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(57) **Abrégé/Abstract:**

The present invention discloses novel method for synthesizing vegan cholesterol and vitamin D3 from inexpensive crude phytosterol.

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(54) Title: SYNTHESIS OF CHOLESTEROL AND VITAMIN D3 FROM PHYTOSTEROLS

(57) Abstract: The present invention discloses novel method for synthesizing vegan cholesterol and vitamin D3 from inexpensive crude phytosterol.

WO 2021/005618 A1

“SYNTHESIS OF CHOLESTEROL AND VITAMIN D3 FROM PHYTOSTEROLS”

Technical filed:

The present invention relates to novel method for synthesizing vegan cholesterol and vitamin D3 from inexpensive crude phytosterols.

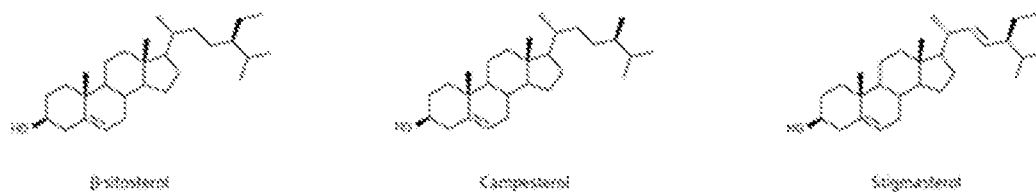
Background and prior art:

Cholesterol is a precursor to vitamin D, inhibiting the synthesis of cholesterol will also inhibit the synthesis of vitamin D. Since sunlight is required to turn cholesterol into vitamin D, avoiding the sun will likewise undermine our ability to synthesize vitamin D.

Phytosterols are natural compounds structurally similar to mammalian cell-derived cholesterol. The best dietary sources of phytosterols are unrefined vegetable oils, seeds, cereals, nuts, and legumes.

Cholesterol and plant sterols are structurally similar, but undergo strikingly different metabolic fates in mammals. Phytosterol is a mixture of sterols i.e. a mixture of stigmasterol, beta-sitosterol, campesterol and stigmasterol, as shown in figure 1.

Figure 1:



Stigmasterol is an unsaturated sterol, having one double bond in the sterol ring structure and one double bond in the side chain. Beta sitosterol is an unsaturated sterol with one double bond in sterol ring structure. Campesterol is structurally

similar to beta sitosterol but has methyl group substituent at C24 position instead of an ethyl group. Stigmastenol is a saturated sterol both in ring structure and in the side chain.

US3920531A discloses a process for the production of hydroxylated derivatives of cholesterol and 7-dehydrocholesterol which comprises subjecting a saturated unsubstituted or substituted derivative of cholestane, dissolved in a suitable solvent, in the presence of peracetic acid, to irradiation with ultraviolet radiation having a wavelength less than 350 nm, separating the reaction products, which if desired, are converted to other derivatives, and recovering the residue of the starting material.

US3822254 discloses a process for the preparation of 25-hydroxycholesterol and its esters starting from the naturally occurring (readily available and inexpensive) starting material, stigmasterol which is isolated commercially from Soybeans. The synthesis encompasses, as key steps, the protection of the 3-hydroxy- Δ^5 -function by formation of an i-steroid, cleavage of the 22, 23-double bond, and introduction of the properly substituted 5-carbon fragment to afford the 25-hydroxycholesterol side chain.

Synthesis of cholesterol from methods such as Robinson synthesis and Woodward synthesis are well reported. WO2016096989A1 discloses method of extracting cholesterol from fish oil, especially from fish oil waste residue by saponification followed by extraction with at least one non-water miscible solvent, at a temperature of above 30°C.

US5128162A discloses methods for removing cholesterol from edible oils.

WO1995014032A1 discloses a process for producing a 25-hydroxycholesterol by hydroxylating a cholesterol at the 25-position using a ruthenium compound as a catalyst.

WO2016096988 discloses method of extracting cholesterol from milk fat.

Synthesis of vitamin D3 from various sources are reported in the prior art. However, there is no report for the synthesis of cholesterol and vitamin D3 from phytosterols as source.

Therefore, it is an objective of the present invention to provide a robust synthesis for the manufacture of cholesterol and vitamin D3 starting from an inexpensive source, i.e., naturally occurring phytosterols.

Summary of the invention:

In line with the above objective, the present invention provides a synthetic process for commercial manufacture of Vitamin D3 from phytosterols.

Accordingly, the synthetic process for commercial manufacture of Vitamin D3 from phytosterols, which comprises;

- a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate (1);
- b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether (2);
- c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane (3);
- d) Tosylating the (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane with a tosylating agent in a base to obtain (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (4);
- e) Subjecting the (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)-3 α ,5-cyclo-5 α -pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me₂S catalyst to obtain i-Cholesteryl methyl ether (5);

- f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol (6) followed by converting into cholesterol acetate (7) by treatment with acetic anhydride in presence of a base at a temperature range of 30 to 70°C;
- g) Subjecting the cholesterol acetate to bromination by treating with a brominating agent in a hydrocarbon solvent to obtain 7-Bromo cholesteryl acetate followed by treating with TBAF in an ether solvent at a temperature range of 10 to 30°C to obtain 7-dehydrocholesterol acetate;
- h) subjecting the 7-dehydrocholesterol acetate to alkaline hydrolysis in an alcoholic solvent at about 30 to 60°C to obtain 7-dehydrocholesterol (8); and
- i) Converting 7-dehydrocholesterol into vitamin D3 by irradiating under high-pressure mercury lamp in a solvent in presence of sensitizer.

In another aspect, the present invention provides a synthetic process for commercial manufacture of cholesterol from phytosterols.

Accordingly, the synthetic process for commercial manufacture of cholesterol from phytosterols, which comprises;

- a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate (1);
- b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether (2);
- c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane (3);

- d) Tosylating the (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane with a tosylating agent in a base to obtain (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (4);
- e) Subjecting the (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me₂S catalyst to obtain i-Cholesteryl methyl ether (5);
- f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol (6).

Detailed description:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and appreciated.

The present invention provides a synthetic process for commercial manufacture of Vitamin D3 from phytosterols.

Phytosterols are globally manufactured by various companies such as ADM, Cargill, BASF, Raisio, DuPont, Arboris, PrimaPharm, FenchChem, DRT and Lipofoods. The phytosterols samples used to carry out the present invention is sourced from USA, Germany and Bangladesh.

Accordingly, the synthetic process for commercial manufacture of Vitamin D3 from phytosterols, which comprises;

- a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate (1);
- b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether (2);
- c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by

treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane (**3**);

- d) Tosylating the (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane with a tosylating agent in a base to obtain (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (**4**);
- e) Subjecting the (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me₂S catalyst to obtain i-Cholesteryl methyl ether (**5**);
- f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol (**6**) followed by converting into cholesterol acetate (**7**) by treatment with acetic anhydride in presence of a base at a temperature range of 30 to 70°C;
- g) Subjecting the cholesterol acetate to bromination by treating with a brominating agent in a hydrocarbon solvent to obtain 7-Bromo cholesteryl acetate followed by treating with TBAF in an ether solvent at a temperature range of 10 to 30°C to obtain 7-dehydrocholesterol acetate;
- h) subjecting the 7-dehydrocholesterol acetate to alkaline hydrolysis in an alcoholic solvent at about 30 to 60°C to obtain 7-dehydrocholesterol (**8**); and
- i) Converting 7-dehydrocholesterol into vitamin D₃ by irradiating under high-pressure mercury lamp in a solvent in presence of sensitizer.

The reaction of step a) can be conducted from room temperature to reflux temperature of the solvent.

The base used in steps a); step b); step d); step f) is an organic base such as pyridine, triethyl amine or DMAP or an inorganic base such as alkali or alkaline earth metals hydroxides, carbonates or bicarbonates. In a preferred embodiment, the base is an organic base selected from pyridine, triethyl amine or DMAP.

The phytosteryl tosylate obtained in step a) is a mixture of stigmasteryl tosylate, sitosteryl tosylate, and campesteryl tosylate.

The reaction of step b) can be conducted at a temperature range of 40°C to reflux temperature of the solvent used.

The phytosteryl i-methyl ether obtained step b) is a mixture of stigmasteryl-i-methyl ether, sitosteryl-i-methyl ether and campesteryl-i-methylether.

The solvent used in step c) is selected from methylene chloride, ethylene chloride, methanol, ethanol, isopropanol, water or mixtures thereof. In a preferred embodiment, the solvent is a mixture of methylene chloride and methanol.

The reducing agent used in step c) is selected from sodium borohydride, lithium aluminium hydride, sodium hydride etc.

In an embodiment, after isolation of (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane (3) obtained in step c) using column chromatography, the fractions containing sitosterol- i-methyl ether are heated in aqueous dioxane in presence of catalytic PTSA at 100°C for 2h, followed by removal of solvent and crystallization in methanol to yield phytosterol as colourless solid which is free from stigmasterol.

The p-toluene sulfonyl halide in step a) and step d) is selected from p-toluene sulfonyl chloride, p-toluene sulfonyl bromide and p-toluene sulfonyl iodide.

The tosylating in step d) may be conducted at a temperature range of -5 to 5°C.

The reaction in step e) may be conducted at a temperature range of 40 to 70°C.

The ether solvent used in step e) and step g) is selected from diethylether, diisopropylether or THF.

The brominating agent used in step g) is selected from bromine, NBS or DDH (1,3-dibromo-5,5-dimethyl hydantoin and the hydrocarbon solvent is pet ether.

In an embodiment, since the 7- bromo cholesterol acetate is unstable; directly used as such for next reaction without isolation or further purification.

The alcoholic solvent in step h) is selected from methanol, ethanol and isopropanol.

The solvent in step i) is selected from an alcoholic solvent like methanol, ethanol, isopropanol; ethers such as diethylether, diisopropylether, THF; esters such as ethylformate, ethylacetate etc.; nitriles such as acetonitrile etc. and hydrocarbon solvents such as methylenechloride, ethylenedichloride, chloroform, benzene, toluene etc.

The sensitizer used in step i) is selected from 5-(3-pyridyl)-2,2'-bithiophene, 2,5-Di(Pyridin-3-yl) thiophene; 2,5-diphenyl thiophene; 5-(2-Pyridyl)-2,2'-Bithiophene; 5-(4-Pyridyl)-2,2'-Bithiophene; 2-Phenyl-5-(2-Pyridyl)Thiophene; 2-Phenyl-5-(3-Pyridyl)-Thiophene; 2-Phenyl-5-(4-Pyridyl)-Thiophene; 2,2'-5',2''-Terthiophene; and 2,5-Di(2-thienyl) Furane.

In yet another preferred embodiment, the invention provides method of irradiation of 7-dehydrocholesterol, which method comprises;

- a) Irradiating 7-dehydrocholesterol in a solvent with a high-pressure mercury lamp;
- b) Adding a sensitizer to convert tachysterol formed during the irradiation into preD3;
- c) Concentrating and storing the irradiated solution at $\leq -10^{\circ}\text{C}$ overnight to crystallize the non-converted 7-dehydrocholesterol followed by filtrating the same;
- d) Dissolving the concentrated filtrate in an organic solvent followed by heating to obtain cholecalciferol; and

e) Purifying the cholecalciferol from mixture of acetone and water.

The solvent used in step a) may be selected from an alcoholic solvent like methanol, ethanol, isopropanol; ethers such as diethylether, diisopropylether, THF; esters such as ethylformate, ethylacetate etc.; nitriles such as acetonitrile etc. and hydrocarbon solvents such as methylenechloride, ethylenedichloride, chloroform, benzene, toluene etc.

The sensitizer used in step i) is selected from 5-(3-pyridyl)-2,2'-bithiophene, 2,5-Di(Pyridin-3-yl) thiophene; 2,5-diphenyl thiophene; 5-(2-Pyridyl)-2,2'-Bithiophene; 5-(4-Pyridyl)-2,2'-Bithiophene; 2-Phenyl-5-(2-Pyridyl)Thiophene; 2-Phenyl-5-(3-Pyridyl)-Thiophene; 2-Phenyl-5-(4-Pyridyl)-Thiophene; 2,2'-5',2''-Terthiophene; and 2,5-Di(2-thienyl) Furane.

The organic solvent in step d) may be a hydrocarbon or halogenated hydrocarbon. Preferably, the organic solvent is hydrocarbon, more preferably toluene.

In another aspect, the present invention provides a synthetic process for commercial manufacture of cholesterol from phytosterols.

Accordingly, the synthetic process for commercial manufacture of cholesterol from phytosterols, which comprises;

- a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate **(1)**;
- b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether **(2)**;
- c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane **(3)**;

- d) Tosylating the (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane with a tosylating agent in a base to obtain (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (**4**);
- e) Subjecting the (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me₂S catalyst to obtain i-Cholesteryl methyl ether (**5**);
- f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol (**6**).

The reaction of step a) can be conducted from room temperature to reflux temperature of the solvent.

The base used in steps a); step b); step d); step f) is an organic base such as pyridine, triethyl amine or DMAP or an inorganic base such as alkali or alkaline earth metals hydroxides, carbonates or bicarbonates. In a preferred embodiment, the base is an organic base. The preferred base is pyridine or DMAP.

The reaction of step b) can be conducted at a temperature range of 40°C to reflux temperature of the solvent used.

The solvent used in step c) is selected from methylene chloride, ethylene chloride, methanol, ethanol, isopropanol, water or mixtures thereof. In a preferred embodiment, the solvent is a mixture of methylene chloride and methanol.

The reducing agent used in step c) is selected from sodium borohydride, lithium aluminium hydride, sodium hydride etc.

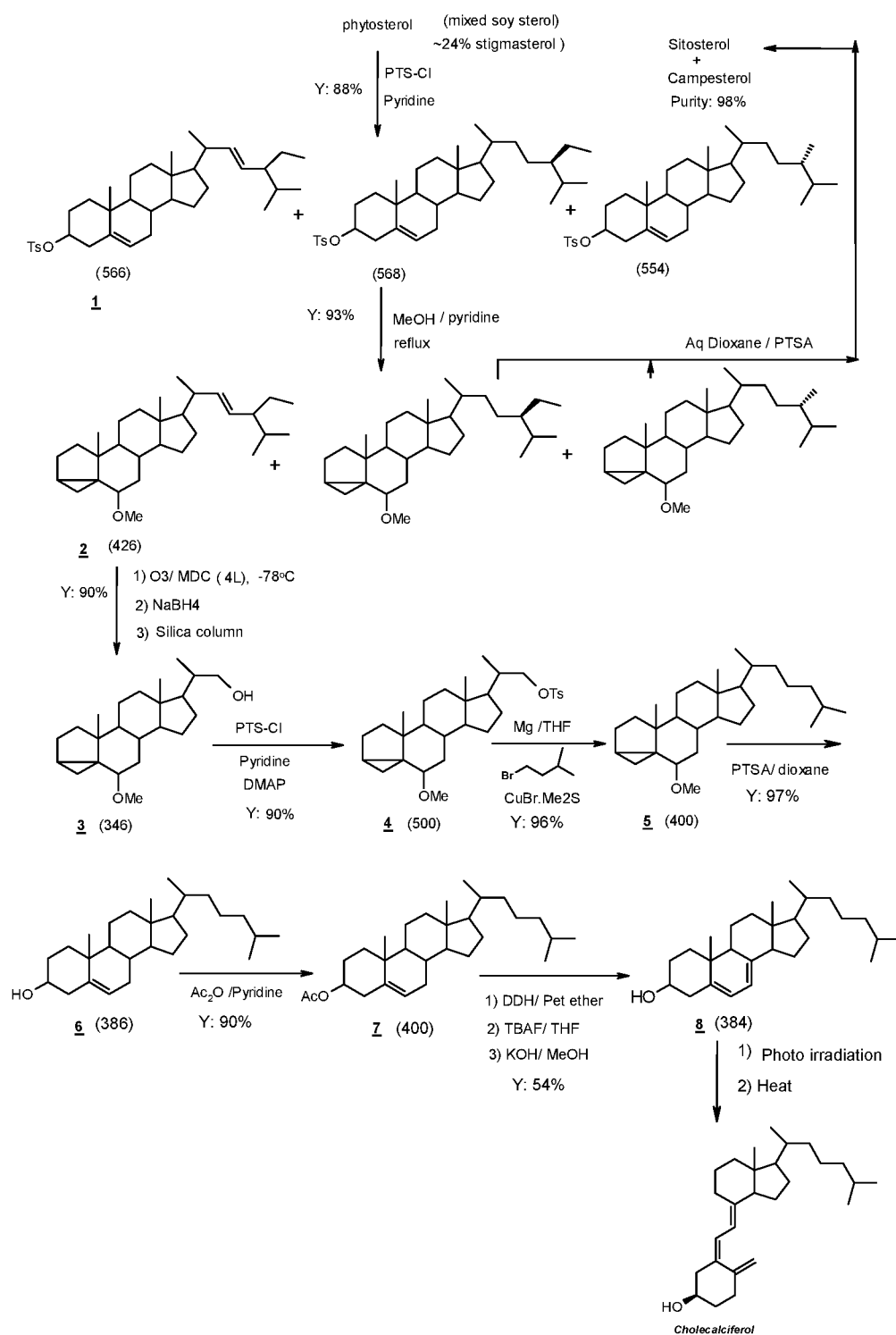
The p-toluene sulfonyl halide in step a) and step d) is selected from p-toluene sulfonyl chloride, p-toluene sulfonyl bromide and p-toluene sulfonyl iodide.

The tosylating in step d) may be conducted at a temperature range of -5 to 5°C.

The reaction in step e) may be conducted at a temperature range of 40 to 70°C.

The ether solvent used in step e) is selected from diethylether, diisopropylether or THF.

Synthesis of Cholesterol / Vitamin D3 from phytosterol according to the present invention is shown in scheme 3.



The present invention is exemplified by the following examples which are provided for illustration only and, should not be construed to limit the scope of the invention.

Example 1

Preparation of Phytosteryl tosylate (1)

To a solution of 500.0 g(1.20mol) of Phytosterol in 5000 ml of dry pyridine was added 500.0 g (2.62 mol) of p-toluene sulfonyl chloride and the mixture was stirred at 25° C for 16 hrs. Pyridine was removed by vacuum distillation and the residue was slowly poured into 10% sodium carbonate solution. The precipitated product was collected by filtration, washed with water followed by methanol and dried in vacuum overnight to yield 600.0 g. of phytosteryl tosylate, which was used for next step without further purification.

Yield: 600 g (88 %)

Appearance: White solid

GC analysis: Stigmasteryl tosylate: 20.37% (RT: 6.10)

Sitosteryl tosylate: 42.29%(RT:6.80)

Campesteryl tosylate: 15.60%(RT:5.76)

Example 2

Preparation of Phytosteryl-i- methyl ether (2)

A mixture of 600.0 g (1.06 mol) of phytosteryl tosylate in 5500 ml of methanol and 300 g (3.79 mol) of pyridine was stirred at 55°C for 5 hrs. The cooled solution was concentrated under reduced pressure. The residue was poured into water and extracted with dichloromethane. The dichloromethane solution was dried over anhydrous sodium sulfate and evaporated to dryness to yield 420.0 g. of colorless thick oil, which was used for next step without further purification.

Yield: 420 g (93 %)

Appearance: colorless thick oil

GC analysis: Stigmasteryl-i-methyl ether: 19.49% (RT: 5.47)

Sitosteryl-i-methyl ether: 48% (RT 6.05)

Campesteryl-i-methylether: 15.08% (RT: 6.34)

Example 3

Preparation of (20S)-20-hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane (3)

A solution of 420.0 g (0.98 mol) of phytosteryl-i-methyl ether in 4000 ml of methylene chloride and 1300 ml of methanol was cooled to -78⁰ C and treated with ozonized oxygen for 3-4 h. The reaction vessel was flushed with nitrogen and 42 g (1.11 mol) of sodium borohydride was added. The mixture was stirred at -50⁰ C for 1 h and then allowed to warm to 0⁰C over a 1 h period. Water was added slowly to decompose the excess hydride and the product was extracted with methylene chloride. The methylene chloride solution was washed with brine solution. The methylene chloride solution was then dried over anhydrous sodium sulfate and evaporated to dryness. The 400 g of crude reaction mass was purified by column chromatography using silica gel to get 60.0 g. of (20S)-20-hydroxy methyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane.

Yield: 60 g (90 %)

Appearance: colorless solid

GC analysis: 93.6% purity (RT 3.35)

Fractions containing sitosterol- i-methyl ether collected separately and concentrated to get thick oil which upon heating in aqueous dioxane in presence of catalytic PTSA at 100⁰C for 2h, followed by removal of solvent and crystallization in methanol gave 230 g of colourless solid as phytosterol free from stigmasterol.

Yield: 60g (90%)

Appearance: colorless solid

GC analysis: >95% purity

Example 4

Preparation of (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (4)

To a solution of 60 g (0.172 mol) of (20S)-20 hydroxymethyl-6 β -methoxy-3 α ,5-cyclo-5 α -pregnane in 600 ml. of pyridine was added slowly 60 g. (0.314 mol) of

p-toluene sulfonyl chloride at 0⁰ C. The mixture was stirred at 0⁰ C for 5 h. Several chips of ice were added, and the mixture was stirred for 5 minutes to decompose the excess p-toluene sulfonyl chloride. The mixture was poured into water and the product was extracted with methylene chloride. The methylene chloride solution was washed with water and brine solution. The solution was dried over anhydrous sodium sulfate and evaporated to dryness to yield 78.0 g white solid of (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)-3 α , 5-cyclo-5 α -pregnane, which was used for next step without further purification.

Yield: 78 g (90 %)

Appearance: colorless solid

Example 5

Preparation of i-Cholesteryl methyl ether (5)

To stirred magnesium turnings (54.0 g, 2.25 mol) in THF (750 ml) few drops of 1,2-dibromoethane were added under Nitrogen atmosphere followed by few drops of isopentyl bromide and heated to 50⁰C for few minutes to initiate reaction then a remaining solution of isopentyl bromide (78.0 g, 0.51 mol) was added drop wise under N₂. After being stirred at the same temperature of 50⁰C for 60 min, the reaction mixture was cooled at 0 ⁰C and a suspension of CuBr·Me₂S (4.0g, 0.01 mol) was added and a solution of (20S)-6 β -methoxy-20-(p-toluene sulfonyl methyl)- 3 α ,5-cyclo-5 α -pregnane (**4**) (78g, 0.156 mol) in THF (300 mL) was added drop wise at 0 ⁰C under N₂. After being stirred at room temperature for 2-3 h, the reaction mixture was poured into saturated aqueous NH₄Cl at 0 ⁰C and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with saturated aqueous NH₄Cl and brine and dried over Na₂SO₄. The obtained mixture was filtered and concentrated in vacuo. The solid product obtained was stirred in methanol at room temperature for 30 min filtered and dried under vacuum to get white solid of IME cholesterol.

Yield: 60 g (96 %)

Appearance: White solid

GC analysis: 94.97% (RT: 5.09)

Example 6:**Preparation of Cholesterol (6)**

The i-cholesteryl methyl ether 60 g (0.15 mol) was dissolved in aqueous dioxane (9:1) 600mL and heated at 80°C in presence of catalytic PTSA until completion of starting material (~3h). Aqueous work up of reaction mixture, followed by extraction with ethyl acetate and removal of solvent yielded cholesterol. Crystallization was carried out in methanol gave cholesterol as white solid (56g).

Yield: 56g (97 %)

Appearance: White solid

M.pt: 149°C

$[\alpha]_{20}^D$: -34° (C=2, CHCl₃)

HPLC analysis: 99.17(RT: 14.22)

GC analysis: 99.03(RT: 7.59)

IR(Neat): 3481,2933,2866,1466,1337,1056,839,799,594.

¹HNMR (400MHz, CDCl₃):

δ 5.35 (m, 1H), 3.55 (m, 1H), 0.94 (s, 3H), 0.94 (d, J = 4.9 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.67 (s, 3H)

¹³C NMR (400 MHz, CDCl₃):

140.7,121.6,71.7,56.7,56.1,50.1,42.3,42.2,39.7,39.5,37.2,36.4,36.1,35.7,31.8,31.6, 28.2,27.9,24.2 23.8,22.8,22.5,21.0,19.3,18.7,11.8.

Example 7:**Preparation of Cholesterol acetate (7)**

Cholesterol (56g, 0.145 mol) was suspended in petroleum ether (400 mL) followed by addition of acetic anhydride (31.36g, 0.30mol) and DMAP (20.0g,0.163 mol) at room temperature and stirred at 40-60°C for 2-4h until completion of starting material. Reaction mixture was washed with water (50 mL X 2) followed by saturated salt solution (100 mL), followed by (50mLX2) 10% sodium bicarbonate solution and finally with water, dried the solvent over anhydrous Na₂SO₄ and removed the solvent to get white solid, which upon crystallization in methanol gave pure cholesterol acetate as white solid (56 g).

Yield: 56g (~90%)

M.Pt: 111-114°C

$[\alpha]_{20}^D$: -44° (C=2, CHCl₃)

Appearance: White solid.

HPLC analysis: >95%

Example 8:

Preparation of 7-Dehydrocholesterol (8)

To a stirred solution of cholesterol acetate (50 g 0.11 mole) in 500 mL pet ether was added DDH (1,3-dibromo-5,5-dimethyl hydantoin, (16.4 g, 0.057 mole) followed by catalytic perkadox as initiator. Reaction mixture was heated to reflux at 65°C, until completion of starting material, monitored by TLC and HPLC. Reaction was quenched by adding water and extracted with pet ether, washed the pet ether layer with water, dried the pet ether layer over anhydrous Na₂SO₄ and removed the solvent to get crude 7- bromo cholesterol acetate as a pale yellow thick oil (~ 50g), used as such for next reaction without further purification.

To a stirred cooled solution of 7-Bromo cholesteryl acetate (50 g, 0.176mole) in dry THF (200 mL) was added anhydrous TBAF (100g, 0.317 mole) dissolved in dry THF (400 mL). Stirred the reaction mixture at 20°C for 2h. Reaction was monitored by HPLC. Reaction was quenched by adding water and extracted with ethyl acetate (3X200mL). Washed the ethyl acetate layer with brine solution (200mL) and dried over anhydrous Na₂SO₄ and removed the solvent to get crude as colourless solid, stirred with methanol and filtered the solid, dried and used for saponification step.

Yield: 30 g

7-Dehydrocholesterol acetate (30 g, 0.07 mole) was suspended in methanol (300 mL) and KOH (20g, 0.36mole) added and heated the reaction mixture at 45°C for 2h. Reaction was monitored by TLC. Solvent removed by distillation and added water to residual mass and extracted with dichloromethane. Dichloromethane layer washed with brine and dried over anhydrous Na₂SO₄, filtered and solvent removed to get crude product, crystallized in methanol/ acetone to get pure 7-dehydrocholesterol as white solid (23g).

Yield: 23 g (54 %, after two steps))

M.pt: 145°C

Appearance: White solid

$[\alpha]_D^{27} = -120^\circ$ (c = 1, CHCl₃)

HPLC analysis: 96.5%(RT:11.13)

GC analysis: 98.5% (RT: 7.44)

¹H NMR (400 MHz, CDCl₃):

δ 5.57 (m, 1H), 5.39(m, 1H), 3.66 (m, 1H), 0.94 (s, 3H), 0.94 (d, J = 4.9 Hz, 3H), 0.87 (d,J = 6.4 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.61 (s, 3H)

¹³C NMR (400 MHz, CDCl₃):

141.1,139.7,119.6,116.2,70.4,55.9,54.5,46.2,42.9,40.7,39.5,39.2,38.3,37.0,36.1,36.1,31.9,28.0,28.0,23.8,23.0,22.8,22.5,21.1,18.8,16.2,11.8.

Example 9:

Preparation of Vitamin D3(Cholecalciferol):

In a 1.5 litre tetrahydrofuran was dissolved 46.8 g of 7-dehydro cholesterol of example 8, and the mixture was stirred in a magnetic stirrer under nitrogen atmosphere. To the mixture was added 1g of butylated hydroxy toluene (BHT). During the irradiation, besides pre D3, tachysterol also formed, hence 1g of sensitizer 5-(3-pyridyl)-2,2'-bithiophene was added to convert the formed tachysterol into preD3 and stirred for a while to obtain clear solution. Falling film apparatus was used to irradiate the solution continuously for 180 minutes at room temperature and the irradiation was carried out using ultra violet rays from the high pressure mercury lamp of wave length 250-360 nm. The content of the previtamin D derivative of intended product was determined and monitored by high performance liquid chromatography (HPLC). After 180 minute irradiation reaction mixture was cooled to 0-5°C for 30 minutes. The solid separated out was filtered as first crop containing 7-Dehydrocholesterol (50-60%).

The filtrate was concentrated to 20% of the original volume, cooled to 0-5°C for 24 hrs and the separated solid was filtered as second crop that contained 7-Dehydrocholesterol (15-20%).

The first and second crops were combined and reused in subsequent batches.

The filtrate was then evaporated under vacuum at 40-45°C to get the crude compound which was then dissolved in 200mL toluene and refluxed for 1-2 hr.

The solvent was concentrated completely under vacuum to get crude reddish orange sticky mass (resin). Yield: 20 g

The residue was purified by column chromatography over silica gel with Toluene: methyl ketone 1:99, 2:98, 4:96 & 5: 95 to isolate pure Vitamin D₃ /cholecalciferol crystals which were recrystallized in acetone/water twice, filtered and dried to get highly pure crystals of Cholecalciferol

The HPLC analysis of the vitamin D₃ crystals are shown in Table 1 as shown below.

Table 1:

S no	Compound	% by HPLC	Potency
1	Vitamin D ₃ /Cholecalciferol	98-99.5%	40 MIU
2	7-Dehydrocholesterol	ND	NA
3	Tachysterol	0.01%	NA
4	Lumisterol	0.01%	NA
5	Trans Vitamin D ₃	0.05%	NA

We claim,

1. A synthetic process for commercial manufacture of Vitamin D3 from phytosterols, which comprises;
 - a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate **(1)**;
 - b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether **(2)**;
 - c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane **(3)**;
 - d) Tosylating the (20S)-20-hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane with a tosylating agent in a base to obtain (20S)-6β-methoxy-20-(p-toluene sulfonyl methyl)- 3α,5-cyclo-5α-pregnane **(4)**;
 - e) Subjecting the (20S)-6β-methoxy-20-(p-toluene sulfonyl methyl)- 3α,5-cyclo-5α-pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me2S catalyst to obtain i-Cholesteryl methyl ether **(5)**;
 - f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol **(6)** followed by converting into cholesterol acetate **(7)** by treatment with acetic anhydride in presence of a base at a temperature range of 30 to 70°C;
 - g) Subjecting the cholesterol acetate to bromination by treating with a brominating agent in a hydrocarbon solvent to obtain 7-Bromo cholesteryl acetate followed by treating with TBAF in an ether solvent at a temperature range of 10 to 30°C to obtain 7-dehydrocholesterol acetate;

- h) subjecting the 7-dehydrocholesterol acetate to alkaline hydrolysis in an alcoholic solvent at about 30 to 60°C to obtain 7-dehydrocholesterol (8); and
- i) Converting 7-dehydrocholesterol into vitamin D3 by irradiating under high-pressure mercury lamp in a solvent in presence of sensitizer.
2. The process as claimed in claim 1, wherein, the reaction of step a) is conducted from room temperature to reflux temperature of the solvent.
 3. The process as claimed in claim 1, wherein, the base used in steps a); step b); step d); step f) is an organic base selected from pyridine, triethyl amine or DMAP or an inorganic base selected from alkali or alkaline earth metals hydroxides, carbonates or bicarbonates.
 4. The process as claimed in claim 3, wherein, the base is an organic base selected from pyridine, triethyl amine or DMAP.
 5. The process as claimed in claim 1, wherein, the phytosteryl tosylate (1) obtained in step a) is a mixture of stigmasteryl tosylate, sitosteryl tosylate, and campesteryl tosylate.
 6. The process as claimed in claim 1, wherein, the reaction of step b) is conducted at a temperature range of 40°C to reflux temperature of the solvent used.
 7. The process as claimed in claim 1, wherein, the phytosteryl i-methyl ether obtained step b) is a mixture of stigmasteryl-i-methyl ether, sitosteryl-i-methyl ether and campesteryl-i-methylether.
 8. The process as claimed in claim 1, wherein, the solvent used in step c) is selected from methylene chloride, ethylene chloride, methanol, ethanol, isopropanol, water or mixtures thereof and the reducing agent is selected from sodium borohydride, lithium aluminium hydride, sodium hydride etc.
 9. The process as claimed in claim 1, wherein, the p-toluene sulfonyl halide in step a) and step d) is selected from p-toluene sulfonyl chloride, p-toluene sulfonyl bromide and p-toluene sulfonyl iodide.

10. The process as claimed in claim 1, wherein, the tosylating in step d) is conducted at a temperature range of -5 to 5°C.
11. The process as claimed in claim 1, wherein, the reaction in step e) is conducted at a temperature range of 40 to 70°C.
12. The process as claimed in claim 1, wherein, the ether solvent used in step e) and step g) is selected from diethylether, diisopropylether or THF.
13. The process as claimed in claim 1, wherein, the brominating agent used in step g) is selected from bromine, NBS or DDH (1,3-dibromo-5,5-dimethyl hydantoin and the hydrocarbon solvent is pet ether.
14. The process as claimed in claim 1, wherein, the alcoholic solvent in step h) is selected from methanol, ethanol and isopropanol.
15. The process as claimed in claim 1, wherein, the solvent in step i) is selected from an alcoholic solvent like methanol, ethanol, isopropanol; ethers such as diethylether, diisopropylether, THF; esters such as ethylformate, ethylacetate etc.; nitriles such as acetonitrile etc. and hydrocarbon solvents such as methylenechloride, ethylenedichloride, chloroform, benzene, toluene etc.
16. The process as claimed in claim 1, wherein, the sensitizer used in step i) is selected from 5-(3-pyridyl)-2,2'-bithiophene, 2,5-Di(Pyridin-3-yl) thiophene; 2,5-diphenyl thiophene; 5-(2-Pyridyl)-2,2'-Bithiophene; 5-(4-Pyridyl)-2,2'-Bithiophene; 2-Phenyl-5-(2-Pyridyl)Thiophene; 2-Phenyl-5-(3-Pyridyl)-Thiophene; 2-Phenyl-5-(4-Pyridyl)-Thiophene; 2,2'-5',2''-Terthiophene; and 2,5-Di(2-thienyl) Furane.
17. A synthetic process for commercial manufacture of cholesterol from phytosterols, which comprises;
 - a) Tosylating Phytosterol by treating with p-toluene sulfonyl halide in a base to obtain phytosteryl tosylate (1);
 - b) Treating the phytosteryl tosylate with methanol in a base to obtain Phytosteryl-i- methyl ether (2);
 - c) Treating the phytosteryl-i-methyl ether with ozonized oxygen in a solvent at a temperature range of -50 to -90°C followed by

treatment with reducing agent at a temperature range of -40°C to 0°C to obtain (20S)-20-hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane (3);

- d) Tosylating the (20S)-20-hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane with a tosylating agent in a base to obtain (20S)-6β-methoxy-20-(p-toluene sulfonyl methyl)- 3α,5-cyclo-5α-pregnane (4);
- e) Subjecting the (20S)-6β-methoxy-20-(p-toluene sulfonyl methyl)- 3α,5-cyclo-5α-pregnane to Grignard reaction by treatment with isopentyl bromide in an ether solvent in presence of CuBr•Me₂S catalyst to obtain i-Cholesteryl methyl ether (5);and
- f) hydrolysing the i-Cholesteryl methyl ether in aq. Dioxane with catalytic amounts of PTSA at a temperature of 70-90°C to obtain cholesterol (6).

- 18. The process as claimed in claim 17, wherein, the reaction of step a) is conducted from room temperature to reflux temperature of the solvent.
- 19. The process as claimed in claim 17, wherein, the base used in steps a); step b); step d); step f) is an organic base selected from pyridine, triethyl amine or DMAP or an inorganic base selected from alkali or alkaline earth metals hydroxides, carbonates or bicarbonates.
- 20. The process as claimed in claim 17, wherein, the reaction of step b) is conducted at a temperature range of 40°C to reflux temperature of the solvent used.
- 21. The process as claimed in claim 17, wherein, the solvent used in step c) is selected from methylene chloride, ethylene chloride, methanol, ethanol, isopropanol, water or mixtures thereof and the reducing agent used is selected from sodium borohydride, lithium aluminium hydride, sodium hydride..

22. The process as claimed in claim 17, wherein, the p-toluene sulfonyl halide in step a) and step d) is selected from p-toluene sulfonyl chloride, p-toluene sulfonyl bromide and p-toluene sulfonyl iodide.
23. The process as claimed in claim 17, wherein, the tosylating in step d) is conducted at a temperature range of -5 to 5°C.
24. The process as claimed in claim 17, wherein, the reaction in step e) is conducted at a temperature range of 40 to 70°C.
25. The process as claimed in claim 17, wherein, the ether solvent used in step e) is selected from diethylether, diisopropylether or THF.
26. A method of irradiation of 7-dehydrocholesterol, which method comprises;
 - a) Irradiating 7-dehydrocholesterol in a solvent with a high-pressure mercury lamp;
 - b) Adding a sensitizer to convert tachysterol formed during the irradiation into preD3;
 - c) Concentrating and storing the irradiated solution at $\leq -10^{\circ}\text{C}$ overnight to crystallize the non-converted 7-dehydrocholesterol followed by filtrating the same;
 - d) Dissolving the concentrated filtrate in an organic solvent followed by heating to obtain cholecalciferol; and
 - e) Purifying the cholecalciferol from mixture of acetone and water.
27. The method as claimed in claim 26, wherein, the solvent used in step a) is selected from an alcoholic solvent like methanol, ethanol, isopropanol; ethers such as diethylether, diisopropylether, THF; esters such as ethylformate, ethylacetate etc.; nitriles such as acetonitrile etc. and hydrocarbon solvents such as methylenechloride, ethylenedichloride, chloroform, benzene, toluene etc.
28. The method as claimed in claim 26, wherein, the sensitizer used in step i) is selected from 5-(3-pyridyl)-2,2'-bithiophene, 2,5-Di(Pyridin-3-yl) thiophene; 2,5-diphenyl thiophene; 5-(2-Pyridyl)-2,2'-Bithiophene; 5-(4-Pyridyl)-2,2'-Bithiophene; 2-Phenyl-5-(2-Pyridyl)Thiophene; 2-

Phenyl-5-(3-Pyridyl)-Thiophene; 2-Phenyl-5-(4-Pyridyl)-Thiophene; 2,2'-5',2''-Terthiophene; and 2,5-Di(2-thienyl) Furane.

29. The method as claimed in claim 26, wherein, the organic solvent in step d) is selected from a hydrocarbon or halogenated hydrocarbon.