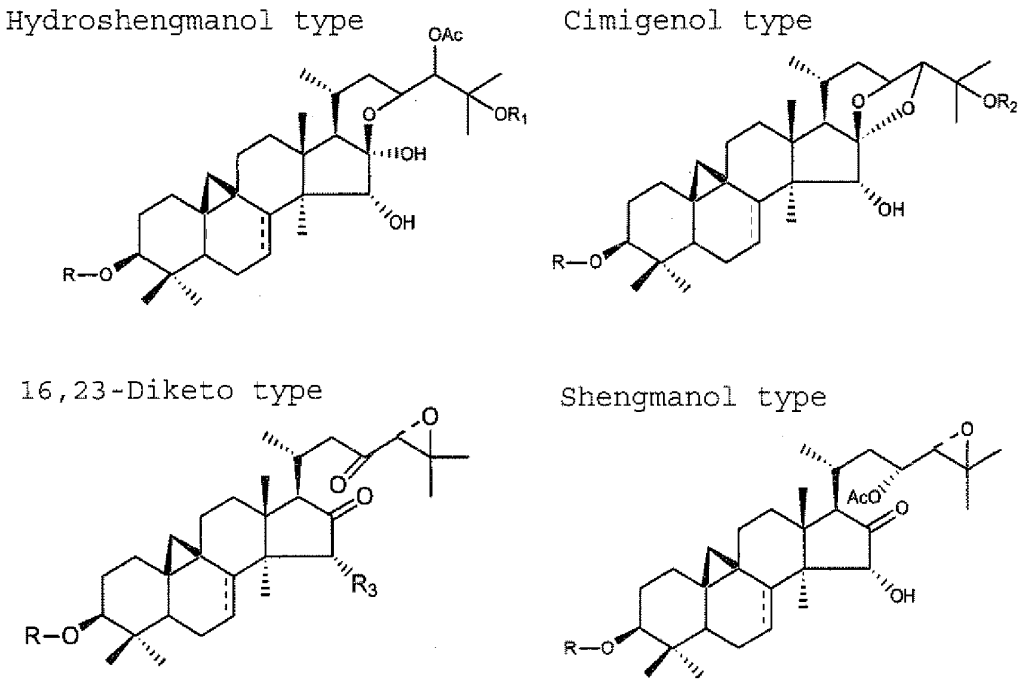
[0012] The present invention relates to the provision of a VEGF production promoter, a hair quality improver, and an external preparation for skin, each of which has a VEGF production promoting activity and can be used as a pharmaceutical agent, a cosmetic, a food, or a material therefor.

The inventors of the present invention have made intensive studies on a substance capable of promoting the production of VEGF. As a result, the inventors have found that a specific cycloartane-type glycoside has an excellent VEGF production promoting activity.

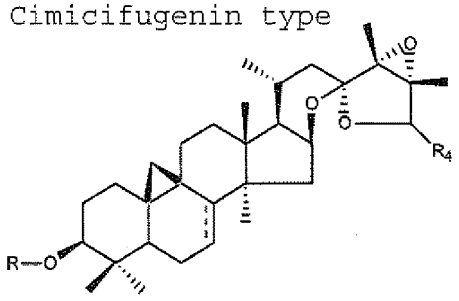
According to the present invention, it is possible to provide a pharmaceutical agent, a quasi-drug, a cosmetic, a food, or the like which has an excellent VEGF production promoting activity and is useful for healing of wound, improvement of skin color, hair growing/hair restoration, improvement of hair firmness and elasticity, and the like.

The cycloartane-type glycoside of the present invention is classified into the following six types based on the type of the ring D in the steroid skeleton.

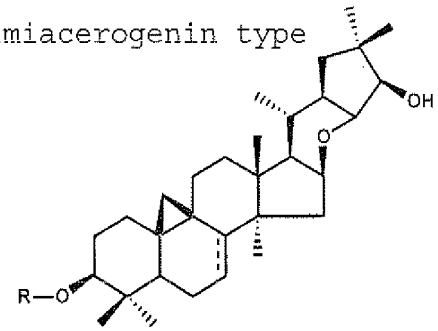
[0013]



Cimicifugenin type



Cimiacerogenin type



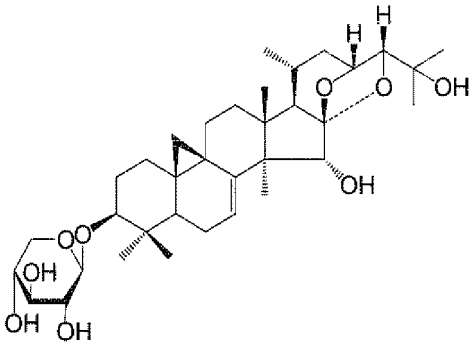
[0014] (In the formulae, R represents a xylose residue or an arabinose residue, R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.)

Of those, from the viewpoint of promotion of VEGF production, cycloartane-type glycosides of a hydroshengmanol type, a cimigenol type, and a 16,23-diketo type are preferred.

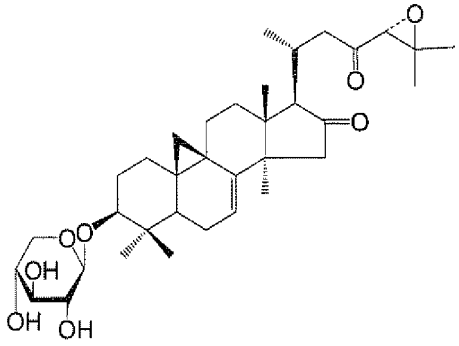
R represents a xylose residue or an arabinose residue, preferably a xylose residue. Further, the compound may have a stereoisomeric form of α-coordination or β-coordination, preferably β-coordination.

[0015] More preferable examples of the cycloartane-type glycoside of the present invention include 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside, 24-epi-7,8-didehydrocimigenol-3-xyloside, and cimicifugoside H-1 represented by the following formulae.

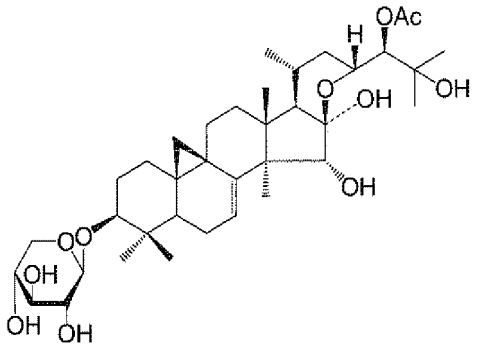
[0016]



24-epi-7,8-Didehydrocimigenol -3-xyloside



Cimicifugoside H-1



7,8-Didehydro-24-O-acetylhydroshengmanol -3-O-β-xyloside

[0017] The cycloartane-type glycoside of the present invention may be obtained by extraction and purification of a plant body. The extraction and purification of a plant body is carried out by, for example, subjecting a root of a plant

belonging to the genus *Cimicifuga* of the family *Ranunculaceae,* specifically, *Cimicifuga simplex, C. japonica, C. acerina, C. dahurica, C. heracleifolia* (*Cimicifuga heracleifolia*), *C. foetida, Cimicifuga racemosa* (black cohosh), or the like to solvent extraction and then separating and purifying the extract by appropriate separation and purification means such as column chromatography, ion-exchange chromatography, or high performance liquid chromatography. Hereinafter, examples of isolation of 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside, 24-epi-7,8-didehydrocimigenol-3-xyloside, and cimicifugoside H-1 described above are shown.

[0018]

1) 99.5% ethanol is added to a shredded product of the root of *Cimicifuga simplex*, and extraction is carried out with stirring, followed by filtration, to thereby obtain an extract of *Cimicifuga simplex*.

2) The solvent of the extract of *Cimicifuga simplex* obtained in 1) is distilled off, and the resultant solid matter is subjected to liquid-liquid distribution with ethyl acetate-water, followed by concentration of the ethyl acetate layer, to thereby obtain solid matter.

3) The solid matter obtained in 2) is charged to a silica gel column, and elution is carried out with a hexane-ethyl acetate mixed solvent and subsequently methanol, to thereby obtain three fractions.

4) The methanol eluted fraction obtained in 3) is charged to a silica gel column again, and elution is carried out with a chloroform-methanol mixed solvent, to thereby obtain 12 fractions.

5) The 12 fractions obtained in 4) are further fractionated by ODS-HPLC (acetonitrile-water mixed solvent), to thereby isolate 24-epi-7,8-didehydrocimigenol-3-xyloside,cimicifugoside H-1, and 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside.

[0019] It should be noted that, according to such extraction and fractionation, the cycloartane-type glycoside may be obtained singly or as a mixture of several types of the compounds. In the VEGF production promoter of the present invention, any of them may be used.

[0020] The resultant cycloartane-type glycoside may be used directly, or may be diluted with an appropriate solvent to prepare a diluted solution or may be prepared into a concentrated extract, driedpowder, orapaste. Further, the resultant may be freeze-dried, and the freeze-dried product may be diluted with a solvent which is usually used in extraction, such as water, ethanol, or a water/ethanol mixed liquid before use. In addition, the resultant may be encapsuled in, for example, a vehicle such as a liposome or a microcapsule.

[0021] As mentioned in Examples to be described later, the cycloartane-type glycoside of the present invention has a VEGF production promoting activity in human epidermal keratinocytes, and hence is considered to exert effects such as improvement of viability of endothelial cells and promotion of vascularization (Heidemarie Rossiter, Caterina Barresi, Johannes Pammer, Michael Rendl, Jody Haigh, Erwin F. Wagner, and Erwin Tschachler. Loss of Vascular Endothelial Growth Factor A Activity in Murine Epidermal Keratinocytes Delays Wound Healing and Inhibits Tumor Formation, Cancer research 64, 3508-3516, 2004) and to be useful for healing of wound, improvement of skin color, hair growing/hair restoration, improvement of hair firmness and elasticity, and the like.

Therefore, the cycloartane-type glycoside of the present invention can be used as a VEGF production promoter or a hair quality improver and can be used for manufacturing a VEGF production promoter or a hair quality improver. The VEGF production promoter may be: a pharmaceutical agent, a quasi-drug, a cosmetic, or a food for humans or animals, which can exert effects such as healing of wound, improvement of skin color, hair growing/hair restoration, and improvement of hair firmness and elasticity; or a material to be blended in the pharmaceutical agent or the like. In addition, in the present invention, the expression "improvement of hair quality" refers to hardening and/or strengthening the nature of hair, and/or imparting firmness and elasticity. The hair quality improver may be a pharmaceutical agent, a quasi-drug, a cosmetic, or a food for humans or animals, or a material to be blended in the pharmaceutical agent or the like.

Further, the cosmetic and food encompass a cosmetic, a food for beauty, a food for a patient, or a functional food such as a food for specified health use which has concepts of promotion of VEGF production, improvement of skin color, hair growing/hair restoration, improvement of hair quality, and the like, and shows the concepts, if necessary.

[0022] The administration form of the pharmaceutical agent containing the cycloartane-type glycoside of the present invention includes, for example, oral administration such as a tablet, a capsule, granules, powder, a syrup, or the like, or parenteral administration such as an intravenous injection, an intramuscular injection, a suppository, an inhalant, a transdermally absorbable drug, an eye drop, a nasal drop, an external preparation, or the like. Further, in order to prepare pharmaceutical preparations of such various dosage forms, the cycloartane-type glycoside of the present invention may be used singly or in appropriate combination with any other pharmaceutically acceptable excipient, binder, extender, disintegrator, surfactant, lubricant, dispersant, buffer agent, preservative, corrigent, flavoringagent, coating, carrier, diluent, or the like. In the case where the cycloartane-type glycoside of the present invention is used as the pharmaceutical preparation, in general, the content of the compound in the preparation is preferably 0.00001 to 50 mass%, more preferably 0.0001 to 10 mass%.

For example, the dose of the pharmaceutical agent per adult per day is preferably 0.0003 to 3,000 mg, more preferably

0.003 to 300 mg in terms of the cycloartane-type glycoside.

[0023] The form of the food containing the cycloartane-type glycoside of the present invention includes the same form as that of the above-mentioned preparation for oral administration (such as a tablet, a capsule, or a syrup).

In order to prepare foods having various forms, the cycloartane-type glycoside of the present invention may be used singly or in appropriate combination with another food material, a solvent, a softener, an oil, an emulsifier, a preservative, a flavoring agent, a stabilizer, a colorant, a UV absorber, an antioxidant, a moisturizer, a thickener, or the like. In general, the content of the cycloartane-type glycoside in the food is preferably 0.00001 to 100 mass%, more preferably 0.0001 to 70 mass%.

[0024] Further, examples of the quasi-drug and cosmetic containing the cycloartane-type glycoside of the present invention include an external preparation for skin, a detergent, a make-up cosmetic, and a cosmetic for scalp hair. According to usage, the quasi-drug and cosmetic may be provided in various dosage forms such as a lotion, an emulsion, a gel, a cream, an ointment, powder, and granules. The quasi-drugs and cosmetics in those various dosage forms may each be prepared using the cycloartane-type glycoside of the present invention singly or in appropriate combination with an oily component, a moisturizer, powder, a dye, an emulsifier, a solubilizer, a detergent, a UV absorber, a thickener, a drug, a flavoring agent, a resin, an anti-bacterial and anti-fungal agent, a plant extract, or an alcohol, which may be blended in a quasi-drug, a skin cosmetic, a cosmetic for scalp hair, or a cleanser. In general, the content of the cycloartane-type glycoside in the quasi-drug or cosmetic is preferably 0.00001 to 100 mass%, more preferably 0.0001 to 70 mass%.

Further, a subj ect to whom the cosmetic, pharmaceutical agent, or quasi-drug is applied is not particularly limited as long as the subj ect requires the product. However, the subj ect is preferably a human who requires promotion of production of VEGF or improvement of hair quality such as hardening and/or strengthening of hair nature and/or imparting firmness and elasticity.

Examples

Example 1 Preparation of cycloartane-type glycoside from root of *Cimicifuga simplex*

[0025] 700 mL of 99.5% ethanol were added to 70 g of a shredded product of the root of *Cimicifuga simplex* (produced in Shaanxi, China), and extraction was carried out at room temperature for 10 days, followed by filtration, to thereby obtain an extract. The extract was concentrated under reduced pressure, and the solvent was distilled off. Subsequently, 2.35 g of the resultant solid matter was subjected to liquid-liquid distribution with ethyl acetate-water, and the ethyl acetate layer was concentrated, to thereby obtain 1.86 g of solid matter. The solid matter was charged to a silica gel column, and elution was carried out with a hexane-ethyl acetate mixed solvent and subsequently methanol, to collect three fractions. 0.9 g of the methanol eluted fraction (fraction 3, 1.39 g) was charged to a silica gel column again, and elution was carried out with a chloroform-methanol mixed solvent, to thereby obtain 12 fractions (3a to 31). Fraction 3e (0.1 g) was fractionated by ODS-HPLC (acetonitrile-water mixed solvent), to collect eight fractions (3e-1 to 8). A component in fraction 3e-6 was isolated as 24-epi-7,8-didehydrocimigenol-3-xyloside (8.6 mg).

In the same manner as above, fraction 3f (0.17 g) was fractionated by ODS-HPLC (acetonitrile-water mixed solvent), to collect ten fractions (3f-1 to 10). Of the fractions, fraction 3f-2 was isolated as cimicifugoside H-1 (7.9 mg), and fraction 3f-8 was isolated as 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside (5.5 mg).

Example 2 Effect on production of VEGF

(1) Materials and method

[0026] Normal human newborn preputial epidermal keratinocytes (Kurabo Industries Ltd.: frozen NHEK (F) Lot. No. 061130-902) were used in a test. The cells were inoculated into three six-well microplates at 2×10^4 cells/mL per well. Culture was carried out in Defined keratinocyte-SFM (SFM medium, Gibco) under conditions of 2% CO_2 and 37°C. After the cell density reached 50 to 60% confluence, the medium was exchanged for additive-free Defined keratinocyte-SFM medium (SFM (-) medium). The cells were conditioned to the SFM (-) medium for 24 hours, and the medium was then exchanged for an SFM (-) medium supplemented with an arbitrary concentration of the triterpenoid saponin obtained in Example 1. Then, the test was started.

It should be noted that a compound-free SFM (-) medium was used as a negative control. 16 hours after the start of the test, the media were collected, and the amounts of VEGF secreted in the supernatants of the cultures were determined using an ELISA kit (R&D Systems). The amount of VEGF produced in the control was defined as 1, and relative values to the control value were used for evaluation.

(2) Results

[0027] Table 1 shows the results of the evaluation.

[0028]

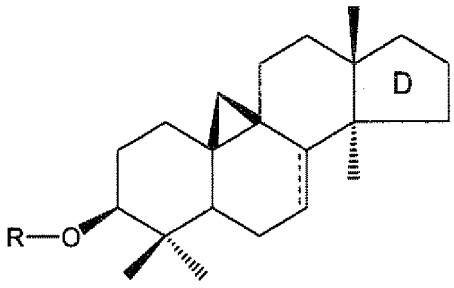
[Table 1]

Compound	Concentration (μg/mL)	Relative value of secreted VEGF amount
Control		1.00
Compound 1	200	5.69
	100	3.65
	20	2.19
Compound 2	200	-
	100	0.40
	20	2.32
Compound 3	200	2.82
	100	2.50
	20	1.19
Compound 1: 7,8-Didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside Compound 2: 24-epi-7,8-Didehydrocimigenol-3-xyloside Compound 3: Cimicifugoside H-1		

[0029] Table 1 shows that all the compounds have excellent effects of promoting production of VEGF.

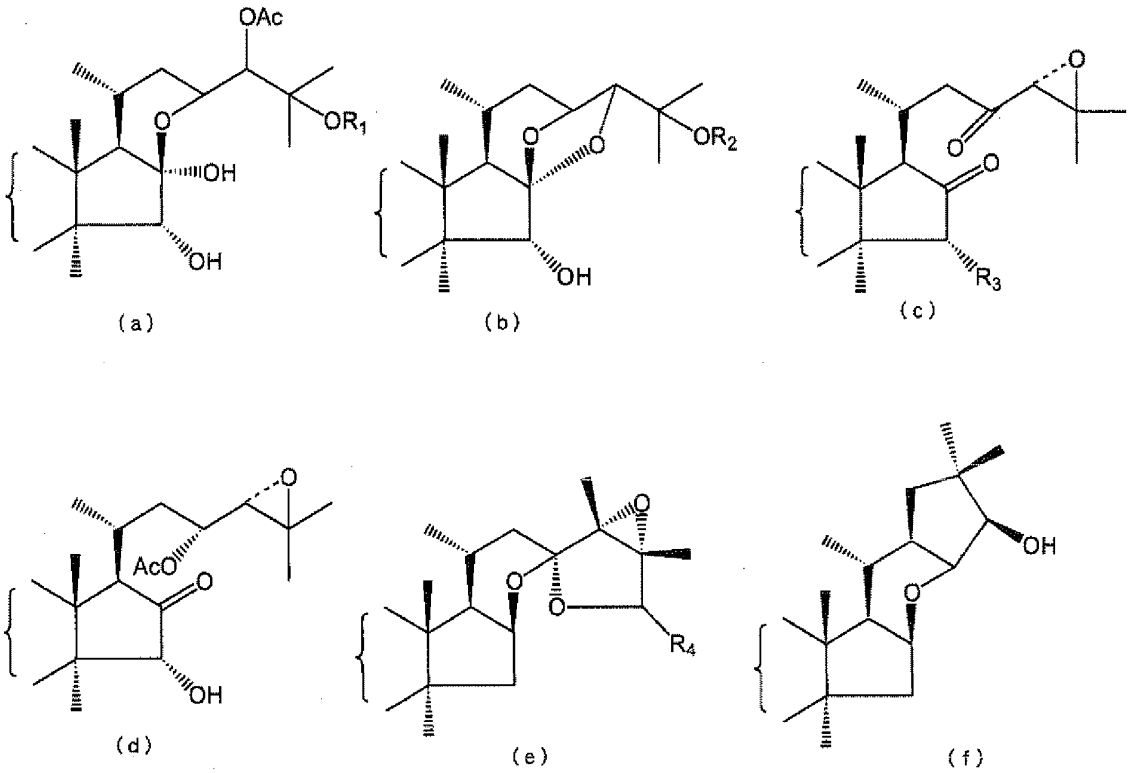
Claims

1. A VEGF production promoter, comprising, as an active ingredient, a cycloartane-type glycoside represented by the following general formula (1):



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



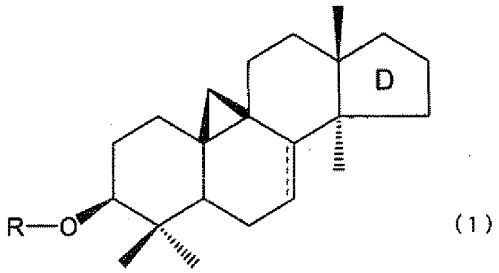
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

2. The VEGF production promoter according to claim 1, wherein the cycloartane-type glycoside is derived from a plant belonging to genus *Cimicifuga* of family *Ranunculaceae*.

3. The VEGF production promoter according to claim 1, wherein the cycloartan triterpenoid saponin is of a hydroshengmanol type, a cimigenol type, or a 16,23-diketo type.

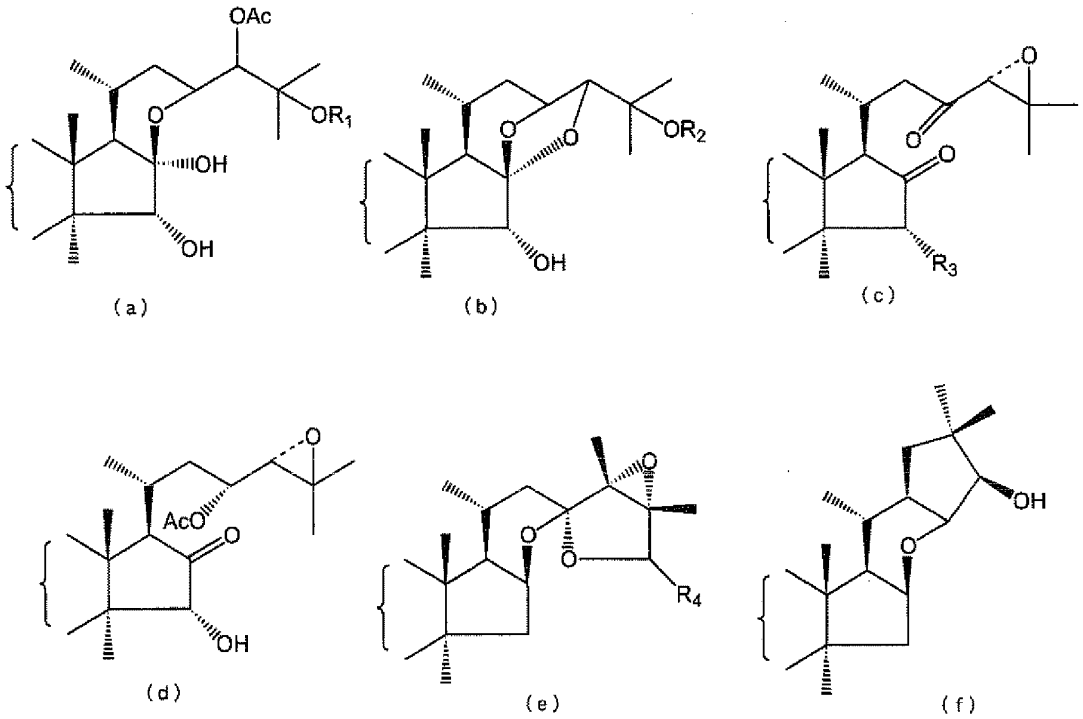
4. The VEGF production promoter according to claim 1, wherein the cycloartane-type glycoside is 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside, 24-epi-7,8-didehydrocimigenol-3-xyloside, or cimicifugoside H-1.

5. Ahair quality improver, comprising, as an active ingredient, a cycloartane-type glycoside represented by the following general formula (1):



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



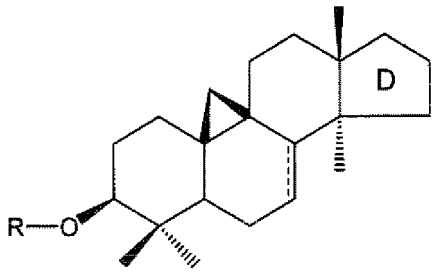
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

6. The hair quality improver according to claim 5, wherein the cycloartane-type glycoside is derived from a plant belonging to genus *Cimicifuga* of family *Ranunculaceae*.

7. The hair quality improver according to claim 5, wherein the cycloartane-type glycoside is of a hydroshengmanol type, a cimigenol type, or a 16,23-diketo type.

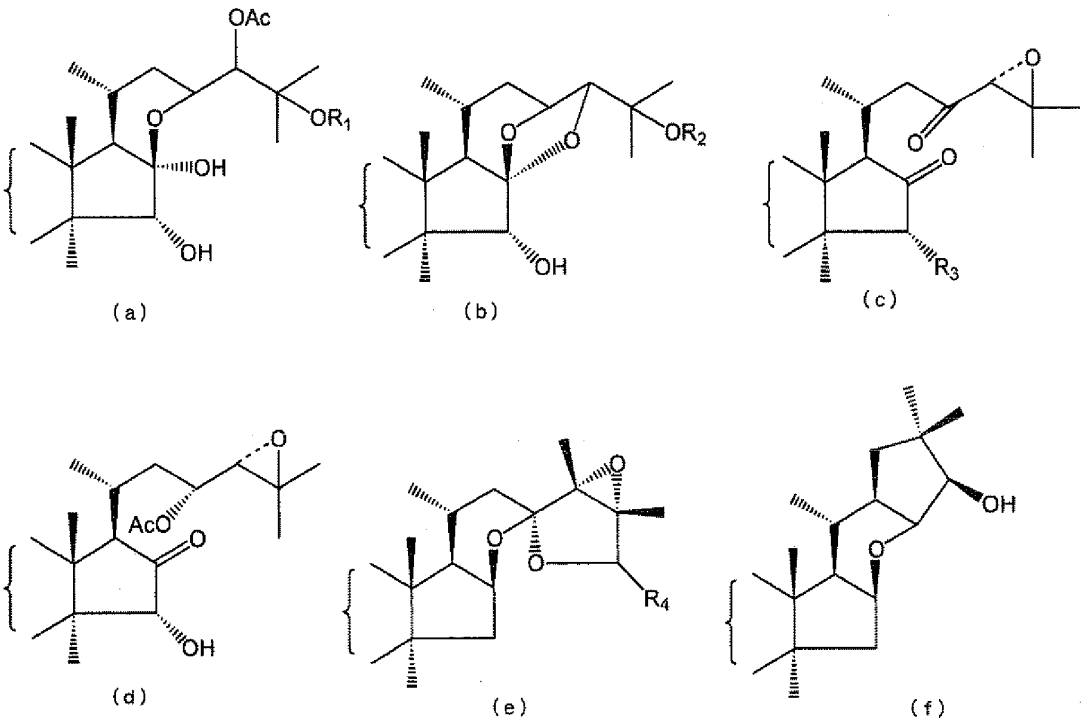
8. The hair quality improver according to claim 5, wherein the cycloartane-type glycoside is 7,8-didehydro-24-O-acetyl-hydroshengmanol-3-O-β-xyloside, 24-epi-7,8-didehydrocimigenol-3-xyloside, or cimicifugoside H-1.

9. An external preparation for skin, comprising a cycloartane-type glycoside represented by the following general formula (1):



(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



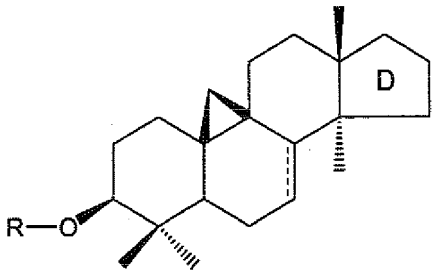
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

10. The external preparation for skin according to claim 9, wherein the cycloartane-type glycoside is derived from a plant belonging to genus *Cimicifuga* of family *Ranunculaceae*.

11. The external preparation for skin according to claim 9, wherein the cycloartane-type glycoside is of a hydroshengmanol type, a cimigenol type, or a 16,23-diketo type.

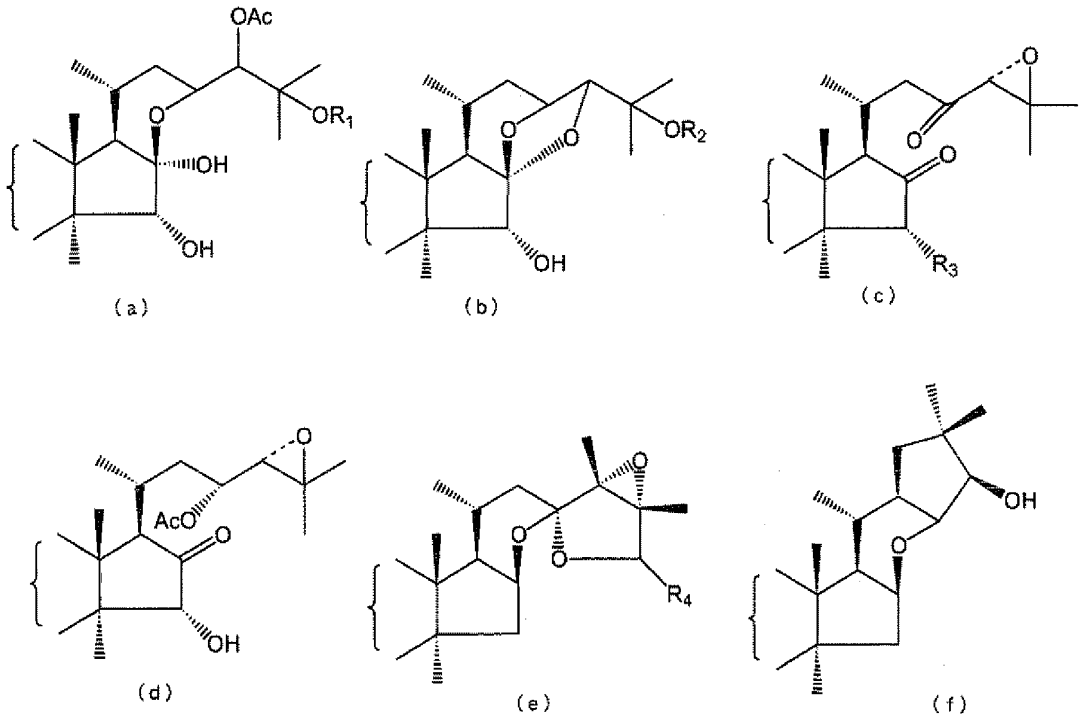
12. The external preparation for skin according to claim 9, wherein the cycloartane-type glycoside is 7,8-didehydro-24-O-acetylhydroshengmanol-3-O-β-xyloside, 24-epi-7,8-didehydrocimigenol-3-xyloside, or cimicifugoside H-1.

13. A method of promoting VEGF production, comprising administering or taking a cycloartane-type glycoside represented by the following general formula (1):



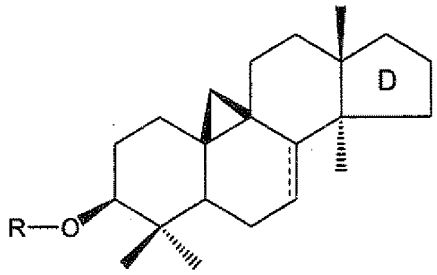
(1)

where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



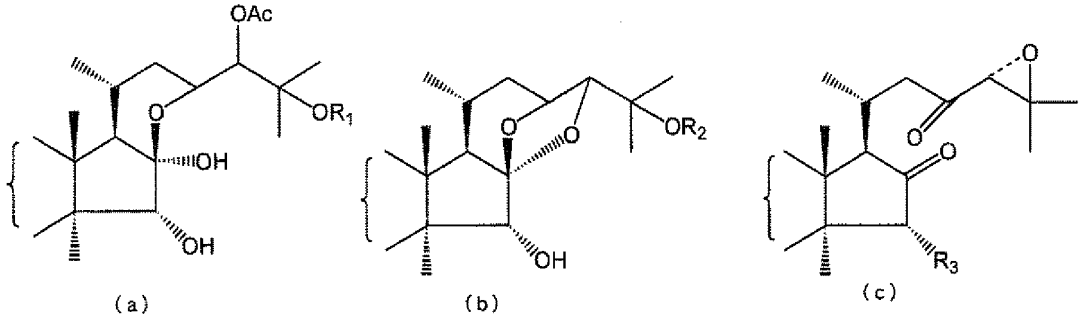
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

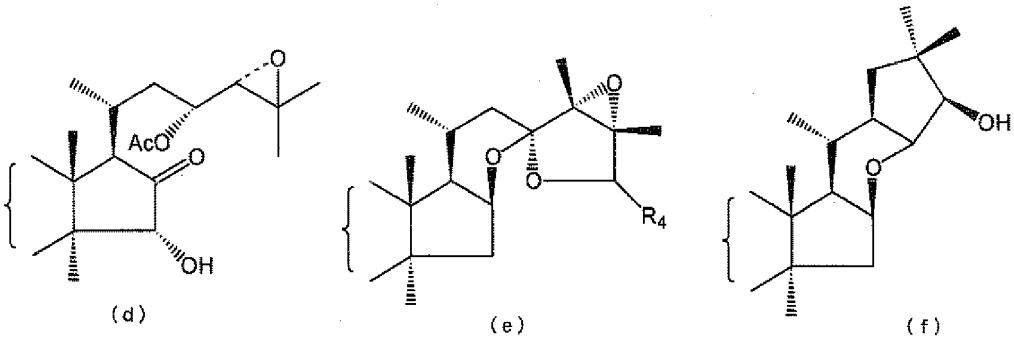
14. A method of improving hair quality, comprising, administering or taking a cycloartane-type glycoside represented by the following general formula (1):



(1)

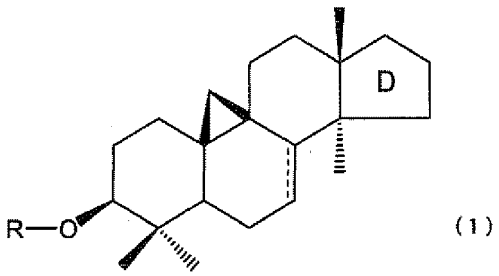
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:





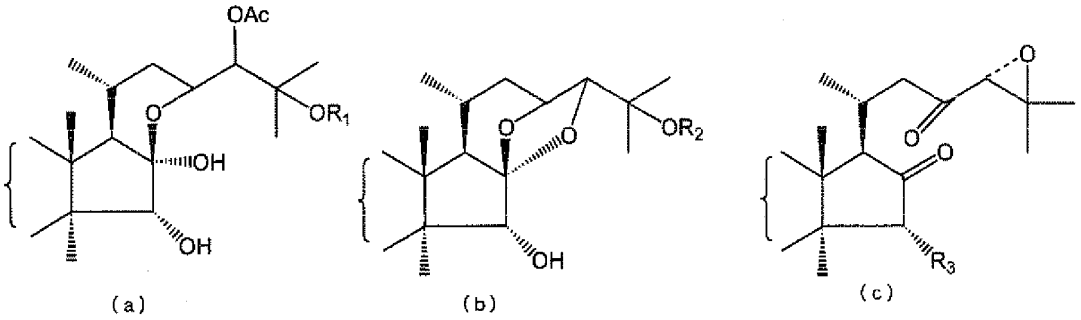
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

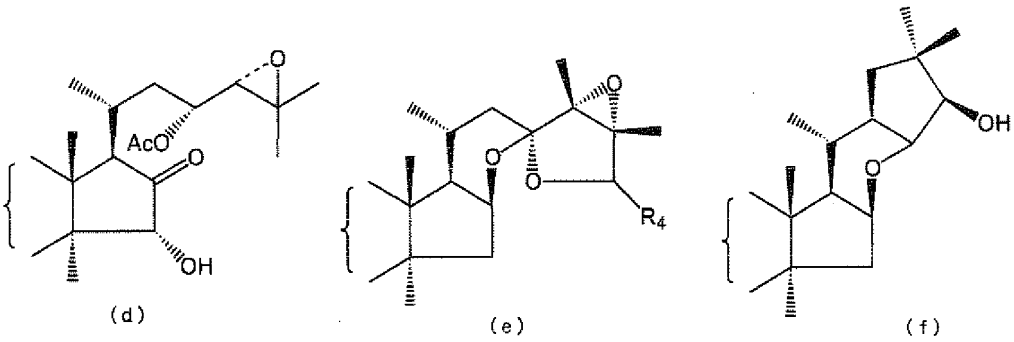
15. Use of a cycloartane-type glycoside represented by the following general formula (1) for manufacturing a VEGF production promoter:



(1)

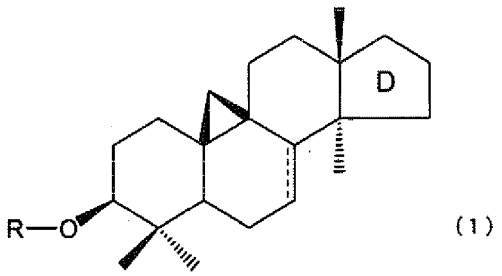
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:





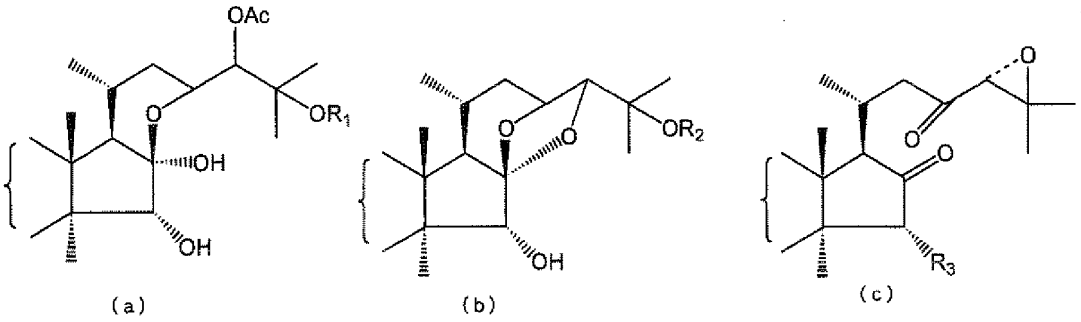
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

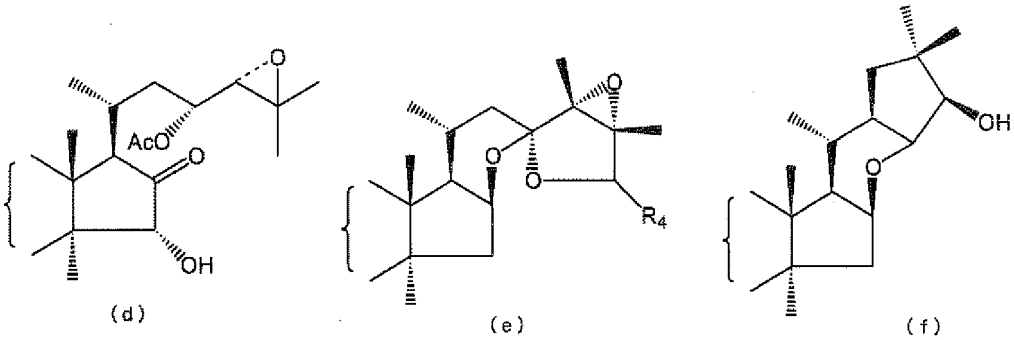
16. Use of a cycloartane-type glycoside represented by the following general formula (1) for manufacturing a hair quality improver:



(1)

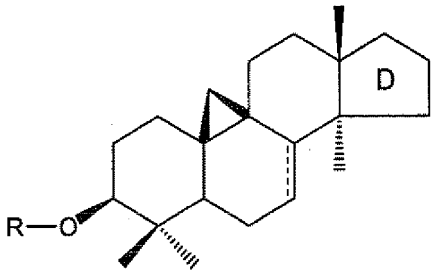
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:





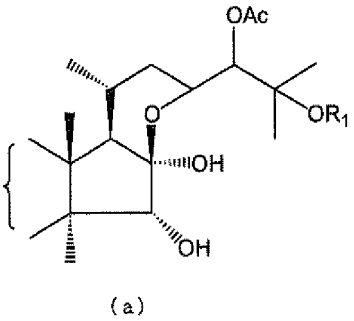
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

17. A cycloartane-type glycoside represented by the following general formula (1) for use in promoting VEGF production:

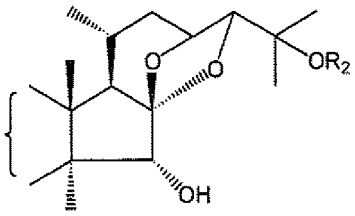


(1)

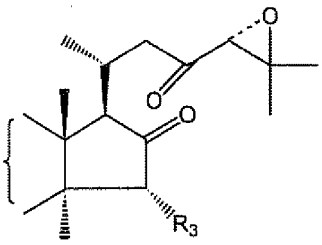
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



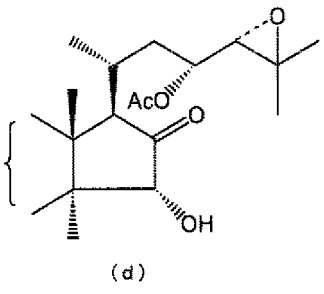
(a)



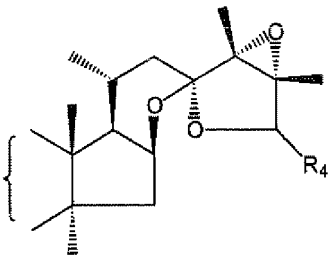
(b)



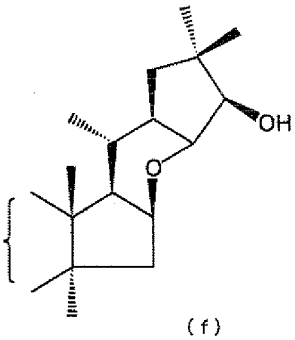
(c)



(d)



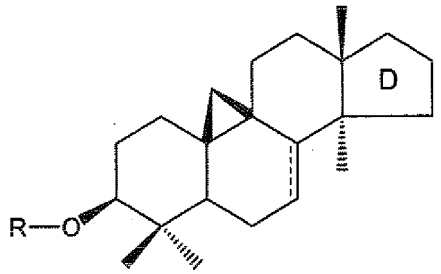
(e)



(f)

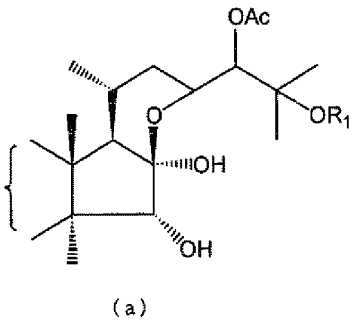
where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

18. A cycloartane-type glycoside represented by the following general formula (1) for use in improving hair quality:

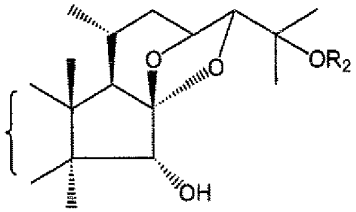


(1)

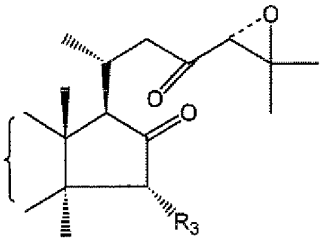
where R represents a xylose residue or an arabinose residue, and the ring D moiety represents any one of the following moieties a to f:



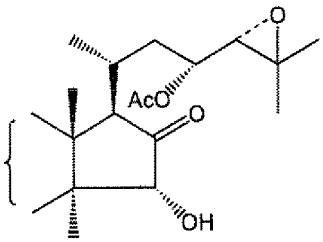
(a)



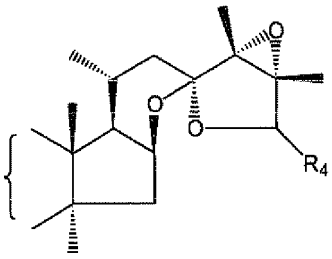
(b)



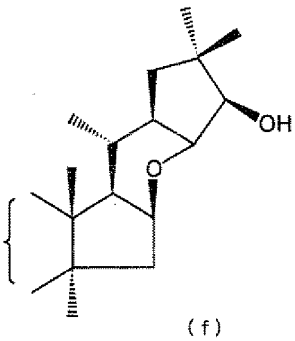
(c)



(d)



(e)



(f)

where R_1 represents a hydrogen atom or a methyl group, R_2 represents a hydrogen atom or an acetyl group, R_3 represents a hydrogen atom or a hydroxyl group, R_4 represents a hydrogen atom or a hydroxyl group, and Ac represents an acetyl group.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/065189

A. CLASSIFICATION OF SUBJECT MATTER
C07J71/00(2006.01)i, *A61K8/63*(2006.01)i, *A61K8/97*(2006.01)i, *A61K31/7048*(2006.01)i, *A61P17/00*(2006.01)i, *A61P43/00*(2006.01)i, *A61Q5/00*(2006.01)i, *A61Q19/00*(2006.01)i
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)
C07J71/00, A61K8/63, A61K8/97, A61K31/7048, A61P17/00, A61P43/00, A61Q5/00, A61Q19/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2011
Kokai Jitsuyo Shinan Koho 1971-2011 Toroku Jitsuyo Shinan Koho 1994-2011

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAplus(STN), REGISTRY(STN), JSTPlus(JDreamII), JMEDPlus(JDreamII), JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP 9-30977 A (Kaken Pharmaceutical Co., Ltd.), 04 February 1997 (04.02.1997), compounds 7a, 16, 19 (Family: none)	17,18 1-12,15,16
X Y	KUSANO, A., Studies on the Constituents of Cimicifuga Species. XXVI. Twelve New Cyclolanostanol Glycosides from the Underground Parts of Cimicifuga simplex WORMSK., Chemical & Pharmaceutical Bulletin, 1999, 47(4), p. 511-516, fig. 2, compounds 1 to 8, 13	17,18 1-12,15,16

☒ Further documents are listed in the continuation of Box C. ☐ See patent family 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 application or patent 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	Date of mailing of the international search report
22 July, 2011 (22.07.11)	02 August, 2011 (02.08.11)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/065189

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	HE, K., Cimicifuga species identification by high performance liquid chromatography-photodiode array/mass spectrometric/evaporative light scattering detection for quality control of black cohosh products, Journal of Chromatography A, 2006, 1112(1-2), p. 241-254, fig. 1, compounds 3 to 7, 9, 23 to 26	17,18 1-12,15,16
X Y	LI, W., High-performance liquid chromatographic analysis of Black Cohosh (Cimicifuga racemosa) constituents with in-line evaporative light scattering and photodiode array detection, Analytica Chimica Acta, 2002, 471(1), p. 61-75, fig. 1, compounds 8, 13, 15 to 18; table 4	17,18 1-12,15,16
Y A	GAUBE, F., Gene expression profiling reveals effects of Cimicifuga racemosa (L.) NUTT. (black cohosh) on the estrogen receptor positive human breast cancer cell line MCF-7, BMC Pharmacology, 2007, 7, 11 (p. 1-19) table 2, fig. 6	1-12,15,16 17,18
Y A	JP 2006-160698 A (Noevir Co., Ltd.), 22 June 2006 (22.06.2006), entire text (Family: none)	1-12,15,16 17,18
Y A	JP 2008-222671 A (Seiren Co., Ltd.), 25 September 2008 (25.09.2008), entire text (Family: none)	1-12,15,16 17,18
Y A	Maruzen Pharmaceuticals Co., Ltd., "Ikumo Sayo Shokubutsu Extract 'Biwaha Extract CA'", Fragrance Journal, 2007, 35(1), pages 124 to 125	1-12,15,16 17,18
Y A	Shigeru MORIWAKI, The present status and prospective problem of hair grower, "A search of molecules relating to hair aging and an effect of eucalyptus extract on VEGF transcription", Fragrance Journal, 2007, 35(12), pages 22 to 27	1-12,15,16 17,18
Y A	JP 2006-342068 A (Ogawa & Co., Ltd.), 21 December 2006 (21.12.2006), claims 1 to 10; paragraphs [0009], [0088] to [0091]; examples 1 to 11, 16 (Family: none)	1-12,15,16 17,18
Y A	JP 2010-143862 A (Sunstar Inc.), 01 July 2010 (01.07.2010), claims 1 to 5; paragraph [0012]; test example 1; examples 6 to 9 (Family: none)	1-12,15,16 17,18

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/065189

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13,14
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 13 and 14 involve methods for treatment of the human body or animal body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- JP 11286432 A **[0009]**
- JP 2000212059 A **[0009]**
- JP 2004043393 A **[0009]**
- JP 2004035443 A **[0009]**

Non-patent literature cited in the description

- **DETMAR M.** The role of VEGF and thrombospondins in skin angiogenesis. *J Dermatol Sci.,* 2000, vol. 24 (1), 78-84 **[0010]**
- **YANO K ; BROWN LF ; DETMAR M.** Control of hair growth and hair follicle size by VEGF-mediated angiogenesis. *J Clin Invest.,* 2001, vol. 107 (4), 409-17 **[0010]**
- **MATSUHASHI, KUSANO.** *Proceedings of the 121st Annual Meeting of the Pharmaceutical Society of Japan,* vol. 4, 54 **[0010]**
- **TAKAHIRA M.** *Bio. Pharm. Bull.,* 1998, vol. 21, 823-828 **[0010]**
- **MORIWAKI, TAGUCHI.** *Fragrance Journal,* 2007 **[0010]**
- **HEIDEMARIE ROSSITER ; CATERINA BARRESI ; JOHANNES PAMMER ; MICHAEL RENDL ; JODY HAIGH ; ERWIN F. WAGNER ; ERWIN TSCHACHLER.** Loss of Vascular Endothelial Growth Factor A Activity in Murine Epidermal Keratinocytes Delays Wound Healing and Inhibits Tumor Formation. *Cancer research,* 2004, vol. 64, 3508-3516 **[0021]**