(19) Japan Patent Office (JP)

(12) Laid-open Patent Application Gazette (A)

(11) Laid-open Application No. S63-23819
(43) Date of Laid-open Publication: Feb. 1, 1988

(51) Int. Cl. [4]	ID Code	Internal Control No.
A 61 K 35/60	ACB	8615-4C

Examination: Not Requested Yet No. of Claims: 1 (Total 4 pages)

(54) Title of Invention: Platelet Aggregation Inhibitor

(21) Application No.: S61-167540
(22) Application Date: July 16, 1986

(72) Inventor: Shoichi MURATA, Sugamata Heights #504, 3-1-1 Imaizumi, Utsunomiya City, Tochigi Prefecture
(72) Inventor: Kenji HARA, 1022-53 Nagashitsu-cho, Utsunomiya City, Tochigi Prefecture
(71) Applicant: KAO Corporation, 1-14-10 Nihonbashi Kayaba-cho, Chuo-ku, Tokyo
(74) Agent: Mitsuyuki ARIGA, Patent Agent, and two others

SPECIFICATION

1. Title of Invention
Platelet Aggregation Inhibitor

2. What Is Claimed Is:
1. A platelet aggregation inhibitor containing a krill organic solvent extract as an active ingredient.
2. The platelet aggregation inhibitor of claim 1, wherein the krill organic solvent extract is a phospholipid fraction.

3. Detailed Description of the Invention

Industrial Field of Application

The present invention relates to a platelet aggregation inhibitor, and more specifically relates to a platelet aggregation inhibitor that has few side effects and high stability and that is obtained by extracting it from krill using an organic solvent.

Prior Art

It is known that when a thrombus forms in a blood vessel, blood flow is greatly impaired and serious injury is caused to tissue. In the pathology of diseases caused by thrombus formation, i.e. thrombosis, three factors act as thrombogenic factors: the condition of the vascular wall, blood flow speed, and blood components; it is known that these interact with one another and play an important role. Among these, the role of platelets, which are one component of blood, is extremely important. The possibility of inducing thrombosis through platelet rebound has been

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

experimentally and epidemiologically verified. Recently, in conjunction with clarifying the causative mechanism of arteriosclerosis, it has become clear that stage one of the onset of arteriosclerosis is platelet aggregation at injured vascular endothelial cells. Therefore, in recent years thrombosis has been treated and prevented by the administration of drugs which are platelet aggregation inhibitors, such as aspirin, indomethacin, phenylbutazone, clofibrate, dextran sulfate, papaverine, and heparin, and polyunsaturated fatty acids such as eicosapentaenoic acid, either orally or by intravenous injection.

Problems the Invention Is to Solve

Nevertheless, all of the abovementioned drugs are not completely satisfactory in some aspect, such as side effects, efficacy, or stability, so there is a need for the development of a platelet aggregation inhibitor with no side effects and improved efficacy and stability to replace these. Also, it is necessary that this platelet aggregation inhibitor can be administered orally so that it can be administered daily.

Means for Solving the Problems

In consideration of these facts, the present inventors performed diligent research in order to obtain a platelet aggregation inhibitor that would satisfy the abovementioned requirements. As a result, they discovered that a krill organic solvent extract has a platelet aggregation inhibition effect, and that this substance has few side effects and improved stability, and moreover can be administered orally, and so completed the present invention.

Therefore, the present invention provides a platelet aggregation inhibitor containing a krill organic solvent extract as an active ingredient.

The krill used in the present invention may be any of the Euphausia genus such as superba, crystallorophias, frigida, triacantha, valentini, longirostris, lucens, similis, spinifera, or recurva, or any of the Thysanoessa genus such as macarura, vicina, or gregaria, and is not limited to a particular species.

Furthermore, the krill that is the starting material is distributed through the oceans of the world, and is plentiful around the South Pole in particular, where its biomass is said to range from hundreds of millions of tons to two billion tons. It appears to be possible to catch 50,000,000 to 70,000,000 tons/year, which is comparable to the current annual catch of fish worldwide, so in terms of supplying the raw material, a stable supply should be possible.

Extraction from krill using an organic solvent is performed in the normal manner. For example, krill or a powder thereof is put in an organic solvent such as a non-polar solvent such as chloroform, benzene, butanol, or ether or a mixture of a non-polar solvent and a polar solvent such as chloroform-methanol, ether-ethanol, or butanol-water. Preferably, it is stirred for 2-24 hours at 15-60°C, and filtered. Preferred organic solvents are chloroform, chloroform-methanol, and butanol-water.

The krill organic solvent extract obtained in this manner can be used as-is as a platelet aggregation inhibitor, or can be concentrated or dried. But it can also be used by obtaining a phospholipid fraction from this extract. Typical methods of obtaining a phospholipid fraction are to condense the abovementioned extract, and then drip a small amount gradually into a large amount of cold acetone, and thereby cause a phospholipid fraction to precipitate, for example, or to separate and collect it by thin-layer chromatography.

The platelet aggregation inhibitor of the present invention is manufactured by combining the krill organic solvent extract obtained as described above or a phospholipid fraction obtained

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

therefrom with a known pharmaceutical carrier as required. Furthermore, when using the krill organic solvent extract, the organic solvent in the extract is preferably removed by replacement with water.

The platelet aggregation inhibitor of the present invention obtained in this manner is preferably administered orally in the amount of 1-20 g/day in terms of the amount of lipids in the extract or phospholipid fraction, for adult males, but it is possible to further increase the dose according to the degree of symptoms.

Furthermore, the extract or phospholipid fraction that is the active ingredient in the platelet aggregation inhibitor of the present invention has extremely high safety, as indicated by the fact that LD_{50} (oral) in rats of the phospholipids contained in these is 25 g/kg.

Operation

The mechanism of the platelet aggregation inhibition effect of the krill organic solvent extract of the present invention has not been explicated in detail, but it appears to derive from the phospholipids having large amounts of polyunsaturated fatty acids such as eicosapentaenoic acid that are contained in the krill organic solvent extract.

Effect of the Invention

As described above, the krill organic solvent extract of the present invention and in particular a phospholipid fraction derived from this exhibit an excellent platelet aggregation inhibition effect in oral administration, and have no toxicity or side effects, and so are an extremely superior platelet aggregation inhibitor.

Embodiments

The present invention is described more specifically in the following embodiments.

Embodiment 1

Freeze-dried krill (Euphausia superba) was finely pulverized, 500g of this pulverized material was added to a butanol-water (65:35) mixture, 1000 ml, and while stirring at 50°C for 20 hours, the lipids were extracted, and a butanol-water extraction solution was obtained by filtering.

The filtered butanol-water extraction solution was concentrated under reduced pressure and the butanol was removed, after which additional concentration was performed to produce 40g of the butanol-water extraction solution (water content 28g).

Embodiment 2

(I) Freeze-dried krill (Euphausia superba) was finely pulverized, 500g of this pulverized material was added to 1000 ml of chloroform, and while stirring at 40°C for 20 hours, the lipids were extracted, and a chloroform extraction solution was obtained by filtering.

(II) The filtered chloroform extraction solution was concentrated under reduced pressure and solidified, and then 50 ml of a water-ethanol (1:1) solution was added, and the dried material was dissolved. This was again concentrated under reduced pressure and the ethanol was removed, producing 37g of the krill chloroform extraction solution (water content 25g).

Embodiment 3

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

A chloroform extraction solution prepared in the same manner as in Embodiment 2(I) was concentrated under reduced pressure to about 10 ml, and then added dropwise to 2000 ml of acetone with stirring, and a phospholipid fraction was precipitated. The precipitate was filtered and collected under reduced pressure, and then dissolved in 30 ml of a water-ethanol 1:1 mixed solution, and the ethanol was removed by reduced-pressure concentration. 20g of a phospholipid fraction of the krill chloroform extraction solution was produced (water content 15g).

Embodiment 4

Tests were performed on the platelet aggregation inhibition effect of the krill extracts and the phospholipid fractions thereof obtained in Embodiment 1, Embodiment 2, and Embodiment 3. Specifically, krill extracts and phospholipid fractions were administered daily for two weeks to male Wistar rats (body weight 200g), 10 rats per group, 0.5 ml per dose, into the stomach using a probe. On the final day of the experiment, under Nembutal anesthesia, blood was collected from the lower abdominal artery, with a 3.8% sodium citrate solution added in the amount 1/10. The collected blood was centrifuged at 500g, 22°C, for 6 hours to make the supernatant a PRP (Platelet Rich Plasma). The remaining blood was centrifuged at 1000g, 22°C, for 15 hours to make the supernatant a Platelet Poor Plasma (PPP). Platelet aggregation activity was measured using an ERMA Aggretec TE-500, and shown as maximum aggregation ratio and maximum aggregation time. Furthermore, platelet aggregation activity was studied with the PRP diluted with PPP so that the number of platelets became 5-7 × 10^7 per 200 μl, and 20 μl of a saline solution with ADP 20 μmol was added as the aggregation initiating substance. Also, rats administered only water were used as the control group. The results are shown in Table 1.

Table 1

Administration group	Maximum aggregation ratio (%)	Maximum aggregation time (min)
Krill butanol-water extract	59.8	3.16
Krill chloroform extract	60.3	3.14
Krill phospholipid fraction	52.8	2.89
Water	68.5	3.48

As shown by these results, it is clear that the phospholipid fractions in the krill organic solvent extracts, etc. have a platelet aggregation inhibition effect.

Formulation Example

Composition:

① Krill phospholipid* 100 mg
② Lactose 100 mg
③ Aluminum silicate 50 mg
④ Magnesium stearate 5 mg

* Powder with extraction solvent completely removed (Embodiment 2)

Manufacturing Method:

Mix ①-④, and press into tablets.

The End

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

⑲日本国特許庁（JP）　⑪特許出願公開

⑫公開特許公報（A）　昭63－23819

⑤1Int.Cl.⁴	識別記号	庁内整理番号	⑬公開　昭和63年(1988)2月1日
A 61 K 35/60	ACB	8615－4C	

審査請求　未請求　発明の数 1　（全4頁）

⑤4発明の名称　血小板凝集抑制剤

⑳特　願　昭61－167540
㉒出　願　昭61(1986)7月16日

⑦2発明者　村田　昌一　栃木県宇都宮市今泉3の1の1　菅又ハイツ504号
⑦2発明者　原　健次　栃木県宇都宮市氷室町1022－53
⑦1出願人　花王株式会社　東京都中央区日本橋茅場町1丁目14番10号
⑦4代理人　弁理士　有賀　三幸　外2名

明　細　書

1. 発明の名称

血小板凝集抑制剤

2. 特許請求の範囲

1. オキアミの有機溶剤抽出物を有効成分として含有する血小板凝集抑制剤。

2. オキアミの有機溶剤抽出物がリン脂質画分である特許請求の範囲第1項記載の血小板凝集抑制剤。

3. 発明の詳細な説明

〔産業上の利用分野〕

本発明は血小板凝集抑制剤に関し、更に詳細にはオキアミを有機溶剤で抽出することにより得られる副作用が少なく安定性の高い血小板凝集抑制剤に関する。

〔従来の技術〕

血管内に血栓が形成されると血流が著しく阻害され、組織に重大な障害をもたらすことが知られている。この血栓形成による疾患、すなわち、血栓症の病態生理において、血栓形成因子として血管壁の性状、血流速度及び血液成分の三つの因子が挙げられ、これらは互いに影響しあい重要な役割を果していることが知られている。このうち、血液成分の1つである血小板の役割は非常に重要であり、血小板の凝集亢進により血栓症の誘発の可能性が実験的に、また疫学的に証明されている。さらに最近、動脈硬化症の発症メカニズムの解明に従い、動脈硬化症の発症第一段階が損傷血管内皮細胞への血小板凝集であることが

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.