



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(51) International Patent Classification <sup>7</sup> :</b><br><b>A61K 31/35, 31/375, A23L 1/302, 1/30</b>   | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 00/12085</b><br><b>(43) International Publication Date:</b> 9 March 2000 (09.03.00)  |
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| <b>(54) Title:</b> ASCORBATE-ISOQUERCETIN COMPOSITIONS<br><br><b>(57) Abstract</b><br><p>The present invention relates to novel compositions containing ascorbic acid and one or more derivatives of quercetin which orally administered conveys in vivo higher protection, longer maintenance of biological activity, higher concentration in tissues and higher biological efficiency to vitamin C in organs in human body. These compositions are useful as pharmaceutical compositions and as food supplements possessing preventive properties against damages of human organs, including skin, tissues and cells due to oxidative stress or damages.</p>  |           |  |

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## Ascorbate-Isoquercetin Compositions

5 The present invention relates to novel compositions containing ascorbic acid with an increased level of its active form. These compositions are useful as food supplements possessing preventive properties against damages of human tissues, including skin cells due to oxidative stress.

In vivo ascorbic acid (vitamin C) exists in three forms:

- 10 a) as an ascorbate in form of an ascorbate monoanion,
- b) as the reversibly oxidised form of a free radical, called semidehydroascorbic acid which could be reversibly oxidised to dehydroascorbic acid or reversibly reduced to ascorbate monoanion, and
- c) as dehydroascorbic acid (oxidised form of semidehydroascorbic acid).

15 Only ascorbate possesses specific vitamin C activity: as a cofactor for enzymes. Observed physiological activities of semidehydroascorbic acid and dehydroascorbic acid formed in vivo from ascorbate are considered to be based on their reversible reductions to ascorbates, (Buettner, 1993- Dharival et al., 1991; Welch et al., 1995 Washko et al., 1993). The second form of ascorbic acid, semidehydroascorbic acid (ascorbate free radical) participates  
20 in univalent redox systems, (Bors et al. 1995), that is in the antioxidant defence activity. This means, semidehydroascorbic acid participates most likely in free radical scavenging activities. According to Gordon (1996, p. 270), "ascorbate appears to be the most important non-protein antioxidant in plasma". Ascorbic acid is absorbed from the gastrointestinal tract in the form  
25 of ascorbic acid. Dehydroascorbic acid is reduced to ascorbic acid for gastrointestinal absorptions (Rose et al., 1988).

30 Structures of body tissues are susceptible to damages caused by the oxidative stress, e.g. by the accumulation of reactive oxygen species during ageing, chronic environmental stress, inflammations or general metabolic dysfunctions. The role of free radicals and reactive oxygen species in aetiology of human diseases (e.g. cancer, atherosclerosis, rheumatoid

arthritis, inflammatory bowel diseases, immune system dysfunctions, brain function decline, connective tissue dysfunctions) is well established (for a recent review see: Gordon, 1996). Uncontrolled generation of free radicals, especially chronic exposure to reactive oxygen species leads to chronic intracellular damages, to oxidative stress and premature ageing. Cells of the human body possess metabolic antioxidant defences which are supported by dietary antioxidants. The early observations of the antioxidant defence metabolic processes involved vitamin C and flavonoids (Bezssonoff, 1926; Bentsath et al., 1936; Bensath et al., 1937; Blanc and Von der Muehl, 1967). Ascorbic acid is not only important non-protein antioxidant in human plasma (Gordon, l.c.) but it increases (Skaper et. al., 1997) the cytoprotective activities of quercetin and rutin. Skaper and co-authors (1997) have shown, for instance, that quercetin protects connective tissue and specifically skin cells (e.g. fibroblasts, keratinocytes, and endothelial cells) from this type of damages. Other authors have demonstrated protective effect of flavonols on cardiovascular and nervous system, their role as chemoprotective agents in carcinogenesis.

Oxidation of the ascorbate in the human body by xenobiotics often leads to the accumulation of semidehydroascorbic acid or dehydroascorbic acid in organs where these forms interfere with the regular metabolism. As ascorbate is a cofactor for eight isolated enzymes (carrying out collagen synthesis, carnitine synthesis, peptide amidation, tyrosine metabolism, and catecholamine synthesis) the decrease of the concentration of ascorbate in body tissues and fluids may leads to seroius metabolic dysfunctions.

The possibilities to protect ascorbic acid in vivo were based on very early observations of Szent-Györgyi group mentioned above that the ascorbic acid activity in humans and guinea pigs is intensified by the great group of "vegetable dyes, the flavons or flavonols". It has been known that flavonoids are contributing to the maintenance of the concentration of the administered ascorbate in adrenals, kidneys, spleen, and the liver of the organisms investigated and improve the antiscorbutic effect of the dosages of

ascorbate used (Papageorge and Mitchell, 1948; Cotereau et al., 1948; Crampton and Lloyd, 1950; Douglas and Kamp, 1959; Blanc and Von der Muehl, 1967; Zloch, 1973).

5 The mechanism of this effect, called "the vitamin C-economising function" of some flavonoids ("facteur d'economie de L'acide ascorbique" of Bezssonoff, 1926 and 1927) has been recognised in many laboratories. For example, Harper et al., 1969, found that, among flavonoids tested, flavonols the have strongest ability to inhibit ascorbic acid oxidation in near neutral solutions (pH 5 - 7) . Harper et al. (1.c.) also pointed out that the presence of free  
10 hydroxyl groups at carbon atoms 3,7, 3', and 4' in a flavonol molecule improves the antioxidative effect of the flavonol molecule, this means, it inhibits ascorbate oxidation more effectively.

15 But there was neither an effective method nor a useful orally applicable formulation leading to an increased level of active ascorbate in human tissue.

20 Accordingly, there was a need for a composition useful for the protection of the orally administered ascorbic acid and enhancement of vitamin activity in the tissues.

Now it has been found that isoquercetin effectively inhibits ascorbate oxidation. The maintenance of the reduced form of ascorbic acid by isoquercetin maintains ascorbic acid level in body tissues and fluids.

25 This effect perhaps may be explained in that isoquercetin not only shows three free hydroxyl groups mentioned by Harper (1.c.), more exactly, hydroxyl groups attached to carbon atoms 7, 3', and 4', but also a glucopyranoside moiety with additional four free hydroxyl groups O-attached to the carbon 3 of isoquercetin. Therefore, the increased effectivity of  
30 ascorbate protection may be caused by the fact that isoquercetin contains a glucose molecule. This glucose molecule seems to be the reason why isoquercetin is able to use the sodium-dependent glucose transport

pathway of the intestinal brush-border membrane in its absorption process (Gee et al., 1998). Experiments have also shown that the absorption of isoquercetin is better than that of pure aglycone.

5 Earlier pharmacokinetic studies with isoquercetin anticipated results obtained and explained by Gee et al., *l.c.*, by having shown excellent absorption rate and bioavailability of isoquercetin (Hollman and Katan, 1997).

10 It has been found that ascorbate is not only able to regenerate oxidised flavonols by reducing them (Yamasaki et al., 1997) but also to protect quercetin (aglycone of the isoquercetin) against oxidative degradation and to maintain the antiviral properties of quercetins (Vrijsen et al., 1988).

15 This means, there is a synergistic effect between isoquercetin and ascorbate in human tissue leading to higher effectivities of both, ascorbate and isoquercetin.

For isoquercetin these activities are as follows:

20 it has shown antihypertensive properties, (Kameda et al., 1987); it inhibits the biosynthesis and release of prostaglandin-like substances (Chanh et al. 1986); it produces dose-dependent protection in oxidative DNA damage (Noroozi et al., 1998), it possesses preventive properties against damages of vascular and connective tissues (especially skin) and it is therapeutically useful in the treatment of dysfunctions of the digestive tract (Seto et al. 25 1992).

Now we have found by experiments that the combination of vitamin C with the most easily bioavailable bioflavonoid, isoquercetin, is most effective in prevention of and in defense against stress dysfunctions, especially against oxidative damages of living tissues including brain, vascular, connective 30 tissues (especially skin).

It has been found that a composition containing ascorbic acid and one or more derivatives of quercetin elected from the group quercetin-3-O-glucoside (isoquercetin), quercetin-4'-glucoside, quercetin-3'-glucoside and acid-quercetin-7-glucoside in a molar ratio of ascorbate to flavonoid in the range of 2:1 to 1 : 2, preferably in the molar ratio of 1 : 1, orally administered conveys in vivo higher protection, longer maintenance of biological activity, higher concentration in tissues and higher biological efficiency to vitamin C in organs of human body. This adduct similarly also provides the properties of higher protection, longer maintenance of biological activity, higher concentration in tissues, and higher biological efficiency in organs of human body to isoquercetin and the other glucosides of the above mentioned group.

Useful compositions may contain in a daily dose 30 - 4000 mg of an active amount of ascorbic acid or preferably of physiologically active ascorbate in form of its sodium salt, calcium, other mineral, or organic cation salts. Usually compositions contain 150 - 1000 mg, but for special treatments the amount is chosen higher between 1000 and 4000 mg, preferably between 1500 and 3000 mg. The compositions according to the present invention may be prepared in form of tablets, capsules or syrups. These application forms may also contain further active ingredients in useful amounts like vitamins, suitable salts of Mg, Ca, K or Fe and perhaps trace elements.

The compositions of the present invention preferably are useful as food supplements, but they may also be administered in a pharmaceutical treatment.

The present invention makes available

- a) a method of maintaining long biological activity and high concentration of ascorbate and isoquercetin in human organs (including skin), tissues and cells,
- b) a method of protection against oxidative damages of human organs, tissues, skin cells,

c) a method of prevention of arteriosclerosis, cardiovascular diseases, and other damages of vascular tissues, of allergic and inflammatory disorders, of bacterial and viral infections, of metabolic dysfunctions involving oxidative damages e.g., premature ageing,

5 d) a method of supporting pharmacological treatments of diseases and dysfunctions caused by oxidative damages,

by orally administration of a composition described above. Generally speaking, compositions that are applicable contain at least ascorbic acid or ascorbate or any other form of this vitamin that would in vivo yield  
10 ascorbate, or semidehydroascorbic acid, or dehydroascorbic acid and isoquercetin. The decision which further ingredients should be components of a composition useful in one of the above mentioned methods depends on the special indication. Usually, if the composition is administered as a way of protection or prevention useful further ingredients may be further vitamins,  
15 salts of Mg, Ca, K, Fe and trace elements in known amounts as used in food supplements. Compositions useful in method of supporting pharmacological treatments may differ from them.

The superiority of isoquercetin and ascorbate used in combination for the  
20 protection of human cells, tissues and organs from the oxidative stress is based on two properties of isoquercetin and of ascorbate. First, on the quick intestinal absorption of orally administered isoquercetin and of ascorbate, and on the rapid and simple passage of both compounds through cytomembranes of human organs; secondly, on the specificity of interaction  
25 of isoquercetin with ascorbate. Specifically, ascorbate maintains isoquercetin in its active oxidised state and isoquercetin maintains ascorbate in its enzymatically active reduced state.

On the basis of our research on the bioavailability and on redox properties of  
30 isoquercetin and ascorbate it has been found that orally administered mixtures of isoquercetin and ascorbate are most effective in protecting the



organs (including skin), tissues, and cells from the chronic intracellular oxidative damages.

5 The uptake of isoquercetin into the human body is facilitated by the sodium-dependent glucose transport system. This type of transport occurring in most animal species (Coady et al., 1990) is active during the uptake of pyranosides as for example described by Hediger for methyl alpha-D-glucopyranoside (Hediger et al., 1987). The sodium-dependent glucose transport system in mammals was studied in many laboratories. Koepsell and Spangenberg (1994) characterised Na(+)-D-glucose cotransport in the 10 intestine. It is a cotransporting system composed of a set of two subunits: transport-mediating proteins and transport-modulating proteins. The first translocates the substrates and the second accelerates the  $V_{max}$  of the Transport. The susceptibility of isoquercetin to be transported using the Na(+)-D-glucose cotransport is suggested to be determined by the manner in 15 which a non-glucose moiety is linked to glucose. More information about this is given in a review of Olson and Pessin, 1996. Direct evidence that isoquercetine uses sodium-dependent glucose transport pathway of the intestinal brush-border membranes was obtained by Gee et al., 1998.

20 Also the uptake of ascorbate by human is caused by a sodium dependent glucose transport system. Interactions between glucose and ascorbate transport activity have been demonstrated in many tissues and cells (Rumsey and Levine, 1998). Apparently ascorbate is absorbed in human intestine by a sodium-dependent active transport system, although in vitro 25 about 10-20% of ascorbic acid moves into cells in the absence of sodium (Kuo et al., 1997). The carrier proteins in the intestinal cell membranes bind and transport the vitamin across the membrane to its intracellular site of action. There are differences in transport kinetics, tissue specificity, Na<sup>+</sup>-dependence and energy dependence (Rumsey and Levine, 1.c.), but in most 30 cases the transport of ascorbate is Na<sup>+</sup>-dependent and requires metabolic energy. Kinetic evidence suggests strongly that ascorbate may be

transported by the same transporter as glucose and, therefore, by the same transporter as isoquercetin.

5 Pharmacokinetic studies with isoquercetin support the present invention as they show excellent absorption rate and bioavailability of isoquercetin. It is absorbed better than rutin and quercetin (Hollman, 1997). Absorbed isoquercetin interacts with ascorbate protecting it and, at the same time, is being protected by ascorbate by being kept in the reduced state (Yamasaki et al., 1997). It has also been shown that ascorbate protects quercetin (aglycone of the isoquercetin) against oxidative degradation and maintains quercetin's antiviral properties (Vrijssen et al., 1988). Effectiveness of isoquercetin in interacting with ascorbate is strengthened by the fact that isoquercetin uses the preferential intestinal Na(+)-D-glucose cotransport discussed above.

15 Therefore, a most powerful dietary antioxidant composition is prepared using among other ingredients ascorbic acid and isoquercetin. The advantageous properties of these compositions are induced by the synergistic effect of Isoquercetin protecting the activity of the orally administered ascorbic acid while maintaining its enzymatically active reduced form, and, on the other side, of ascorbate maintaining isoquercetin in its active oxidised state.

20 Surprisingly it was found that in contrast to other quercetin glucosides, isoquercetin shows far better absorption rates in human intestinal tract than rutin or the quercetin aglycone and that it acts as a specific and most powerful dietary antioxidant at the same time.

25 This positive result was unexpected because mixtures of ascorbic acid and quercetin or quercetin glucosides other than isoquercetin were considerably less effective .

30

Subject of this invention is that in humans the oral administration of a mixture or combination of ascorbic acid and isoquercetin (quercetin-3-O-glucoside); or of any of mixtures of ascorbic acid and quercetin-4'-glucoside; of ascorbic acid and quercetin-3'-glucoside; of ascorbic acid and quercetin-7-glucoside, with a suitable molar ratio, preferably equimolar ratio, of ascorbate to flavonoid, conveys efficient protection against oxidative damages, due to long maintenance of biological activity of each of the ingredients and due to maintenance of high concentration of both ascorbate and isoquercetin in organs, tissues, and cells.

The invention of this application includes especially compositions containing the above mentioned ingredients useful for the prevention and treatment of atherosclerosis and other cardiovascular disorders, certain forms of cancer, allergic and inflammatory disorders, bacterial and viral infections, a number of metabolic dysfunctions, e.g. premature ageing and other pathological conditions that involve oxidative damages.

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## CLAIMS

- 5 1. Orally applicable composition containing ascorbic acid or ascorbate or its derivative in combination with one or more derivatives of quercetin elected from the group quercetin-3-O-glucoside (isoquercetin), quercetin-4'-glucoside, quercetin-3'-glucoside, and quercetin-7-glucoside.
- 10 2. Composition according to claim 1 containing isoquercetin in combination with ascorbic acid or of a physiologically active ascorbate in form of its sodium, calcium, other mineral or organic salts.
3. Composition according to claims 1 or 2 containing a combination of isoquercetin and ascorbic acid or their mineral or organic salts and additionally other ingredients.
- 15 4. Composition according to claim 3 wherein other ingredients are vitamins.
5. Composition according to claims 1 - 4 wherein other ingredients are suitable salts of Mg, Ca, K, and Fe.
- 20 6. Composition according to claims 1 - 5 wherein other ingredients are trace elements.
7. Composition according to claims 1 - 6 containing ascorbic acid or ascorbate and isoquercetin in a molar ratio in the range of 2 : 1 to 1 : 2.
8. Composition according to claims 1 - 6 containing ascorbic acid or ascorbate and isoquercetin in a molar ratio in the range of 1 : 1.
- 25 9. Compositions according to claims 1 - 8 containing 30 - 4000 mg ascorbic acid or ascorbate in daily dose, preferably 150 - 1000 mg.
10. Compositions according to claims 1 - 8 containing 1500 - 3000 mg ascorbic acid or ascorbate in daily dose.
- 30 11. Use of compositions according to claims 1 - 10 as a food supplement.

12. Method of maintaining long biological activity and high concentration of ascorbate and isoquercetin in human organs, especially skin, tissues and cells by orally administration of a composition according to claims 1 - 10.
- 5 13. Method of protection against oxidative damages of organs, including skin, tissues and cells by orally administration of a composition according to claims 1 - 10.
- 10 14. Method of prevention of arteriosclerosis, cardiovascular diseases, allergic and inflammatory disorders, bacterial and viral infections, metabolic dysfunctions, e.g. premature ageing, and of other pathologic conditions involving oxidative damages by orally administration of compositions according to claims 1 - 10.
- 15 15. Method of supporting pharmacological treatments of diseases and dysfunctions caused by oxidative damages by orally administration of compositions according to claims 1 - 10.
- 20 16. Pharmaceutical composition containing a compositions according to claims 1 - 9.
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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/06166

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 A61K31/35 A61K31/375 A23L1/302 A23L1/30

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A23L

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

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

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | PATENT ABSTRACTS OF JAPAN<br>vol. 016, no. 338 (C-0965),<br>22 July 1992 (1992-07-22)<br>& JP 04 099771 A (SAN EI CHEM IND LTD),<br>31 March 1992 (1992-03-31)<br>abstract<br>---                                   | 1                     |
| X          | DATABASE WPI<br>Section Ch, Week 199433<br>Derwent Publications Ltd., London, GB;<br>Class B05, AN 1994-269369<br>XP002123121<br>& JP 06 199693 A (KATO K),<br>19 July 1994 (1994-07-19)<br>abstract<br>---<br>-/-- | 1                     |

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Date of the actual completion of the international search

26 November 1999

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Caturla Vicente, V

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/06166

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