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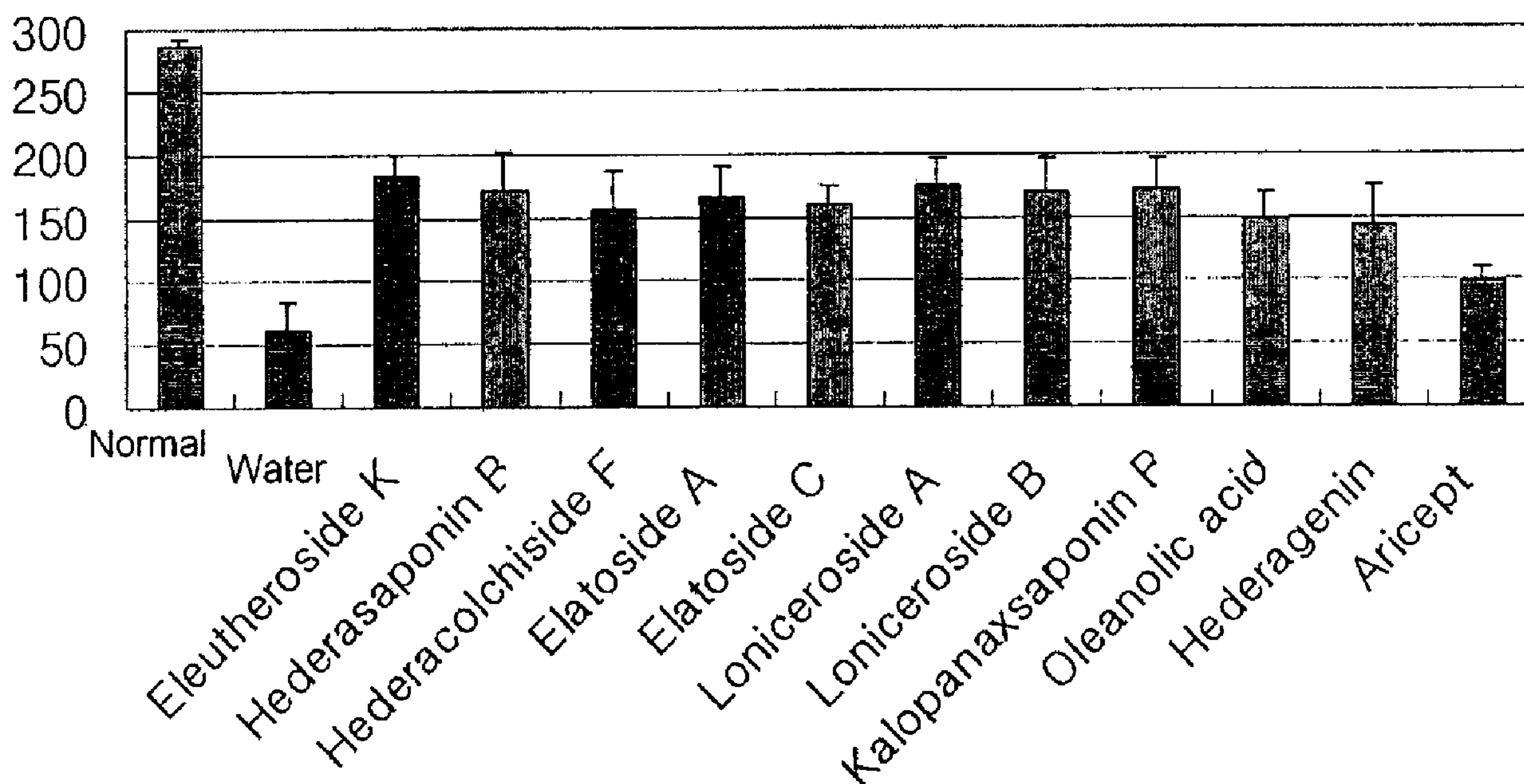
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(54) Titre : COMPOSES DE SAPONINE DE TRITERPENE DE TYPE OLEANANE PERMETTANT DE TRAITER EFFICACEMENT LA DEMENCE ET DE FAIBLES TROUBLES COGNITIFS ET D'AMELIORER LA FONCTION COGNITIVE

(54) Title: OLEANANE TRITERPENE SAPONIN COMPOUNDS WHICH ARE EFFECTIVE ON TREATMENT OF DEMENTIA AND MILD COGNITIVE IMPAIRMENT (MCI), AND IMPROVEMENT OF COGNITIVE FUNCTION



(57) Abrégé/Abstract:

The present invention relates to the use of oleanane-type triterpene saponin compounds, which are effective for improving memory and learning ability, as an effective ingredient of drugs for the treatment and prevention of dementia and mild cognitive impairment and health foods for the improvement of brain functions including cognitive function.

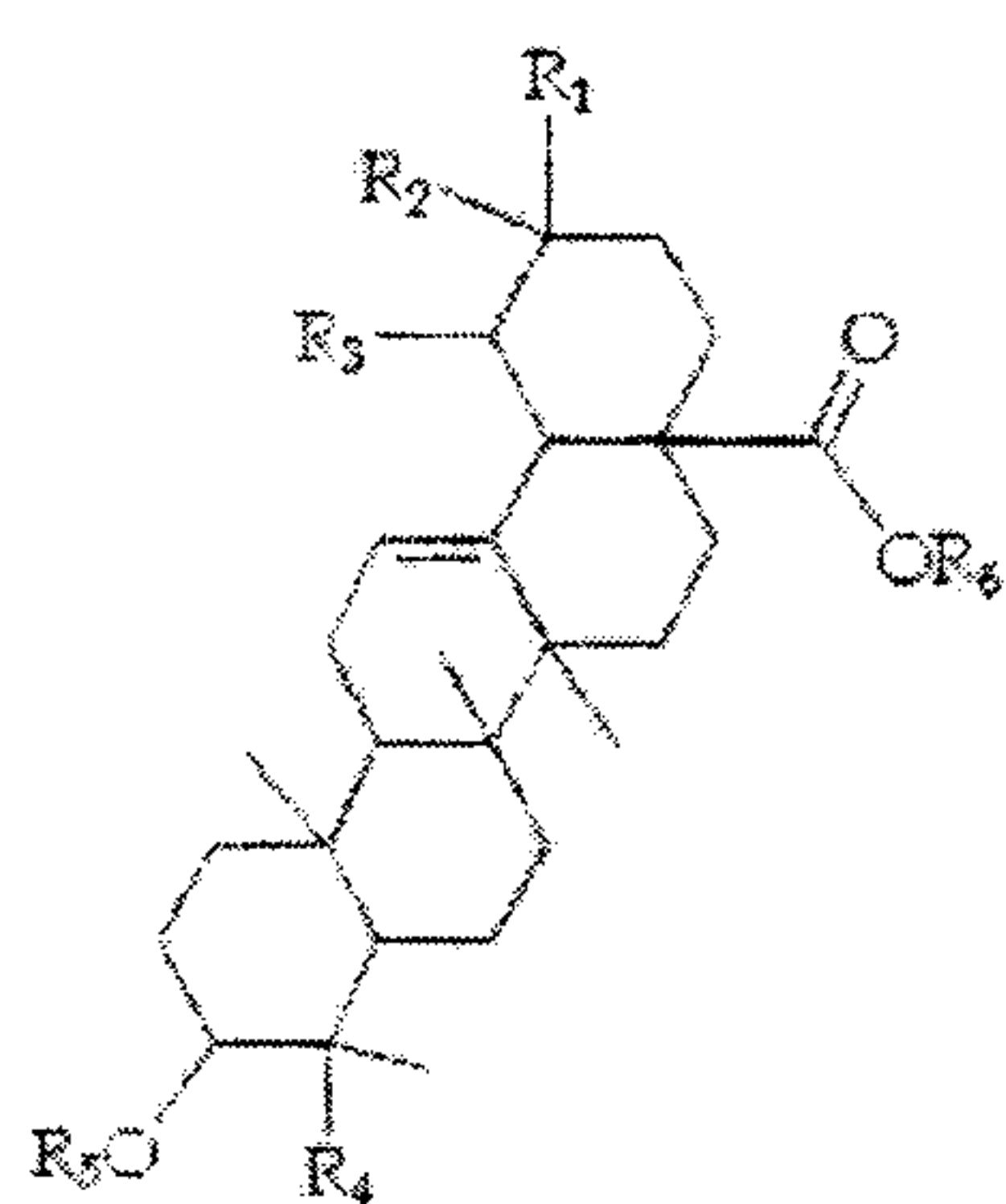
【ABSTRACT】

The present invention relates to the use of oleanane-type triterpene saponin compounds, which are effective for improving memory and learning ability, as an effective ingredient of drugs for the treatment and prevention of dementia and mild cognitive impairment and health foods for the improvement of brain functions including cognitive function.

1 **OLEANANE TRITERPENE SAPONIN COMPOUNDS WHICH ARE EFFECTIVE**
 2 **ON TREATMENT OF DEMENTIA AND MILD COGNITIVE IMPAIRMENT (MCI),**
 3 **AND IMPROVEMENT OF COGNITIVE FUNCTION**

4
 5 **【Technical Field】**

6 The present invention relates to a use of an oleanane-type triterpene saponin
 7 compound represented by the formula (1) below, which is effective for improving
 8 memory and learning abilities, as an effective ingredient of health food for the
 9 treatment and prevention of dementia and mild cognitive impairment and the
 10 improvement of brain functions including a cognitive function:



(1)

16 wherein each of R₁, R₂ and R₃ is hydrogen or C₁-C₄ alkyl; R₄ is C₁-C₄ alkyl or C₁-C₄
 17 hydroxyalkyl; and each of R₅ and R₆ is hydrogen or sugar, wherein at least one of R₅ and
 18 R₆ is sugar which is selected from glucose, galactose, rhamnose, xylose, arabinose and
 19 glucuronic acid.

1 **【Background Art】**

2 Triterpene saponin refers to a sapogenin (aglycone) compound having a triterpene
3 group and forming a glycoside bond or ester bond with a sugar or sugar chains.

4 Triterpene saponins are classified based on the type of sapogenins. Typical
5 examples of triterpene saponins are saponins having pentacyclic triterpene groups, for
6 example, oleanane, ursane, lupane, hopane, taraxerane, and the like. Also,

7 saponins are classified depending on their binding sites with sugar chains. To take
8 oleanolic acid for example, one bonded with a sugar chain at either the C-3 or C-28

9 position is called monodesmoside while one bonded with sugar chains at both C-3 and
10 C-28 positions is called bisdesmoside. Glycoside bonding predominates at the C-3

11 position and ester bonding predominates at the C-28 position. The sugars that
12 typically bind with saponin are glucose, galactose, rhamnose, xylose, arabinose,

13 glucuronic acid, and the like.

14 The absorption rate of saponins by intestines is generally low when they are
15 present in the form of bisdesmoside. However, the ester bond present at C-28

16 position of saponins is easily hydrolyzed by the enzymes of intestinal bacteria. As

17 the sugar chain is removed, the sugar chain terminal connected to the C-3 position by
18 glucoside bond is partially hydrolyzed beginning from the terminal and then absorbed

19 by the body [Kim DH, Bae EA, Han MJ, Park HJ, Choi JW. Metabolism of

1 Kalopanaxsaponin K by human intestinal bacteria and antirheumatoid arthritis activity
2 of their metabolites. *Biol Pharm Bull.* 2002 Jan; **25(1)**: 68-71.]. In contrast, the
3 triterpene sapogenin itself, not being bound to sugar, has low solubility and shows
4 reduced activity in animal tests because of its relatively low intestinal absorption,
5 compared with that of the glucoside counterpart [Yoshikawa M, Matsuda H.
6 Antidiabetogenic activity of oleanolic acid glycosides from medicinal foodstuffs.
7 *BioFactors.* 2000; **13(1-4)**: 231-7].

8 As the population of aged people increases throughout the world various kinds of
9 degenerative geriatric diseases have been raised as social as well as economic issues.
10 According to the statistics released by the Alzheimer's Association and the National
11 Institute on Aging, about 4 million Americans are suffering from dementia.
12 Although it is common that dementia develops at the age of 60 or later, in rare cases, it
13 begins even in the 50s. Of all Americans of 65 years or older, 10.3 % are suffering
14 from dementia and as much as 95 billion dollars is spent each year to treat dementia.

15 According to a report from the Korea Institute for Health and Social Affairs, the
16 population of dementia patients has been increasing rapidly along with the increase in
17 the number of aged people. The population of dementia patients aged 65 or older
18 is expected to increase to 9 % in 2020, 0.7 % up from 8.3 % in 1995. And, according
19 to the estimate by the Korea National Statistical Office, the population of the aged

1 people suffering from dementia is expected to increase from 277,048 (8.3 % of the
2 people aged 65 years or older) in 2000 to 527,068 (9 %) in 2015 and 619,132 (9 %) in 2020.
3 Since dementia is an intractable illness that devastates the patient's life and destroys the
4 lives of the patient's family, it is becoming a serious social, economical problem and has
5 to be overcome.

6 Dementia may develop due to a variety of causes, and its main symptom is
7 characterized by having markedly reduced learning ability and memory.

8 Tacrine, the first treatment for dementia approved by the FDA in 1993, can
9 postpone the loss of cognitive function in about 30 % of patients with Alzheimer's
10 disease at its early or middle stage by inhibiting the breakdown of acetylcholine (ACh)
11 in the brain. However, it is hardly used these days because it generates a lot of
12 adverse reactions against the liver. Aricept, which was approved by the FDA in
13 1996, is used to increase the level of acetylcholine. This drug can be taken once a
14 day before sleeping. Its common side effects include nausea, diarrhea and
15 tiredness, which do not last long. Accordingly, development of a new treatment
16 for dementia with good efficacy and few side effects is required.

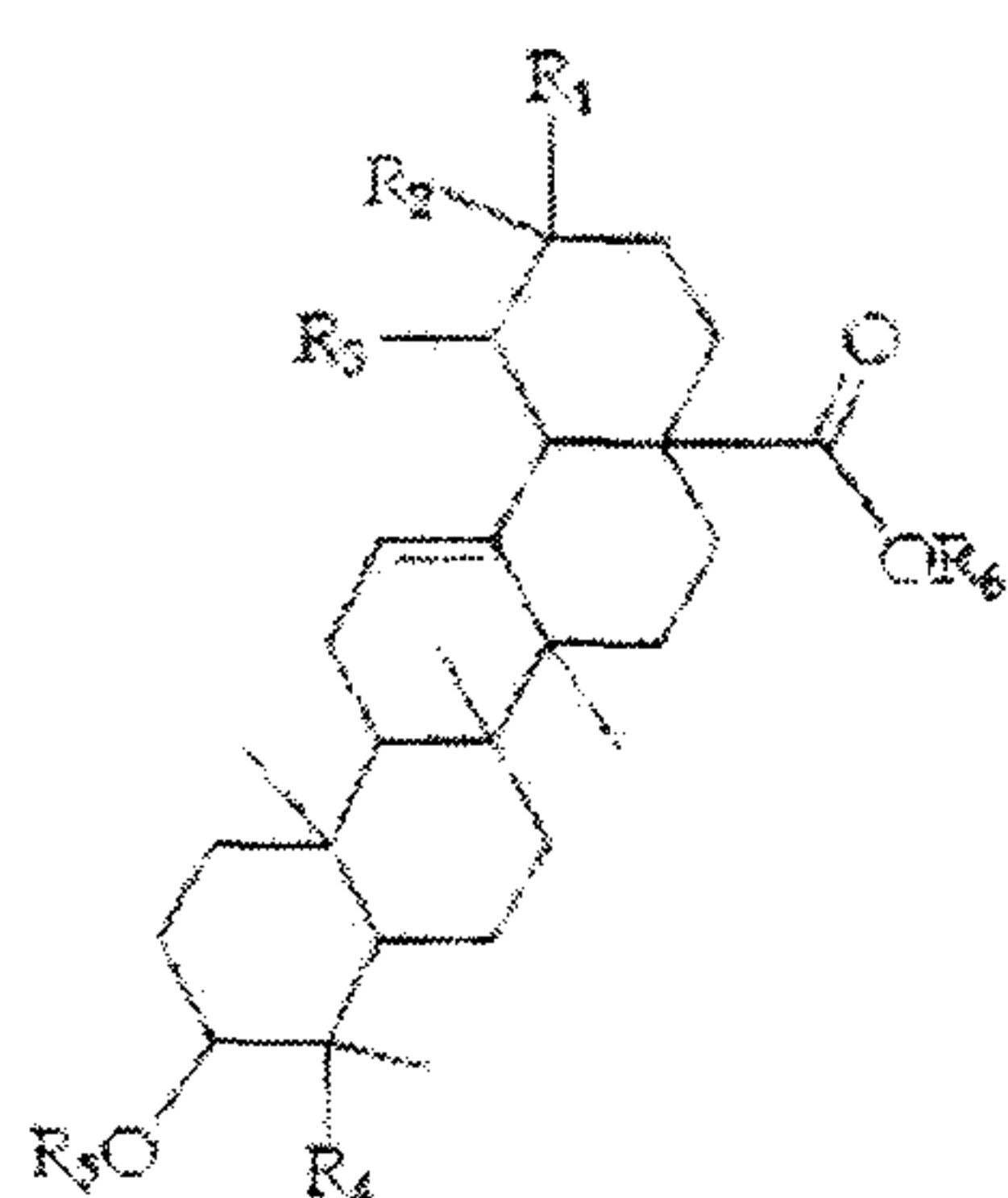
17 The present inventors have performed intensive researches to develop a substance
18 effective for improving memory and learning abilities with little toxicity. As a
19 result, they discovered that an oleanane-type triterpene saponin compound is effective

for treating dementia and mild cognitive impairment and improving cognitive function.

Accordingly, an object of the present invention is to provide a therapeutic pharmaceutical drug and a health food for the improvement of brain functions comprising an oleanane-type triterpene saponin compound as an effective ingredient.

【Disclosure】

The present invention relates to a therapeutic pharmaceutical drug for treating dementia and mild cognitive impairment or a health food for the improvement of brain functions comprising the oleanane-type triterpene saponin compound represented by the following formula (1):



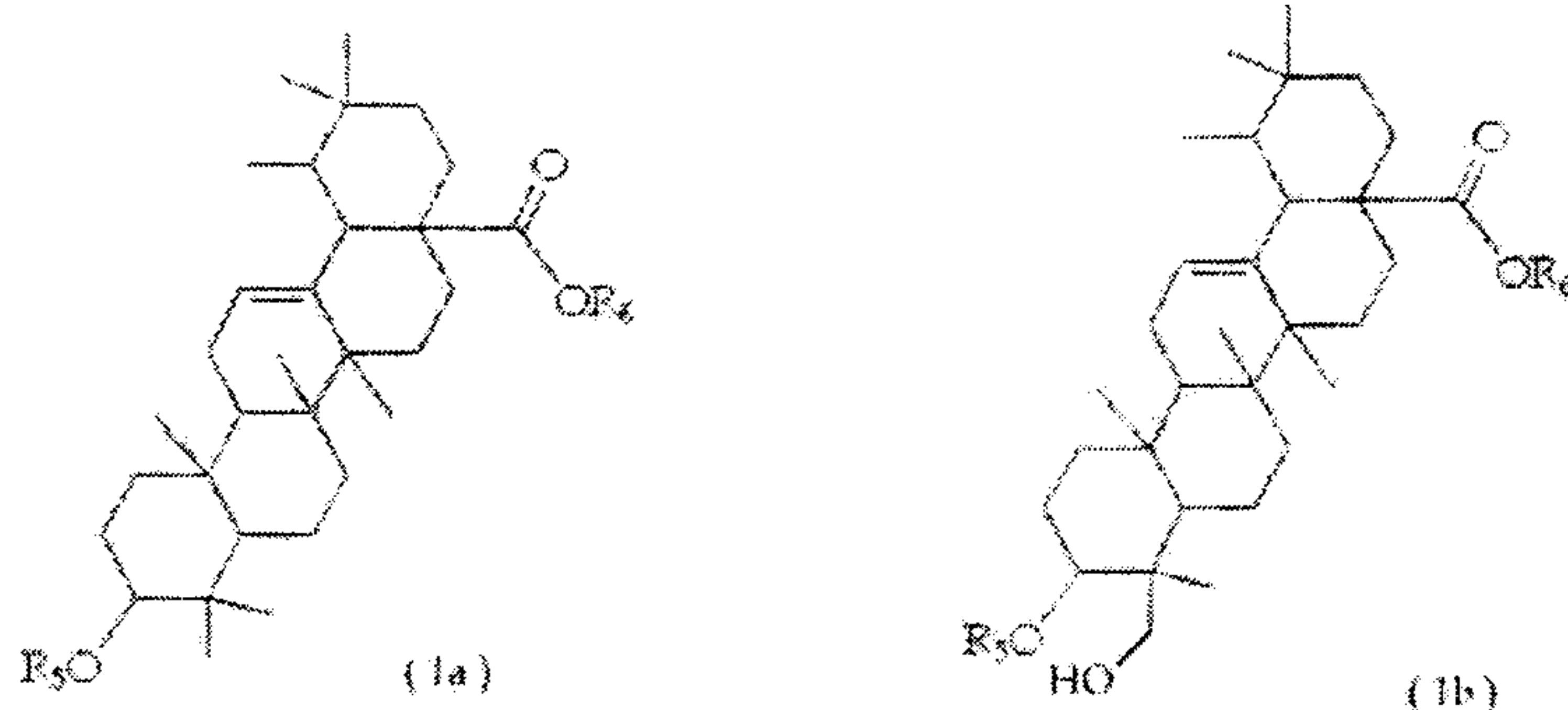
(1)

wherein each of R₁, R₂ and R₃ is hydrogen or C₁-C₄ alkyl; R₄ is C₁-C₄ alkyl or C₁-C₄ hydroxyalkyl; and each of R₅ and R₆ is hydrogen or sugar, wherein at least one of R₅ and R₆ is sugar which is selected from glucose, galactose, rhamnose, xylose, arabinose and glucuronic acid. In an embodiment of the present invention, R₅ is arabinose and C₄

of the pyranose ring is not substituted.

Hereunder is given a more detailed description of the present invention.

Of the oleanane-type triterpene saponin compounds represented by the formula (1), oleanolic acid saponin represented by the formula (1a) below and hederagenin saponin represented by the formula (1b) below are well known in the related art:



The oleanolic acid represented by the formula (1a) is a saponin compound having oleanolic acid as sapogenin and is known to have anticancer as well as anti-inflammatory effects [Li J, Guo WJ, Yang QY. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. *World J Gastroenterol.* 2002 Jun; 8(3): 493-5], cerebral nerve protecting effect [Qian YH, Liu Y, Hu HT, Ren HM, Chen XL, Xu JH. The effects of the total saponin of *Dipsacus asperoides* on the damage of cultured neurons induced by β -amyloid protein 25-35], antiviral effect [Kapil A, Sharma S. Effect of oleanolic acid on complement in adjuvant- and carrageenan-induced inflammation in rats. *J. Pharm Pharmacol.* 1995 Jul; 47(7): 585-7], antihyperlipidemic effect [Lee KT, Sohn IC, Kim DH, Choi JW, Kwon SH, Park HJ. Hypoglycemic and hypolipidemic effects of

1 tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their
2 antioxidant activity *in vitro*. *Arch Pharm Res*. 2000 Oct; **23**(5): 461-6], antiallergenic effect
3 [Park KH, Park J, Koh D, Lim Y. Effect of saikosaponin-A, a triterpenoid glycoside,
4 isolated from *Bupleurum falcatum* on experimental allergic asthma. *Phytother Res*. 2002
5 Jun; **16**(4): 359-63], immune control effect [Ju DW, Zheng QY, Cao X, Fang J, Wang HB.
6 Esculentoside A inhibits tumor necrosis factor, interleukin-1, and interleukin-6
7 production induced by lipopolysaccharide in mice. *Pharmacology*. 1998 Apr; **56**(4): 187-
8 95] and anti-angiogenic effect [Korean Patent No. 101,480].

9 And, the hederagenin saponin represented by the formula (1b) is a compound
10 having hederagenin as sapogenin and is known to have anti-inflammatory effect [Kwak
11 WJ, Han CK, Chang HW, Kim HP, Kang SS, Son KH. Loniceroside C, an
12 antiinflammatory saponin from *Lonicera japonica*. *Chem Pharm Bull (Tokyo)*. 2003 Mar;
13 **51**(3): 333-5], pain-alleviating effect [Choi J, Huh K, Kim SH, Lee KT, Park HJ, Han YN.
14 Antinociceptive and anti-rheumatoidal effects of *Kalopanax pictus* extract and its saponin
15 components in experimental animals. *J Ethnopharmacol*. 2002 Feb; **79**(2): 199-204],
16 antioxidation effect [Choi J, Huh K, Kim SH, Lee KT, Lee HK, Park HJ.
17 Kalopanaxsaponin A from *Kalopanax pictus*, a potent antioxidant in the rheumatoidal rat
18 treated with Freund's complete adjuvant reagent. *J Ethnopharmacol*. 2002 Jan; **79**(1): 113-
19 8] and blood sugar lowering effect [Kim DH, Yu KW, Bae EA, Park HJ, Choi JW.

1 Metabolism of Kalopanaxsaponin B and H by human intestinal bacteria and
2 antidiabetic activity of their metabolites. *Biol Pharm Bull.* 1998 Apr; **21(4)**: 360-5].

3 But, until now, nothing is known about the effect of the oleanane-type triterpene
4 saponin compound represented by the formula (1), which comprises oleanolic acid
5 saponin or hederagenin saponin, in treating dementia and mild cognitive impairment
6 and improvement of brain functions.

7 Japanese Patent Laid-Open No. 2000-247993 recognizes the high affinity of
8 oleanolic acid to sigma receptors and discloses that it is effective in treating various
9 brain diseases related with the sigma receptors, including schizophrenia, depression,
10 anxiety, cerebrovascular diseases, behavior disorder in the aged people, Alzheimer's
11 disease, Parkinson's disease, Huntington's disease, drug addiction, stress, etc.
12 However, this patent presents no *in vivo* animal test or *in vitro* nerve cell test results
13 directly related with the treatment of the diseases.

14 The oleanane-type triterpene saponin compounds having oleanolic acid or
15 hederagenin as sapogenin may be in the form of a monodesmoside bound to sugar at C-
16 3 or C-28 position or in the form of a bisdesmoside bound to sugars at both C-3 and C-28
17 positions. There exist more than 150 saponin compounds from the various
18 combinations of the sugar chain [Kang Sam Sik, Triterpenoid saponin, Seoul National
19 University Press, 1996, TradiMed Database].

Oleanolic acid saponins:

Achyranthoside C, acutosides A-G, akeboside Stj, anemoside A, araliasaponins XII-XVIII, araloside D, arvensosides A-B, betavulgarosides IV-V, *Bupleurum chinense* triterpene glycoside S1, caraganoside A, chikusetsusaponins IB, IV, ciwujianosides A1, C4, C3, D1, clematichinenosides A, C, *Clematis chinensis* prosapogenins CP9, CP9a, CP7a, CP2b, clemontanosides E, F, saffron (*Crocus sativus*) oleanolic acid saponin, elatosides B, D, eleutheroside K, fatsiaside A1, hederacolchiside E, hederasaponin B, huzhangosides A, B, C, lablaboside A, lucyoside H, 6'-methylmomordin I, momordins I, IC, IIB, 3-O- α -L-arabinopyranosyloleanolic acid, 3-O- α -L-arabinopyranosyloleanolic acid-28-O- β -D-glucosyl(1 $\rightarrow$ 6) β -D-glucoside, 3-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid 28-O- β -D-xylopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester, 3-O- β -D-galactopyranosyl(1 $\rightarrow$ 2)- β -D-glucolopyranosyloleanolic acid, 3-O- β -D-glucopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid, 3-O- β -D-glucopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid-28-gentiobiocide, 28-O- β -D-glucopyranosyloleanolic acid, 3-O- β -D-glucopyranosyloleanolic acid, 3-O- β -D-glucolopyranosyloleanolic acid, 3-O- β -D-ribopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid, 3-O- β -D-xylopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid, 3-O- β -D-xylosyl(1 $\rightarrow$ 4)- β -D-glucosyl(1 $\rightarrow$ 4)- α -L-

1 rhamnosyl(1→3)-β-D-glucosyl(1→3)-α-L-rhamnosyl(1→2)-α-arabinosyloleanolic acid
 2 28-O-β-D-glucosyl(1→6)-β-D-glucosyl ester, 2'-O-glucopyranosylmomordin Ic, 2'-O-
 3 glucopyranosylmomordin Ic, 3-O-β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl(1→3)-
 4 α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosideoleanolic acid 28-O-β-D-
 5 glucopyranosyl(1→6)-β-D-glucopyranosyl ester, oleanolic acid 3-O-neohesperidoside,
 6 oleanolic acid 3-O-α-L-arabinopyranosyl-28-O-β-D-glucopyranosyl(1→6)-β-D-
 7 glucopyranosyl ester, oleanolic acid-3-O-β-D-glucopyranosyl(1→2)-α-L-
 8 arabinopyranoside, pericarpsaponin J3, prosapogenin CP4, quinatose D, raddeanins
 9 A, C, D, E, F, raddeanosides R10, R11, rivularinin, spinacosides C, D, spinasaponin A,
 10 tarasaponins II, III, VI, *Tetrapanax papyriferum* saponins R-3, R-1-a, R-4b, udosaponin
 11 methyl esters A-C.

12 Hederagenin saponin:

13 Akebia saponins A-G, akeboside, asperosaponins F, H1, calcoside D, caulosides D,
 14 F, *Clematis chinensis* prosapogenins CP10, CP10a, Cp8a, CP3b, CP0, CP3a, CP2a, 3-O-β-D-
 15 D-xylopyranosyl(1→3)-α-L-arabinopyranosylhederagenin-28-O-α-L-
 16 rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside,
 17 hederagenin-3-O-α-L-arabinoside, hederagenin-3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-
 18 arabinopyranosyl]-28-O-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl ester,
 19 hederagenin-3-O-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-O-[3-O-acetyl-

- 1 β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside, hederagenin-3-O-[α -L-
- 2 rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyl]-28-O-[3-O-acetyl- β -D-
- 3 xylopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside, hederagenin-3-O- α -L-
- 4 rhamnopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-
- 5 arabinopyranosyl 28-O- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester,
- 6 hederagenin-3-O- β -D-glucopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-
- 7 arabinopyranosyl 28-O- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester,
- 8 hederagenin-3-O-(4-O-acetyl)- α -L-arabinopyranosyl 28-O- β -D-glucopyranosyl(1 $\rightarrow$ 6)-
- 9 β -D-glucopyranosyl ester, kalopanaxsaponins B, G, JLa, JLb, leontosides A, B,
- 10 lucyosides A, E, mukurozisaponins EI, G, X, Y2, YI, 4'-O-acetylakebiasaponin D, 3-O-[α -
- 11 L-arabinosyl]hederagenin-28-O-[β -D-glucosyl]ester, 3-O- α -L-
- 12 rhamnopyranosyl(1 $\rightarrow$ 3)- β -D-glucosyl(1 $\rightarrow$ 3)- α -D-rhamnosyl(1 $\rightarrow$ 2)- α -
- 13 arabinosylhederagenin-28-O- β -D-glucosyl(1 $\rightarrow$ 6)- β -D-glucosyl ester, 3-O- β -D-
- 14 glucopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosylhederagenin,
- 15 3-O- β -D-glucopyranosylhederagenin, 3-O-(β -D-glucosyl(1 $\rightarrow$ 4)- α -L-rhamnosyl(1 $\rightarrow$ 3)- β -
- 16 D-glucosyl(1 $\rightarrow$ 3)- α -D-rhamnosyl(1 $\rightarrow$ 2)- α -arabinosylhederagenin-28-O- β -D-
- 17 glucosyl(1 $\rightarrow$ 6)- β -D-glucosyl ester, 3-O-(β -D-glucosyl(1 $\rightarrow$ 4)- α -L-rhamnosyl(1 $\rightarrow$ 3)- β -D-
- 18 glucosyl(1 $\rightarrow$ 3)- α -L-rhamnosyl(1 $\rightarrow$ 2)- α -arabinosylhederagenin, 3-O-(β -D-
- 19 xylosyl(1 $\rightarrow$ 4)- β -D-glucosyl(1 $\rightarrow$ 4)- α -L-rhamnosyl(1 $\rightarrow$ 3)- β -D-glucosyl(1 $\rightarrow$ 3)- α -L-

- 1 arabinosyl(1→2)- α -arabinosylhederagenin, 3-O-[(2'-O-acetyl)- α -L-
- 2 arabinopyranosyl(1→6)- β -D-glucopyranosyl]hederagenin, 3-O-(2'-O-acetyl)- α -L-
- 3 arabinopyranosylhederagenin-28-O-[β -D-glucopyranosyl(1→6)- β -D-glucopyranoside],
- 4 percarpsaponins C, J2, G, K, pulsatilosides A, B, C, quinquatosides A, B, C, sapindosides A,
- 5 B, C, staunosides A, B, D, E, tauroside G3, udosaponin methyl esters D, E, F.

6 The oleanane-type triterpene saponin compounds comprising oleanolic acid
 7 saponin and hederagenin saponin are extracted from the following plants: plants in the
 8 genus *Amaranthus*, e.g., *Achyranthes* sp., *Amaranthus* sp., etc.; plants in the *Araliaceae*
 9 family, e.g., *Acanthopanax* sp., *Aralia* sp., *Fatsia* sp., *Kalopanax* sp., *Panax* sp., *Tetrapanax*
 10 sp., etc.; plants in the *Basellaceae* family, e.g., *Boussingaultia* sp., etc.; plants in the
 11 *Berberidaceae* family, e.g., *Caulophyllum* sp., etc.; plants in the *Boraginaceae* family, e.g.,
 12 *Anchusa* sp., etc.; plants in the *Caprifoliaceae* family, e.g., *Lonicera* sp., etc.; plants in the
 13 *Chenopodiaceae* family, e.g., *Chenopodium* sp., etc.; plants in the *Cucurbitaceae* family, e.g.,
 14 *Actinostemma* sp., *Luffa* sp., *Momordica* sp., etc.; plants in the *Dipsacaceae* family, e.g.,
 15 *Dipsacus* sp., etc.; plants in the *Euphorbiaceae* family, e.g., *Putranjiva* sp., etc.; plants in the
 16 *Hippocastanaceae* family, e.g., *Aesculus* sp., etc.; plants in the *Lardizabalaceae* family, e.g.,
 17 *Akebia* sp., etc.; plants in the *Leguminosae* family, e.g., *Acacia* sp., *Albizia* sp., *Swartzia* sp.,
 18 etc.; plants in the *Opiliaceae* family, e.g., *Opilia* sp., etc.; plants in the *Phytolaccaceae* family,
 19 e.g., *Phytolacca* sp., etc.; plants in the *Ranunculaceae* family, e.g., *Anemone* sp., *Clematis* sp.,

1 *Hedera* sp., *Pulsatilla* sp., etc.; plants in the *Rubiaceae* family, e.g., *Randia* sp., *Xeromphis*
2 sp., etc.; plants in the *Sapindaceae* family, e.g., *Pometia* sp., *Spindus* sp., *Thinouia* sp., etc.;
3 plants in the *Valerianaceae* family, e.g., *Patrinia* sp.

4 The present inventors performed animal tests and the result reveals that, as
5 compared to the control group where no drug was administered, the group where
6 scopolamine (1 mg/kg), which is known to decline the memory function by inhibiting
7 the transfer of neurotransmitters, was administered and the group where the oleanane-
8 type triterpene saponin compound represented by the formula (1) was administered 1
9 hour after the administration of scopolamine, the effect of a significant memory
10 improvement was observed.

11 Accordingly, the oleanane-type triterpene saponin compound represented by the
12 formula (1) can be used as an effective ingredient of a pharmaceutical drug for treating
13 dementia and mild cognitive impairment or as a health food for improving cognitive
14 function.

15 The drug comprising the oleanane-type triterpene saponin compound represented
16 by the formula (1) as an effective ingredient may be presented in the form of general
17 drug capable of oral or non-oral administration. In the preparation of the drug for
18 oral or non-oral administration, a commonly used pharmaceutically or sitologically
19 acceptable diluent or an excipient such as a filler, an expander, a binder, a wetting agent,

1 a disintegrator, a surfactant, and the like may be used.

2 Examples of solid drugs for oral administration are a tablet, a pill, powder, a
3 granule, a capsule, and the like. The solid drugs are prepared by mixing at least
4 one excipient, such as starch, calcium carbonate, sucrose, lactose, gelatin, etc., with
5 lignan, lactone or a derivative thereof. Further, such surfactant as magnesium
6 stearate and talc may be added in addition to the excipient. Examples of liquid
7 drugs for oral administration are suspension, liquid medicine for internal use, emulsion,
8 syrup, etc. In addition to simple diluent such as water and liquid paraffin, various
9 excipients, for example, wetter, sweetener, fragrance, preservative, etc., may be
10 included.

11 Examples of drugs for non-oral administration are sterilized aqueous solution,
12 water-insoluble solution, suspension, emulsion, lyophilized drug and suppository.
13 The water-insoluble solution or suspension includes propylene glycol, polyethylene
14 glycol, vegetable oil such as olive oil, injectable ester such as ethyl oleate, etc.
15 Witpsol, macrogol, Tween 61, cacao butter, laurin fat, glycerol gelatin, etc. may be used
16 as the base of the suppository.

17 The content of the effective ingredient in the drug may be adequately selected
18 considering the degree of absorption in the body, degree of inactivation, rate of
19 excretion, age, sex, physical conditions of the user, etc. A recommended dosage is

1 0.1-10 mg/kg/day, based on the oleanane-type triterpene saponin compound
2 represented by the formula (1), more preferably 0.5-5 mg/kg/day. The drug may
3 be administered 1 to 3 times a day.

4 Also, the oleanane-type triterpene saponin compound represented by the formula
5 (1) may be provided in the form of health food comprising the compound as an active
6 ingredient. In this description, the term "health food" refers to a general food or
7 one prepared in the form of a capsule, powder, a suspension, etc. in which the
8 oleanane-type triterpene saponin compound represented by the formula (1) has been
9 added. When it is intaken, the health food provides a particular health-related
10 effect. But, unlike normal medicines, it does not cause any side effects even after a long-
11 term use because it is prepared by means of food.

12

13 **【Description of Drawings】**

14 Fig. 1 is a graph showing the effect of the oleanane-type triterpene saponin
15 compounds of the present invention in improvement of memory ability after they were
16 administered once orally.

17

18 **【Best Mode】**

19 Practical and preferred embodiments of the present invention will be illustrated as

1 shown in the following examples. However, it will be appreciated that those
2 skilled in the art may, in consideration of this disclosure, make modifications and
3 improvements within the spirit and scope of the present invention.

4

5 **Example 1: Extraction of Oleanane-type Triterpene Saponin**

6 Oleanolic acid saponin and hederagenin saponin were isolated as follows from the
7 following herbs known to contain lots of oleanolic acid saponin and hederagenin
8 saponin.

9 Each 1 kg (dry weight) of *Aralia elata*, *Pulsatilla chinensis*, *Lonicera japonica* and
10 *Kalopanax pictus* was extracted with 7 L of 50 % ethanol for 4 hours while refluxing.
11 This procedure was repeated for 2 times. The extract was filtered and concentrated
12 under reduced pressure at 50 °C using a rotary evaporator. Water was added to
13 the resultant condensate to a volume of about 5 equivalents (V/W). The resultant
14 suspension was mixed with water saturated *n*-butanol of equal volume, put in a
15 separation funnel, stirred and let alone for 24 hours. The above butanol layer was
16 isolated. After 2-3 times of re-fractionation, the butanol fraction was concentrated
17 using an evaporator and the solvent was completely removed in a vacuum oven.

18 Column chromatography was performed on the *n*-butanol fraction using
19 octadecylsilylated silica resin (YMC*GEL ODS-A 12 nm, S-150 m). The amount of

1 the resin was 250 g, or 25 equivalents of the sample amount (10 g). The step-
2 gradient method of stepwise increasing the methanol content of solvent from 10 %
3 (V/V) methanol, which amounts to 2-3 volume equivalents of the resin, by 10 % (V/V)
4 was employed. Only the fractions isolated by 70 %, 80 % and 90 % methanol
5 solvents (V/V) were taken to concentrate the saponin compound as much as possible.

7 **Example 2: Isolation and Structure Analysis of Oleanane-type Triterpene Saponin**

8 High performance liquid chromatography (HPLC) was performed on the
9 methanol fractions using acetonitrile/water mixture solvent. Using a PDA detector,
10 major peaks having maximum absorptivity at 210 nm and showing the specific
11 triterpene absorption spectra were selected. Subsequently, preparative HPLC was
12 performed using acetonitrile/water mixture solvent of 9.5 mL/min to separate the
13 selected peaks. After concentration using an evaporator, the solvent was
14 completely removed by drying in a vacuum oven. YMC J'Sphere ODS-H80 column
15 was used and component analysis was performed at 210 nm. The isolated peak
16 components were hydrolyzed in acidic condition by heating at 100 °C in 2N H₂SO₄ for
17 60 minutes to remove sugar from saponin. The resultant product was analyzed by
18 HPLC along with oleanolic acid and hederagenin standard substances purchased from
19 Sigma. The peak components confirmed as oleanolic acid saponin or hederagenin

1 saponin were further separated to obtain the 8 substances listed in Table 1 below.

2 ^1H -NMR (500 MHz), ^{13}C -NMR (125 MHz), DEPT and 2D NMR (^1H - ^1H COSY,
3 HMQC, HMBC, TOCSY, NOESY) analyses were performed to determine the structure
4 of the isolated substances. And, the hydrolyzed sugars were analyzed with the
5 carbohydrate analysis system and analyzed by GC after TMS-derivatization, in order to
6 identify the identity and number of the sugars. The structure of each sugar was
7 performed by 2D NMR (^1H - ^1H COSY, HMQC, HMBC, TOCSY) analysis. The
8 binding of the sugars was analyzed by 2D NMR (HMBC, NOESY).

9 100-500 mg of 8 oleanolic acid saponin or hederagenin saponin substances were
10 obtained as given in Table 1.

1

【Table 1】

Category	Scientific Names	Herbs	Molecular formula	M.W.
Substance ①	Eleutheroside K 3-O- α -L-Rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid	<i>Pulsatilla chinensis</i>	C ₄₁ H ₆₆ O ₁₁	735.0
Substance ②	Hederasaponin B 3-O- α -L-Rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosyloleanolic acid-28-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 4)- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester	<i>Pulsatilla chinensis</i>	C ₅₉ H ₉₆ O ₂₅	1205.4
Substance ③	Hederacolchiside E 3-O- α -L-Rhamnopyranosyl(1 $\rightarrow$ 2)- β -D-glucopyranosyl(1 $\rightarrow$ 4)- α -L-arabinopyranosyloleanolic acid-28-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 4)- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester	<i>Pulsatilla chinensis</i>	C ₆₅ H ₁₀₆ O ₃₀	1366.6
Substance ④	Elatoside A 3-O- β -D-Xylopyranosyl(1 $\rightarrow$ 2)- β -D-galactopyranosyl(1 $\rightarrow$ 3)- β -D-glucolopyranosyloleanolic acid	<i>Aralia elata</i>	C ₄₇ H ₇₄ O ₁₈	927.1
Substance ⑤	Elatoside C 3-O- β -D-Xylopyranosyl(1 $\rightarrow$ 2)- β -D-galactopyranosyl(1 $\rightarrow$ 3)- β -D-glucolopyranosyloleanolic acid-28-O- β -D-glucopyranosyl ester	<i>Aralia elata</i>	C ₅₃ H ₈₄ O ₂₃	1089.2
Substance ⑥	Loniceroside A 3-O- α -L-Arabinopyranosylhederagenin-28-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- β -D-xylopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester	<i>Lonicera japonica</i>	C ₅₂ H ₈₄ O ₂₁	1045.2
Substance ⑦	Loniceroside B 3-O- α -L-Rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosylhederagenin-28-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- β -D-xylopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester	<i>Lonicera japonica</i>	C ₅₈ H ₉₄ O ₂₅	1191.4
Substance ⑧	Kalopanaxsaponin B, Pericarpsaponin Pk 3-O- α -L-Rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranosylhederagenin-28-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 4)- β -D-glucopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranosyl ester	<i>Kalopanax pictus</i>	C ₅₉ H ₉₆ O ₂₆	1221.4

2

Example 3: Passive Avoidance Test

Passive avoidance test was performed to identify the memory improvement effect of the above 8 oleanolic acid saponin or hederagenin saponin substances in the body. Also, oleanolic acid and hederagenin, aglycons of the saponin compounds, were included in the test.

A shuttle box measuring 50 cm × 15 cm × 40 cm was used. The box was divided into two compartments by a guillotine door. One compartment was brightly illuminated and the other was covered by black cloth.

A mouse was placed in the illuminated compartment. Then, when the guillotine door was open, the mouse moved into the dark compartment within 20 seconds because it prefers darkness. The guillotine door was closed automatically as soon as the mouse entered the dark compartment. In this way, the latency time, or the time that elapsed until a mouse left the illuminated compartment and entered the dark compartment, was determined. On the first day, this training trial was performed until all the mice entered the dark compartment within 20 seconds.

On the following day, the trained mice were placed in the illuminated compartment, one at a time, and were allowed to move into the dark compartment. When the mice entered the dark compartment, a foot-shock (0.8 mA, 3 seconds) was delivered through the electronic grid equipped on the floor of the dark compartment.

1 24 hours after this acquisition trial, the mice were placed in the illuminated
2 compartment and were allowed to move into the dark compartment. Then, normal
3 mice hesitated to move into the dark compartment remembering the shock of the day
4 before. The time elapsed until the mice entered the dark compartment was
5 measured up to 300 seconds.

6 Retention time was measured to evaluate the memory improvement effect.
7 Neither scopolamine nor the drug was administered to the control group.
8 Scopolamine (1 mg/kg), which is known to decline memory function by inhibiting the
9 transfer of neurotransmitters, was administered. An hour later, saponin (30
10 mg/kg) and aglycon (30 mg/kg), Aricept (donepezil, 1 mg/kg, positive control) and
11 water (negative control) were administered.

12 The negative control group to which Aricept had been administered orally
13 showed 1.6 times longer retention time than the negative control group to which water
14 had been administered. Although there were some variations depending on the
15 compositions of aglycon and sugar, all the 8 oleanolic acid saponin or hederagenin
16 saponin substances in accordance with the present invention showed about 2.5-3.0
17 times longer retention time than that of the negative control group.

18 Further, these saponins showed a better cognitive function improvement effect
19 than the aglycons oleanolic acid (2.4 times) and hederagenin (2.3 times) [see Table 2 and

1 Fig. 1].

2 Thus, it can be concluded that oleanolic acid saponin and hederagenin saponin are
 3 effective for improving memory which is declined in dementia and mild cognitive
 4 impairment. They are superior in the effect not only to Aricept, which is used to
 5 treat dementia, but also to oleanolic acid and hederagenin, which are aglycons of
 6 saponin.

7 **【Table 2】**

Treatment		Retention time (sec)
Normal		286.9 $\pm$ 5.8
Water		61.5 $\pm$ 22.4
Oleanolic acid		149.4 $\pm$ 19.7
Hederagenin		142.8 $\pm$ 32.6
Aricept		98.5 $\pm$ 10.6
Oleanolic acid saponins	Eleutheroside K	182.6 $\pm$ 15.9
	Hederasaponin B	172.3 $\pm$ 29.1
	Hederacolchiside E	155.7 $\pm$ 31.2
	Elatoside A	166.0 $\pm$ 24.8
	Elatoside C	160.1 $\pm$ 15.6

Hederagenin saponins	Loniceroside A	175.3 $\pm$ 21.1
	Loniceroside B	169.7 $\pm$ 25.4
	Kalopanaxsaponin B	170.5 $\pm$ 24.6

1

2 Presented below are the examples of preparing therapeutic pharmaceutical drugs
3 or health foods that comprise the oleanane-type triterpene saponin compound
4 represented by the formula (1) as an active ingredient. However, they should not
5 be construed as limiting the scope of the present invention.

6

7 **Preparation Example 1: Preparation of Powder and Capsule**

8 50 mg of the oleanane-type triterpene saponin compound was mixed with 74 mg
9 of lactose, 15 mg of crystalline cellulose and 1 mg of magnesium stearate to obtain
10 power. The resultant powder was filled into a No. 5 gelatin capsule using an
11 adequate apparatus.

12

13 **Preparation Example 2: Preparation of Liquid Drug**

14 50 mg of oleanane-type triterpene saponin compound was added to 20 g of sugar,
15 20 g of isomerized sugar and adequate amount of lemon flavor. Sterilized purified

1 water was added to a total volume of 100 mL. The resultant liquid was filled into a
2 brown bottle and sterilized.

4 **Preparation Example 3: Preparation of Health Food**

5 100 mg of the oleanane-type triterpene saponin compound represented by the
6 formula (1) were mixed with 100 mg of ginseng extract, 100 mg of green tea extract, 100
7 mg of vitamin C, 120 mg of powdered vitamin E, 2 mg of ferrous lactate, 2 mg of zinc
8 oxide, 20 mg of nicotinamide, 5 mg of vitamin A, 2 mg of vitamin B1, 2 mg of vitamin
9 B2, 200 mg of cornstarch and 20 mg of magnesium stearate (for one day).

11 **【Industrial Applicability】**

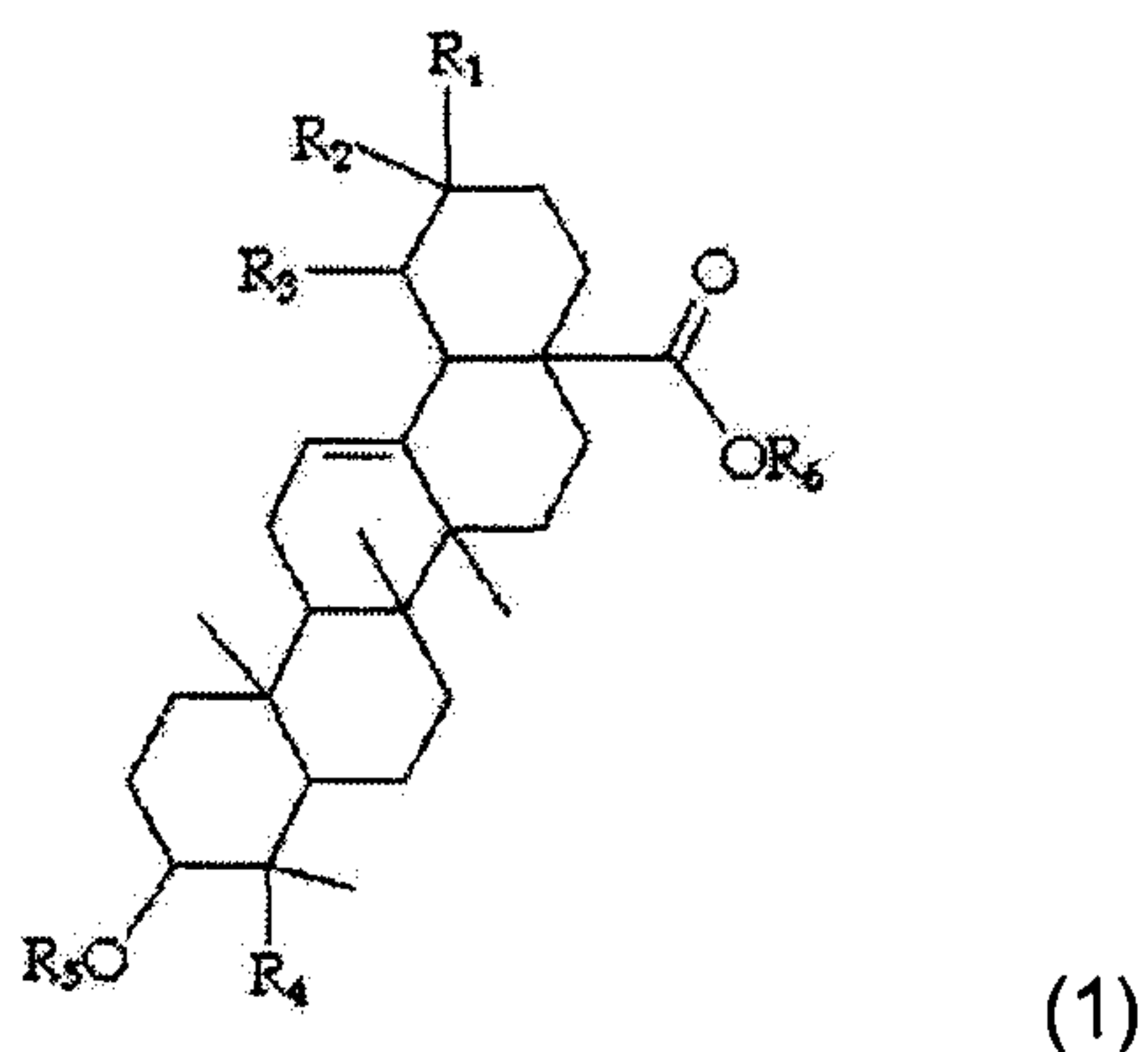
12 As apparent from the above description, the oleanane-type triterpene saponin
13 compound represented by the formula (1) has superior memory improvement effect
14 and, thus, is useful for drugs for treating dementia and mild cognitive impairment and
15 health foods for the improvement of brain functions, including cognitive function.

17 Those skilled in the art will appreciate that the concepts and specific embodiments
18 disclosed in the foregoing description may be readily utilized as a basis for modifying
19 or designing other embodiments for carrying out the same purposes of the present

- 1 invention. The scope of the claims should not be limited by the preferred
- 2 embodiments set forth in the examples, but should be given the broadest interpretation
- 3 consistent with the description as a whole.

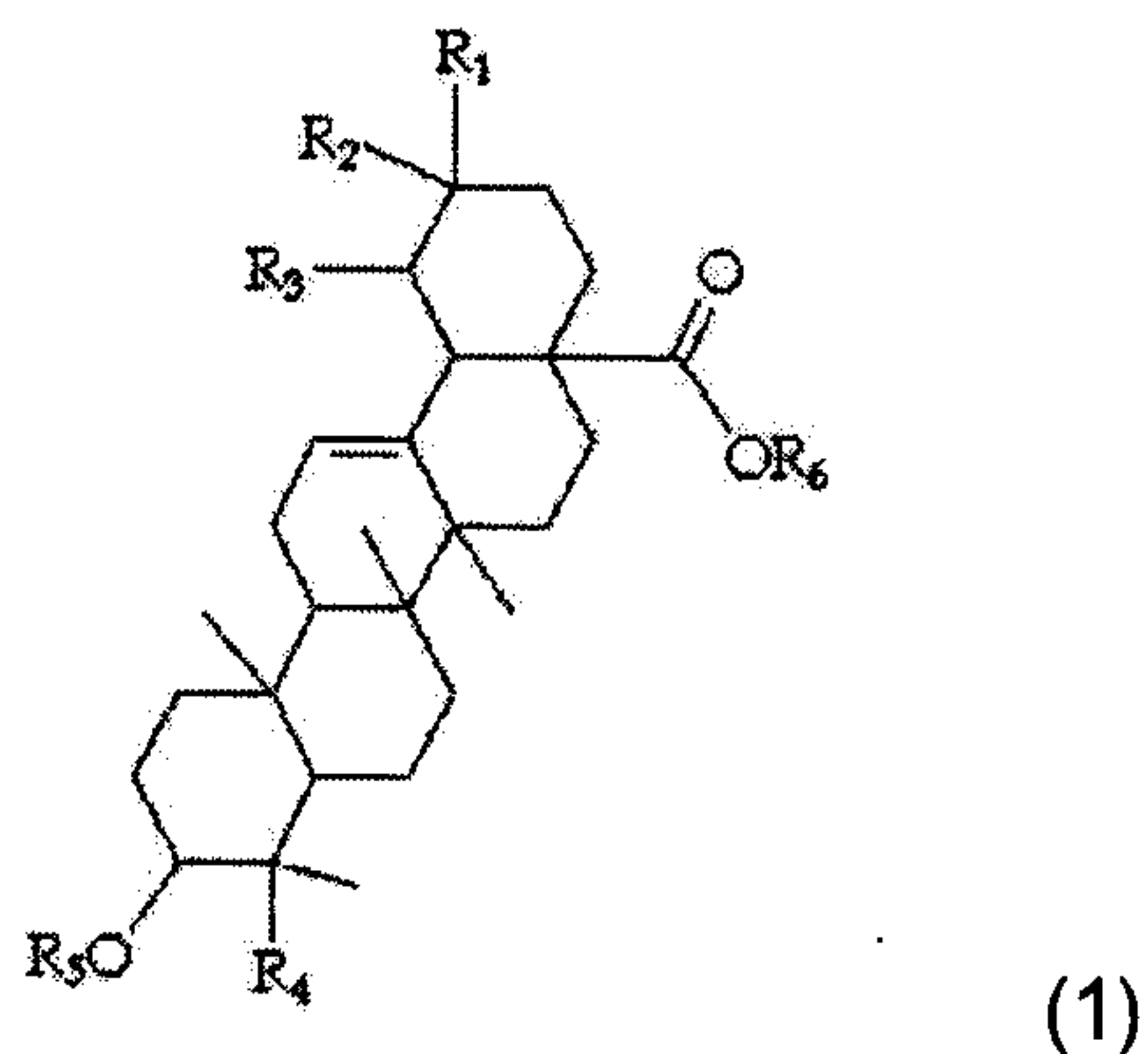
CLAIMS

1. A drug effective for the treatment and prevention of dementia and mild cognitive impairment which comprises an oleanane-type triterpene saponin compound represented by the formula (1):



- wherein each of R_1 , R_2 and R_3 is hydrogen or C_1 - C_4 alkyl; R_4 is C_1 - C_4 alkyl or C_1 - C_4 hydroxyalkyl; R_5 is galactose, rhamnose, xylose, arabinose or glucuronic acid and R_6 is hydrogen, glucose, galactose, rhamnose, xylose, arabinose or glucuronic acid.
2. The drug as set forth in Claim 1, wherein the oleanane-type triterpene saponin compound represented by the formula (1) is eleutheroside K, hederasaponin B, hederacolchiside E, Ioniceroside A, Ioniceroside B or kalopanaxsaponin B.
 3. A health food effective for the improvement of brain functions which comprises

an oleanane-type triterpene saponin compound represented by the formula (1):



wherein each of R_1 , R_2 and R_3 is hydrogen or C_1 - C_4 alkyl; R_4 is C_1 - C_4 alkyl or C_1 - C_4 hydroxyalkyl; R_5 is galactose, rhamnose, xylose, arabinose or glucuronic acid and R_6 is hydrogen, glucose, galactose, rhamnose, xylose, arabinose or glucuronic acid.

4. The health food as set forth in Claim 3, wherein the oleanane-type triterpene saponin compound represented by the formula (1) is eleutheroside K, hederasaponin B, hederacolchiside E, Ioniceroside A, Ioniceroside B or kalopanaxsaponin B.

1/1
Drawings

Fig. 1

