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(54) Title: DIETARY SUPPLEMENT INCORPORATING BETA-SITOSTEROL AND PECTIN

(57) Abstract

A composition for inhibiting absorption of fat and cholesterol from the gut and a method for making and using the composition. The composition comprises β-sitosterol bound irreversibly to pectin to form a β-sitosterol/pectin complex.

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DIETARY SUPPLEMENT INCORPORATING BETA-SITOSTEROL AND PECTIN

Field of the Invention

This invention relates to a composition and method for the reduction of serum cholesterol and weight reduction.

Background of the Invention

Cholesterol is an essential molecule in animals, required for the synthesis of cell membranes and as a precursor of steroid hormones and bile salts. However, high levels of serum cholesterol, especially low density lipoprotein (LDL), and serum lipids have been correlated with an increased risk of coronary heart disease. Deposition of lipid plaques on the intima of arteries (atheroma), resulting in atherosclerosis, appears to occur most readily when serum lipids are elevated in concentration.

The average diet in the United States provides about 40 percent of calories as lipids and approximately 57% of adult Americans have borderline high cholesterol (greater than 200 mg/dl). Coronary heart disease constitutes a leading cause of mortality and morbidity in the United States. Its principle underlying cause, atherosclerosis, is responsible for a large percentage of deaths and disabilities.

Dietary management is generally recommended to lower total and LDL cholesterol concentrations by reducing the intake of saturated fats and cholesterol and weight reduction for those overweight, by eliminating excess calories. However, many people at moderate to high risk for coronary heart disease are unwilling to change to a low risk diet or find that such a change is not feasible. As an alternative several medications and dietary supplements such as cholestyramine resin, probucol, colestipol HCl, nicotinic acid, mevinolin,

pectin, guar gum and oat bran have been proposed for use to control serum cholesterol levels. However, for the most part these medications are unpleasant to take and may have undesirable side effects. Brans and fibers as dietary supplements must be taken in large volumes to be minimally effective. Even if these dietary supplements are effective in reducing serum cholesterol they will not affect weight reduction unless fat intake is limited.

Another naturally occurring compound reported to be effective in lowering serum cholesterol is a plant steroid, β-sitosterol. β-sitosterol has been shown to impair adsorption of cholesterol. However the compound can be absorbed and result in xanthomatous deposits and can have an estrogen like activity.

There is a need for a dietary supplement which lowers cholesterol, especially low density lipoprotein, by altering dietary fat and cholesterol absorption. Such a supplement should be palatable and non-toxic for extended use.

Summary of the Invention

A composition for inhibiting absorption of fat and cholesterol from the gut is described. The composition comprises β-sitosterol bound irreversibly to pectin to form a β-sitosterol/pectin complex.

The β-sitosterol/pectin complex is prepared by a method comprising mixing β-sitosterol and pectin and suspending the β-sitosterol/pectin mixture in water. The suspension is then heated to complex β-sitosterol and pectin and then acidified to esterify the β-sitosterol to the pectin. The esterified β-sitosterol/pectin is then heated to form a β-sitosterol/pectin complex.

The composition is useful for weight reduction when administered to a patient desiring a reduction in body weight and for reducing serum cholesterol levels in a patient suffering from elevated serum cholesterol.

Detailed Description

The present invention relates to a complex of pectin and β-sitosterol. The compounds are both known to be effective in reducing cholesterol, however, to be effective they are required in large quantities. Also β-sitosterol has toxic or at least undesirable side effects.

β-sitosterol is derived from soy and rice and has a structure similar to cholesterol, except for the substitution of an ethyl group at C-24 of its side chain. Pectin is a polysaccharide derived primarily from citrus rind. It has a molecular weight of 20,000-40,000, of partial methyl ester of α-(1→4) linked D-galacturonate sequences interrupted with (1→2)-L-rhamnose residues. Neutral sugars, D-galactose, L-arabinose, D-xylose and L-fructose, form side chains of the pectin molecule. Pectin is not broken down by enzymes of the gastrointestinal tract and is, therefore, a dietary fiber.

For use in the present invention pectin is irreversibly complexed with β-sitosterol to form a complex which is not degraded by pH changes or enzymes of the gut, thus rendering the β-sitosterol non-absorbable by the gut.

To prepare the β-sitosterol/pectin complex, about 300 mg of β-sitosterol (Sigma Chemical Co. of St Louis MO, Cat. No. S9889) is mixed with about 30 mg pectin (Sigma Chemical Co., Cat. No. P2157) the mixture is dried at room temperature for about five minutes. About 30 ml of distilled water is added to the mixture and the suspension is stirred at room temperature for about 5 minutes to hydrate the β-sitosterol and pectin. The suspension is then heated at about 93°C for about 2 minutes to complex the β-sitosterol with the pectin. The complexed suspension is then cooled at room temperature for about 5 minutes and esterified by the addition of about 15 ml of rice wine vinegar (4.3% v/v acetic acid). The esterified complex is stirred at room temperature for about 5 minutes, heated at about 93°C for about 6 minutes and then sized by pushing the esterified suspension through a 20 mesh sieve.

Excipients are then added. About 335 mg of calcium carbonate and about 2 ml of water are added and the mixture stirred at about 40°C for about 10 minutes. About 18 mg of calcium silicate, about 18 mg of silicon dioxide and about 18 mg of croscarmellose sodium are then added and the mixture is stirred at about 40°C for about 10 minutes. About 10 mg of magnesium stearate is then added and the mixture is stirred for about 5 minutes. The mixture is pressed into about 700 mg tablets with about 20,000 psi pressure.

One tablet of about 700 mg is taken about 30 minutes before a meal.

Example 1

300 mg of β-sitosterol was mixed with 30 mg pectin and dried at room temperature for five minutes. 30 ml of distilled water was added to the mixture and the resultant suspension was stirred at room temperature for 5 minutes to hydrate the β-sitosterol and pectin. The suspension was then heated at 93°C for 2 minutes to complex the β-sitosterol with the pectin. The complexed suspension was cooled at room temperature for 5 minutes and esterified by the addition of 15 ml of rice wine vinegar. The esterified complex was stirred at room temperature for 5 minutes, heated at 93°C for 6 minutes and sized by pushing the β-sitosterol/pectin complex through a 20 mesh sieve.

334 mg of calcium carbonate and 2 ml of water were added and the mixture was stirred at 40°C for 10 minutes. 18 mg of calcium silicate, 18 mg of silicon dioxide and 18 mg of croscarmellose sodium were then added and the mixture was stirred at 40°C for 10 minutes. 10 mg of magnesium stearate was then added and the mixture was stirred for an additional 5 minutes. The resultant mixture was pressed into 727 mg tablets with 20,000 psi pressure.

The resultant pills were placed in distilled water, pH 7, and broke down to a soft, whitish, pleasant smelling flocculent within 30 minutes.

Example 2

Pills prepared as described in Example 1 were crushed and suspended in 100 ml of distilled water, adjusted to pH 3 with acetic acid. The suspension was heated at 36°C for 30 minutes with vigorous agitation. The suspension was then cooled for 5 minutes and a suspension sample was collected. The remaining suspension was filtered through 100 micron filter paper and the filtrate collected. The suspension sample and the filtrate were analyzed by infrared Fourier resonance spectroscopy (IFRS). Samples were also dried and the residue weight determined. Solutions of β-sitosterol alone, pectin alone and an uncomplexed mixture of β-sitosterol and pectin where used for comparison in the IFRS analysis.

The dry weight of the β-sitosterol/pectin complex and the filtrate indicated that less than 1% w/w of the complex was solubilized by the solubilization conditions at pH 3. The IFRS analysis indicated that the β-sitosterol/pectin complex retained double and single carbon-carbon bonds different from either β-sitosterol and pectin.

The pH of the stomach is normally about pH 3, therefore, the β-sitosterol/pectin complex retains its structure and is expected to pass through the stomach intact.

Example 3

Pills prepared as described in Example 1 were crushed and suspended in 100 ml of distilled water, adjusted to pH 11 with sodium hydroxide. The suspension was heated at 36°C for 60 minutes with vigorous agitation. The suspension was cooled for 5 minutes and a suspension sample was collected. The remaining suspension was filtered through 100 micron filter paper and the filtrate collected. The suspension sample and the filtrate were analyzed by IFRS. Samples were also dried and the residue weight determined. Solutions of β-sitosterol alone, pectin alone and an uncomplexed mixture of β-sitosterol and pectin where used for comparison in the IFRS analysis.

The dry weight of the β-sitosterol/pectin complex and the filtrate indicated that about 50% w/w of the β-sitosterol/pectin complex was solubilized by the solubilization conditions at pH 11. The IFRS analysis indicated that the β-sitosterol/pectin complex retained double and single carbon-carbon bonds different from either β-sitosterol and pectin.

The pH of the distal small intestine is about pH 11. Therefore, the β-sitosterol/pectin complex is expected to be solubilized in the small intestine where it will be available for complexing cholesterol and fat receptors.

Example 4

Five patients were given a 727 mg tablet, prepared as described in Example 1, about 30 minutes before each meal for two weeks. At the end of the two week period serum samples were obtained and analyzed for β-sitosterol by gas chromatography as described by Vanhanen *et al.*, Circulation Supplement II, 2390, (1991), incorporated herein by reference.

No β-sitosterol was detected in the samples, indicating that β-sitosterol was not absorbed from the gut and remains complexed to the pectin.

Example 5

Fourteen patients with mixed lipoproteinemia, on a diet of 500 mg of cholesterol/day, were given a 727 mg tablet of β-sitosterol/pectin complex, prepared as described in Example 1, about 30 minutes before each meal for eight weeks in a double blind, cross-over study. Total cholesterol, LDL and HDL were

performed prior to beginning the administration of the β-sitosterol/pectin complex and at monthly intervals thereafter, were determined enzymatically in an enzyme linked assay using cholesterol esterase, cholesterol oxidase and peroxidase, by methods well know to those skilled in the art and as described in Sigma procedure No. 352 (Sigma Chemical Co., St Louis MO), incorporated herein by reference. Body weight was also determined. The results are set out in Tables I-IV.

Table I

TOTAL CHOLESTEROL				
"Normal" = 100-200[1]	Pre-Admin.	1 Month Admin.	2 Months Admin.	3 Months Admin.
Average	277	231	241	241
Range	192-352			

[1] mg/dl

After administration for 1 month a reduction of 16.4% was observed in the serum cholesterol of the patients in the study. This reduction was maintained over three months.

Table II

LDL CHOLESTEROL				
"Normal" = > 130[1]	Pre-Admin.	1 Month Admin.	2 Months Admin.	4 Months Admin.
Average	223	157	175	156[2]
Range	120-252	118-218	128-224	

[1] mg/dl
[2] results for 2 patients whose LDL cholesterol began at 389 mg/dl

A decrease of 15.2% was observed in LDL cholesterol levels of the patients in the study at 1 month and this decrease was maintained over 4 months.

Table III

HDL RATIOS				
"Normal" =	Pre-Admin.	1 Month Admin.	2 Months Admin.	4 Months Admin.
Average	6.8	6.8	6.7	7.6[1]

[1] for 3 patients

No significant difference was observed in the HDL ratio.

Table IV

BODY WEIGHT			
	Pre-Admin.	1 Month Admin.	2 Months Admin.
Average	82.1[1]	86.6	76.2
Range	57.2-113.9	68.5-100.2	57.2-78

[1] kg

A body weight increase of 5.5% was observed after one month of administration of the β-sitosterol/pectin complex. However, after two months administration there was a decrease of 7.2% from the pre-administration average weight.

The patients in the study were also monitored for liver and kidney function and interference of the β-sitosterol/pectin complex with current medication. No change, during the course of the study, was attributed to the administration of the complex. Additionally, no adverse reactions such as nausea, vomiting, diarrhea, constipation, skin rash, headache, etc. were suffered by the patients during the period they were taking the β-sitosterol/pectin complex.

Therefore, the β-sitosterol/pectin complex is effective in reducing total cholesterol and LDL cholesterol. The β-sitosterol/pectin complex is also effective in body weight reduction.

Example 6

Fat was tagged with retinoic acid and given to a patient with a measured meal, as described in See *et al.*, Clinical Research (April, 1991) incorporated herein by reference. A second patient was given ox bile (cholic acid) in addition

to the tagged fat and a third patient was given β-sitosterol/pectin complex prepared as described in Example 1, in addition to tagged fat, 30 minutes prior to the ingestion of the meal. The serum levels of the retinoic acid tag were determined for each patient at two hourly intervals. The results are summarized in Table V.

Table V

Retinoic Acid Absorption				
	0	2 hr.	4 hr.	6 hr.
Tagged Fat	45[1]	62	67	52
Tagged fat + ox bile	52	55	78	77
Tagged fat + β-sitosterol/pectin	38	47	43	42

[1] μg/dl retinoic acid

The results indicate that tagged fat is taken up and reaches a maximum in 2-4 hours after ingestion. By 6 hours the serum levels have dropped to about baseline. The addition of ox bile results in a greater increase in fat absorption and serum fat levels which remain at an elevated level after 6 hours. In contrast β-sitosterol/pectin complex results in a lower absorption of the retinoic acid tag, about half the amount absorbed when tagged fat was given alone, after two hours. By 4 hours the fat absorption had dropped to baseline indicating that β-sitosterol/pectin complex inhibits dietary fat absorption.

The present invention is not limited to the specific embodiment given. It will be obvious to one skilled in the art that other doses and timing of the dosage could be used to advantage to suit the needs of different individuals and that other excipients could be used. Therefore, the present invention is not intended to be limited to the working embodiments described above. The scope of the invention is defined in the following claims.

WHAT IS CLAIMED IS:

1. A composition for inhibiting absorption of fat and cholesterol from the gut comprising β-sitosterol bound irreversibly to pectin to form a β-sitosterol/pectin complex.

2. A composition as recited in claim 1 wherein β-sitosterol and pectin are present in a ratio of 10 β-sitosterol:1 pectin, by weight.

3. A composition as recited in claim 1 wherein the β-sitosterol/pectin complex is pressed into pills for administration.

4. A composition as recited in claim 3 wherein the pills further comprise an excipient.

5. A composition as recited in claim 4 wherein the excipient are selected for the group consisting of calcium carbonate, silicon dioxide, croscarmellose sodium, magnesium stearate and mixtures thereof.

6. A method for preparing β-sitosterol/pectin complex comprising:
mixing β-sitosterol and pectin;
suspending the β-sitosterol/pectin mixture in water;
heating the suspension to complex β-sitosterol and pectin;
acidifying the complexed β-sitosterol and pectin to esterify the β-sitosterol to the pectin; and
heating to form a β-sitosterol/pectin complex.

7. A method as recited in claim 6 wherein the β-sitosterol and pectin are mixed in a of 10 β-sitosterol:1 pectin, by weight.

8. A method as recited in claim 6 wherein the β-sitosterol/pectin mixture is suspended in 1 volume, with respect to the weight of pectin, of water.

9. A method as recited in claim 6 wherein the β-sitosterol and pectin are heated at 93°C to complex the β-sitosterol and pectin.

10. A method as recited in claim 6 wherein the complexed β-sitosterol and pectin is acidified by the addition of a 0.5 volume, with respect to the weight of pectin, of 4.3% acetic acid.

11. A method as recited in claim 6 wherein the esterified β-sitosterol and pectin is heated at 93°C.

12. A method as recited in claim 6 further comprising sizing the β-sitosterol/pectin complex by pushing the complex through a 20 mesh sieve.

13. A method for reducing serum cholesterol levels comprising administering β-sitosterol bound irreversibly to pectin to a patient suffering from elevated serum cholesterol.

14. A method as recited in claim 13 wherein the β-sitosterol bound irreversibly to pectin is administered orally.

15. A method as recited in claim 13 wherein the β-sitosterol bound irreversibly to pectin is administered about 30 minutes before each meal.

16. A method as recited in claim 13 wherein the β-sitosterol bound irreversibly to pectin is administered in a dose of about 700 mg before each meal.

17. A method for weight reduction comprising administering β-sitosterol bound irreversibly to pectin to a patient desiring a reduction in body weight.

18. A method as recited in claim 17 wherein the β-sitosterol bound irreversibly to pectin is administered orally.

19. A method as recited in claim 17 wherein the β-sitosterol bound irreversibly to pectin is administered about 30 minutes before each meal.

20. A method as recited in claim 17 wherein the β-sitosterol bound irreversibly to pectin is administered in a dose of about 700 mg before each meal.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) A61K 37/00, 31/715; C07G 17/00
US CL :514/26, 54, 824; 536/5, 123.1
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)

U.S. : 514/26, 54, 824; 536/5, 123.1

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 practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,881,005 (THAKKAR ET AL.) 29 APRIL 1975, see col. 1.	1-20
X	US, A, 4,602,003 (MALINOW) 22 JULY 1986, see entire document.	1-20
X	US, A, 4,602,005 (MALINOW) 22 JULY 1986, see entire document.	1-20
A	US, A, 5,112,815 (AMRUS ET AL.) 12 MAY 1992.	
Y,P	US, A, 5,244,887 (STRAUB) 14 SEPTEMBER 1993, see entire document.	1-20
A	Medical Clinics of North America, Volume 66, Number 2, issued March 1982, Kane et al., "Treatment of Hypercholesterolemia", pages 537-550.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Clinical Cardiology, Volume 11, issued 1988, Cerda et al., "The Effects of Grapefruit Pectin on Patients at Risk for Coronary Heart Disease Without Altering Diet or Lifestyle", pages 589-594, see entire document.	1-20
Y	Clinical Cardiology, Volume 11, issued 1988, Baekey et al., "Grapefruit Pectin Inhibits Hypercholesterolemia and Atherosclerosis in Miniature Swine", pages 595-600, see entire document.	1-20
Y	Nutrition Research, Volume 9, No. 3, issued March 1989, Lu et al., "Effects of Dietary Fibers in Early Weaning on Later Response of Serum and Fecal Steroid Levels to High-Cholesterol Diet in Rats", pages 345-352, see entire document.	1-20
Y	Biochimica et Biophysica Acta, Volume 732, issued 1983, Ikeda et al., "Some Aspects of Mechanism of Inhibition of Cholesterol Absorption by β-Sitosterol", pages 651-658, see entire document.	1-20