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(21) International Application Number: PCT/CA00/00357 (22) International Filing Date: 5 April 2000 (05.04.00) (30) Priority Data: 2,267,929 6 April 1999 (06.04.99) CA (71) Applicant (for all designated States except US): B.C. CHEMICALS LTD. [CA/CA]; P.O. Box 6000, Prince George, British Columbia V2N 2K3 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): WONG, Alfred [CA/CA]; 3047 West 6th Avenue, Vancouver, British Columbia V6K 1X4 (CA). BOGUSKI, Wilfred, Nicholas [CA/CA]; P.O. Box 452, Dalmeny, Saskatchewan S0K 1E0 (CA). (74) Agent: TURLOCK, Lance, A.; Davis & Company, 2800 Park Place, 666 Burrard Street, Vancouver, British Columbia V6C 2Z7 (CA).		(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PREPARATION OF SATURATED PHYTOSTEROLS (57) Abstract An end product mixture substantially comprising saturated phytosterols is prepared from a raw material mixture of phytosterols derived from tall oil. At least a substantial portion of the raw material mixture consists of unsaturated phytosterols. The method includes the step of hydrogenating the raw material mixture in a solvent substantially comprising ethyl acetate while in the presence of a hydrogenation catalyst, then filtering and drying the hydrogenation product to separate the mixture of saturated phytosterols from the solvent and to leave an amount of solvent residue in the mixture that is not greater than a desired amount. Hydrogenation is carried out at a temperature greater than 35 deg. C., preferably 40 deg. C or higher, and at a pressure greater than standard pressure, preferably 20 psi or higher. The resulting mixture of saturated phytosterols may be used for various purposes including the manufacture of food additives or dietary supplements with a view to facilitating the reduction of serum cholesterol levels.		

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PREPARATION OF SATURATED PHYTOSTEROLS

FIELD OF THE INVENTION

The present invention relates to the preparation of saturated phytosterols and, more particularly, to a method of preparing an end product mixture of saturated phytosterols, at least a substantial of the mixture consisting of saturated phytosterols, from a raw material mixture of phytosterols derived from tall oil, at least a substantial portion of the raw material mixture consisting of unsaturated phytosterols.

BACKGROUND TO THE INVENTION

It has been known for many years that plant sterols or phytosterols can serve to effectively reduce serum cholesterol levels. In various studies, β -sitosterol and, even more so, its hydrogenated form (β -sitostanol), has been recognized as particularly efficacious.

One important source of phytosterols is tall oil, and there are known methods for isolating phytosterols and their saturated or hydrogenated (stanol) forms either from tall oil soap (sometimes referred to as soap skimmings) or from tall oil pitch.

When phytosterols are isolated from tall oil, a portion of the isolated mix (normally including β -sitosterol and campesterol) will typically include at least some hydrogenated forms of the sterols (viz. β -sitostanol and campestanol). However, given the higher efficacy of β -sitostanol compared to β -sitosterol, it is desirable that substantially all β -sitosterols present in any such mix should be converted to β -sitostanols.

The conversion of a sterol to a stanol is, by definition, hydrogenation. Generally, it is known to hydrogenate a phytosterol or a phytosterol mixture by mixing the phytosterol with a solvent in a reactor in the presence of an appropriate catalyst. However, while hydrogenation is mandated, relatively little attention has been paid to how hydrogenation

may best be achieved, including which solvent may be best suited to the hydrogenation process.

From an economic or commercial point of view, it is highly desirable to effect the hydrogenation of a phytosterol or a phytosterol mix as quickly as possible. In this regard, it is recognized that one possible way to accelerate the hydrogenation process is to carry out the process at an elevated temperature or at an elevated temperature and pressure. However, if the temperature is excessive, then the phytosterols will degrade or break down.

Further, and again from an economic or commercial point of view, it is highly desirable to be able to produce high purity saturated phytosterols while recycling or reusing the hydrogenation solvent with many successive batches of raw material phytosterols. However, the ability to do so can be impaired if the execution of the hydrogenation process introduces impurities or contaminants either as the result of phytosterol break down or otherwise. Impurities or contaminants that are carried with the saturated phytosterols may, at added cost, require removal. Likewise, to extend the lifetime of the hydrogenation solvent, any impurities or contaminants that are carried with the solvent may require removal before the solvent is recycled.

A further important factor that contributes to the overall cost of producing saturated phytosterols is the time that it takes to dry or sufficiently remove the presence of solvent from the end product. When saturated phytosterols are to be used in the preparation of food additives or dietary supplements, government regulations typically will permit the presence of only a very small amount of solvent residue (for example, 50 parts per million).

Accordingly, a primary object of the present invention is to provide a new and improved method for efficiently preparing a high purity end product mixture of saturated phytosterols from a raw mixture of phytosterols derived from tall oil - and one which converts a high percentage of unsaturated phytosterols present in the raw material mixture to corresponding saturated phytosterols.

SUMMARY OF THE INVENTION

In accordance with a broad aspect of the present invention, there is provided a method for the preparation of an end product mixture of phytosterols, at least a substantial portion of the mixture consisting of saturated phytosterols, from a raw material mixture of phytosterols derived from tall oil, at least a substantial portion of the raw material mixture consisting of
5 unsaturated phytosterols, such method comprising the steps of

- (a) hydrogenating the raw material mixture in a solvent substantially comprising ethyl acetate while in the presence of a hydrogenation catalyst to produce a hydrogenation product comprising the end product mixture in the solvent; and,
- 10 (b) filtering and drying the hydrogenation product to separate the end product mixture from the solvent and to thereby leave an amount of solvent residue in the end product mixture that is not greater than a predetermined amount.

During hydrogenation, the temperature is greater than 35 deg. C. (preferably about 40 deg. C. or higher) and the pressure is greater than standard pressure (preferably about 20 psi
15 or higher). Suitable catalysts include platinum dioxide and palladium. However, by reason of its lower cost, the latter is preferred.

After filtering and drying to reduce the amount of solvent and solvent residue, the resulting end product mixture of saturated phytosterols may be used for various purposes including the manufacture of food additives or dietary supplements with a view to facilitating
20 the reduction of serum cholesterol levels.

Herein, and in the claims, it is to be understood that pressures when expressed as "psi" are pressures relative to standard pressure. Thus, for example, 20 psi as used above means 20 psi in excess of standard pressure. Standard pressure is about 14.7 pounds per square inch absolute.

Solvents other than ethyl acetate may be used to prepare a mixture of saturated phytosterols from a mixture of unsaturated phytosterols derived from tall oil. Such other solvents include ethanol and isopropanol. However, ethyl acetate has been found to produce surprisingly unique results. With ethyl acetate, it is possible not only to achieve conversion ratios above 97% and approaching 99% or higher but, in so doing, to produce a resulting saturated phytosterol mixture that has particularly high purity. Moreover, it has been found that a saturated phytosterol mixture produced with ethyl acetate as the solvent can be filtered and dried (e.g. to less than 50 ppm solvent residue) substantially more efficiently than when produced with solvents such as ethanol or isopropanol.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

The FIGURE is a representation of a gas chromatograph (GC) trace obtained upon hydrogenation of a raw material mixture of phytosterols as described below, in part serving to illustrate results that are achieved when (in accordance with the present invention) ethyl acetate is used as the hydrogenation solvent, and also serving to illustrate results that are achieved when (not in accordance with the present invention) ethanol or isopropanol is used as the hydrogenation solvent.

DETAILED DESCRIPTION

As noted above, raw material phytosterol mixtures that are isolated from tall oil typically will include both unsaturated and saturated phytosterols. In more detail, Table 1 below indicates characteristics of raw material crystalline mixes that may be derived from softwood trees indigenous to northern British Columbia (including spruce, lodgepole pine and sub-alpine fir).

TABLE 1

		<u>Typical Values</u>
Phytosterols:		> 95% by weight
5	β -sitosterol	55 - 60% by weight
	β -sitostanol	12 - 18% by weight
	campesterol	12 - 17% by weight
	campestanol	2 - 4% by weight
	other sterols	< 3% by weight
Melting Point		132 - 140 deg. C.

10 Phytosterol mixtures as indicated in Table 1 may be used in the manufacture of cholesterol lowering agents without any hydrogenation step. However, hydrogenation is desirable because β -sitostanol is recognized as having an efficacy significantly higher and an absorption rate significantly lower than β -sitosterol.

15 Raw material mixtures as indicated in Table 1 are themselves considered useful to be used directly for the manufacture of food additives or dietary supplements that have serum cholesterol lowering properties. However, hydrogenation is desirable because β -sitostanol is recognized as having particularly high efficacy and because β -sitosterol is the most plentiful unhydrogenated phytosterol.

20 Several tests were conducted under various conditions of temperature and pressure utilizing raw material (RM) mixes like that indicated in Table 1. In each case, the raw material mixture was first mixed with ethyl acetate (ETAC), then introduced together with a hydrogenation catalyst (CAT) to a stirred reactor where hydrogenation took place under controlled conditions of temperature and pressure. The catalyst used was 5% palladium on a carbon support. Thereafter, the hydrogenation product (viz. the end product mixture of

phytosterols in the solvent) was filtered, cooled, separated and dried by conventional means well known to those skilled in the art.

GC analysis was then used to analyze the end product mixture and to calculate the percentage of stanols present therein. Table 2 summarizes the test conditions and results.

5

TABLE 2

Test	RM/SOL (wt %)	CAT/RM (wt %)	H ₂ press. (psi)	Temp. (°C)	Run Time (min.)	Conversion (%)
2.01	8	10	20	20	120	50
2.02	8	10	20	30	120	73
2.03	8	10	20	40	120	98
2.04	8	10	20	50	120	99
2.05	8	10	40	50	120	99
2.06	8	10	40	65	120	99
2.07	8	10	65	65	120	99
2.08	8	6	75	100	120	97
2.09	8	6	75	100	120	97
2.10	8	6	75	100	120	98
2.11	8	9	75	100	120	97
2.12	8	10	75	100	120	99
2.13	15	10	75	100	120	99
2.14	15	10	75	100	240	100
2.15	8	10	75	100	120	98

In Table 2, the column headed "Conversion" is a calculation of 100% times the amount of saturated phytosterols present in the end product mixture divided by the sum of such amount and the amount of unsaturated phytosterols present in the end product mixtures. The amounts were determined in mg/g from the analysis of the GC traces.

10

From Table 2, it will be noted that there is a rapid decrease in percent conversion as the temperature of hydrogenation drops below 40 deg. C. For this reason, the temperature

used in accordance with the invention is greater than 35 deg. C, and preferably about 40 deg. C. or higher where the percent conversion is in the high 90% range.

Further, although a relatively high percent conversion is shown at 40 deg. C and a hydrogen pressure of 20 psi, it is noteworthy that the percent conversion remains high at elevated temperatures and pressures. This indicates a substantial absence of phytosterol break down under such elevated conditions. Further, since conversion efficiency is approximately the same at 75 psi and 100 deg. C as it is at 20 psi and 40 deg. C, and since the conversions taking place may be accelerated at higher temperatures and pressures, it follows that comparable results should be achieved with run times less than 120 minutes as used in most of the tests.

Apart from the high conversion efficiencies that can be achieved while using ethyl acetate as the hydrogenation solvent, it has also been found that required drying times are significantly less than is required with other solvents such as ethanol and isopropanol. By way of example, a sample of raw material like that shown in Table 1 that was hydrogenated in ethyl acetate in the manner described above was dried for 2 hours at 105 deg. C under an absolute pressure of 25 mmhg. The result was fine white phytosterol crystals containing less than 50 ppm ethyl acetate. In comparison, the amount of solvent residue remaining in like samples that were hydrogenated in ethanol and in isopropanol exceeded ten times this amount after drying for twice as long.

Reduced drying time represents a significant advantage that ethyl acetate provides over solvents such as ethanol and isopropanol. However, it has also been found the use of ethyl acetate mitigates against impurities that tend to appear in the end product mixture when ethanol or isopropanol is used.

More particularly, reference is now made to the FIGURE. Except as noted below, the FIGURE is generally representative of GC traces that will be obtained upon hydrogenation of a raw material mixture like that indicated in Table 1. As shown, the trace includes three major peaks:

Peak 1	Internal Standard (cholesterol)
Peak 2	Campestanol
Peak 3	β -sitostanol

Smaller peak 4 to the right of peak 3 is representative of other sterols. Peak 5 to the
5 left of peak 1 represents undesirable impurities. Although there is a degree of uncertainty, it
is thought to be generated by dehydroxylated forms of sterols (campestane and stigmastane).

In relation to the present invention, the most significant observation to be made with
respect to the FIGURE is that peak 5 indicating the presence of impurities does not appear in
the case of hydrogenation with ethyl acetate whereas it does appear in the cases of
10 hydrogenation with either ethanol or isopropanol.

WE CLAIM:

1. A method for the preparation of an end product mixture of phytosterols, at least a substantial portion of said mixture consisting of saturated phytosterols, from a raw material mixture of phytosterols derived from tall oil, at least a substantial portion of said raw material mixture consisting of unsaturated phytosterols, said method comprising the steps of:
 - (a) hydrogenating said raw material mixture in a solvent substantially comprising ethyl acetate while in the presence of a hydrogenation catalyst to produce a hydrogenation product comprising said end product mixture in said solvent, said hydrogenating step being carried out at a temperature greater than 35 deg. C. and a pressure greater than standard pressure; and,
 - (b) filtering and drying said hydrogenation product to separate said end product mixture from said solvent and to thereby leave an amount of solvent residue in said end product mixture that is not greater than a desired amount.
2. A method as defined in claim 1, wherein said hydrogenating step is carried out at a temperature of about 40 deg. C.
3. A method as defined in claim 1, wherein said hydrogenating step is carried out at a temperature of at least 40 deg. C.
4. A method as defined in claim 1, wherein said hydrogenating step is carried out at a temperature of about 100 deg. C.
5. A method as defined in claim 1, wherein said hydrogenating step is carried out at a temperature of at least 100 deg. C.
6. A method as defined in any one or more of claims 1 to 5, wherein said hydrogenating step is carried out at a pressure of about 20 psi.

7. A method as defined in any one or more of claims 1 to 5, wherein said hydrogenating step is carried out at a pressure of at least 20 psi.
8. A method as defined in any one or more of claims 1 to 5, wherein a substantial portion of said raw material mixture is β -sitosterol.
- 5 9. A method as defined in any one or more of claims 1 to 5, wherein a substantial portion of said raw material mixture is β -sitosterol, and wherein said hydrogenating step is carried out at a pressure of about 20 psi.
10. A method as defined in any one or more of claims 1 to 5, wherein a substantial portion of said raw material mixture is β -sitosterol, and wherein said hydrogenating step is
10 carried out at a pressure of at least 20 psi.
11. A method as defined in any one or more of claims 1 to 10 wherein said hydrogenation catalyst is palladium.
12. A mixture of phytosterols prepared from tall oil in accordance with the method as defined in any one or more of the preceding claims.

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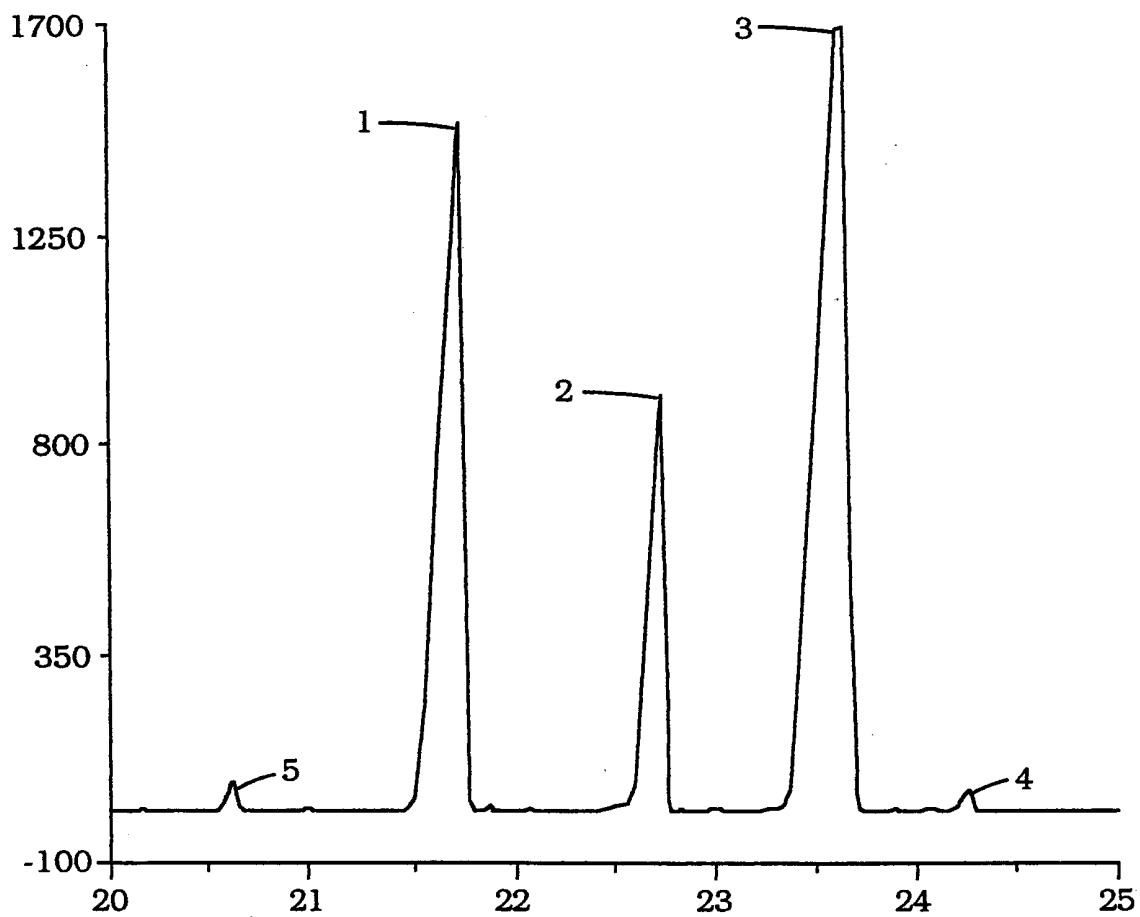


FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 00/00357

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07J9/00 C11B13/00 A61K31/575 A61P9/10

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 C07J C11B A61K

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)

EPO-Internal, CHEM ABS Data, PAJ, WPI Data, PAPERCHEM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 38206 A (EKBLÖM JARI ; RAISIO BENECOL LTD (FI)) 3 September 1998 (1998-09-03)	12
Y	examples 1,5	1-11
X	US 5 244 887 A (STRAUB CARL D) 14 September 1993 (1993-09-14)	12
Y	column 3, paragraph 3 column 4, line 25 - line 30 column 5, paragraph 2 - paragraph 3	1-11
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

24 August 2000

Date of mailing of the international search report

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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	CHEMICAL ABSTRACTS, vol. 70, no. 24, 16 June 1969 (1969-06-16) Columbus, Ohio, US; abstract no. 109125, ALLAIS, JEAN P. ET AL: "Preparation of pure.beta.- sitosterol by thin-layer chromatography on silver nitrate impregnated alumina" XP002145680 abstract & QUAL. PLANT. MATER. VEG. (1968), 16(1-4), 215-22 ,	1-11
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P,Y	examples 1,7	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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