Chiang Mai J. Sci. 2006; 33(1) : 109-116
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Stanol Synthesis from Palm Oil Distillate

Sarunya Chuanphongpanich* [a], Nipon Tuwanon [a], Duang Buddhasukh [b], Pichai Pirakitikulr [c] and Sukon Phanichphant [b]

[a] Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.

[b] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

[c] Reverse Brain Drain Project, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

*Author for correspondence, e-mail: sarunyag4470135@yahoo.com

Received : 12 April 2005
Accepted : 12 September 2005

ABSTRACT

The goal of this study is to synthesize stanol from palm oil distillate. Sterols as a by product from cooking oil distillation were extracted from a saponification reaction with organic solvent. The isolated sterols were 22-dihydrobrassicasterol (13.7%), stigmasterol (6.27%) and sitosterol (80%). The yield of sterols from palm oil distillate was 82 percent. The sterols were converted to stanols via a hydrogenation reaction by dissolving sterols in isopropyl alcohol in a ratio of 1: 2.5 w/w with 2% palladium on carbon as a catalyst for a period of 1 h at 85-90 °C. These stanols were epiergostanol (13.7%) and stigmastanol (86.27%). The final yield of stanol from sterol was 99.97 percent. Chemical structure of these sterol and stanol compounds were confirmed by infrared spectrometry and gas chromatography with mass spectrometry.

Keywords: palm oil distillate, stanols, sterols.

1. INTRODUCTION

Coronary heart disease (CHD) is the most common cause of morbidity and mortality in the United States and reaching epidemic proportion in Asia [1]. Hyperlipidemia is defined as an abnormal elevation in blood cholesterol, cholesteryl esters, triglycerides or phospholipid, a common modifiable risk factor for CHD [2]. In most cases, CHD results from atherosclerosis which is a complex process of long-term accumulation of lipids deposits in various parts of circulatory system. The cholesterol-lowering properties of plant sterols (phytosterols) have been known for more than 50 years and beta-sitosterol was reported to be the key active component [3]. It is well known that a strictly-controlled diet would provide significant reduction of elevated cholesterol levels. However, the idea of eating tasty nutritious food which can provide comparable cholesterol lowering quality has gained considerable consumer interest. Through the selection of functional foods, the free-living life style of the health-conscious consumer can effectively be undisturbed. As a result, there is a renewed interest in phytosterols for a new cholesterol-lowering food ingredient [4]. A related class of compounds, plant stanols (saturated plant sterols), have also been found to be more effective in reducing cholesterol absorption than plant sterols [5-10]. Stanols, are found in small quantities in nature in many plants such as wheat, rye and corn [11]. This is not a good source of large quantities of stanol due to the large cost with extraction of sufficient quantities of stanol. As a result, a more cost effective method for large quantities

of stanol is by hydrogenation of the much more abundant plant sterols. Many hydrogenation reaction for plant sterols are well-known. Plant sterols can be converted into stanols by hydrogenation technique that employ Pd/C catalyst in organic solvents [12]. Furthermore, sterols are found in vegetable oils and more in vegetable oil distillate, vegetable oil sludge and other plant oil sources such as tall oils.

In the southern part of Thailand, the farming of palm plantation is one of the most significant agricultural professions. Palm oil is consumed in large quantities in many countries of the world where it is used as a major cooking oil for various types of dishes or in the manufacture of margarine and soaps. The by-products of palm oil processing have been used in animal feeds [13]. The purpose of this study is to extract sterol from the palm oil sludge and/or distillate and followed by the synthesis of stanols from sterols. The outcome of stanols synthesis eventually enhances the value of palm oil.

2. MATERIALS AND METHODS

2.1 Palm Oil Distillate

The palm oil distillate used in this study was kindly provided from Pamola Co., Ltd., Kratumbaen, Samutsakorn, Thailand.

2.2 Extraction and Isolation of Sterol from Palm Oil Distillate

2.2.1 Saponification

Palm oil distillate (55 g) was weighed into a 500 ml round-bottomed flask, and 330 ml of 95% ethyl alcohol and 55 ml of 26.73 M aqueous potassium hydroxide (60 g of potassium hydroxide in 40 ml of water) with boiling chips were added to the flask. The mixture was refluxed for 2 h on a steam bath.

2.2.2 Extraction and Separation

After the completion of saponification process, the mixture was cooled to room temperature and the mixtures were transferred to a 500 ml separatory funnel. The flask was washed with two 25 ml portions of hot distilled water and two 25 ml portions of room-temperature distilled water, then the washing liquid was added to the separatory funnel. The solution was extracted five times with 50 ml of ethylene dichloride. Each 50 ml portion was collected into another separatory funnel. The combined extracts were washed with three 30 ml portions of a 10% ethyl alcohol aqueous solution. The extracts were added with anhydrous sodium sulfate and then filtered. The extracts were concentrated to a minimal amount of solvent by a rotary evaporator apparatus.

2.2.3 Crystallization and Filtration

A portion of the residue (20 ml) -the unsaponifiable material- was dissolved in the minimal volume of hot ethylene dichloride. The solution was then allowed to stand overnight. The resulting crystals were collected by vacuum filtration. When all the crystals have been transferred, they were washed with a small amount of the cold ethylene dichloride and the solvent was completely removed *in vacuo*. The sterol yield was 16.40 g or 82 percent.

2.2.4 Recrystallization

The crystals (0.038 g) were dissolved in hexane, after which water was added. The water reacts with the sterols to form a semihydrate (one water molecule per two sterol molecules), which is insoluble in hexane, and will form a precipitate. The resulting slurry was filtered to remove the crystals from the solution. The crystals were washed with cold hexane and the solvent was completely removed giving yellow crystals; the yield was 0.0282 g.

2.2.5 Identification of the Isolated Sterol

The isolated sterol was identified by infrared absorption analysis and gas chromatography/mass spectrometry (GC-MS). GC-MS analyses were performed on a Hewlett Packard model 6890 series II Gas Chromatography coupled with an Agilent 7685 series injector fitted with HP 59864B ionization gauge controller equipped with

5973 mass detector. The column used was Hewlett Packard HP5 capillary column (30m x 250m x 0.25mm id). The operating conditions were as follows: initial oven temperature at 140 °C, and then raised to 280 °C at a rate of 10 °C /min and maintained at this temperature for a further 36 min. Determination of mass spectra were recorded at an electron energy of 70 eV, oven temperature 255 °C (isothermal) and the ion source temperature was 160 °C. Helium was the carrier gas at a pressure of 15.22 p.s.i. and as a make-up gas with a flow rate of 20 ml/min (1 p.s.i = 6894.76 Pa). Identification was based on sample condition time data, electron impact-mass spectra (EI-MS) and with an HP ChemStation software (PA, USA) data.

2.2.6 Hydrogenation of Sterol Compounds

The sterols were converted to stanols via a hydrogenation process. The apparatus for this experiment consists of basically three parts: the hydrogen generator, the reaction flask, and the mineral-oil trap. In the hydrogen generator using zinc metal reacts with dilute sulfuric acid. The hydrogen generator was charged with about 30 g of zinc pieces in the 250 ml round-bottom flask, and 150 ml of 6 M sulfuric acid was in a rate control funnel and on top of the zinc flask. Acid was slowly added. The reaction can be demonstrated as a classical equation below :

$$Zn\ (s) + H_2SO_4 \rightarrow H_2\ (g) + ZnSO_4$$

In the reaction flask; sterols (2 g) were weighed into the 500 ml two neck-round bottom flask, a 30 ml of isopropyl alcohol solution, 2% palladium on carbon (0.04 g) were added and one magnetic stirrer was carefully lower to this flask. When the reaction was initiated, the mixture was gradually heated to 85-90 °C and maintained at this temperature for 1 h. The magnetic stirrer was then turned to high speed to agitate the mixture vigorously. After the reaction was complete, the catalyst was removed by filtration. The reaction mixture was cooled in an ice bath (5 °C) for 10 h, white stanol crystals were obtained. The crystals were filtered and dried and the yield was 1.941 g.

2.2.7 Identification of Stanol Compound

Stanol compound was identified with similar techniques as described in sterol analysis.

3. Results and Discussion

It has been known that the sterols can be separated after saponification of the total lipids. In this study, it was discovered that palm oil distillate have abundance of sterols compounds and the yield was 82 %. After saponification reaction, the mixture was washed with a minimum of distilled water to remove the alkali. Ethylene dichloride was used for extraction and separation of sterols from unsaponifiable materials such as free fatty acid, steryl ester and methyl ester. The IR spectrum of this product, (Figure not show), shows the presence of hydroxy group of alcohols (νO-H at 3434.56 cm^{-1} and νC-O at 1055 cm^{-1}) and cyclic alkane (νC-H at 2930.28, 2871.73 cm^{-1} ; σC-H 1475.35, 1375.60 cm^{-1}) and olefinic group (νC=C at 1640 cm^{-1}). The isolated sterol was finally confirmed by the analysis of GC-MS. The three peaks from gas chromatogram (Figure 1) which was detected by the retention time at 18.87, 19.37 and 20.36, were identified with mass spectra (Figure 2) as a known ergogst-5-en-3β-ol (22-dihydrobrassicasterol, 13.7%), (22E)-stigmasta-5,22-dien-3β-ol (stigmasterol, 6.27%) and stigmast-5-en-3β-ol (sitosterol, 80%), respectively. The chemical constituents of sterol from palm oil distillate were shown in Table 1.

From the mass spectrum of 22-dihydro-brasicasterol, Figure 2(a), the important peaks of mass-to-charge ratio (m/z) appear at 315, 289, 273, 255. From the mass spectrum of stigmasterol, Figure 2(b), the important peaks of mass-to-charge ratio (m/z) appears at 300, 271. The base peak appears at 255 m/z. From the mass spectrum of sitosterol, Figure 2(c), the important peaks of mass-to-charge ratio

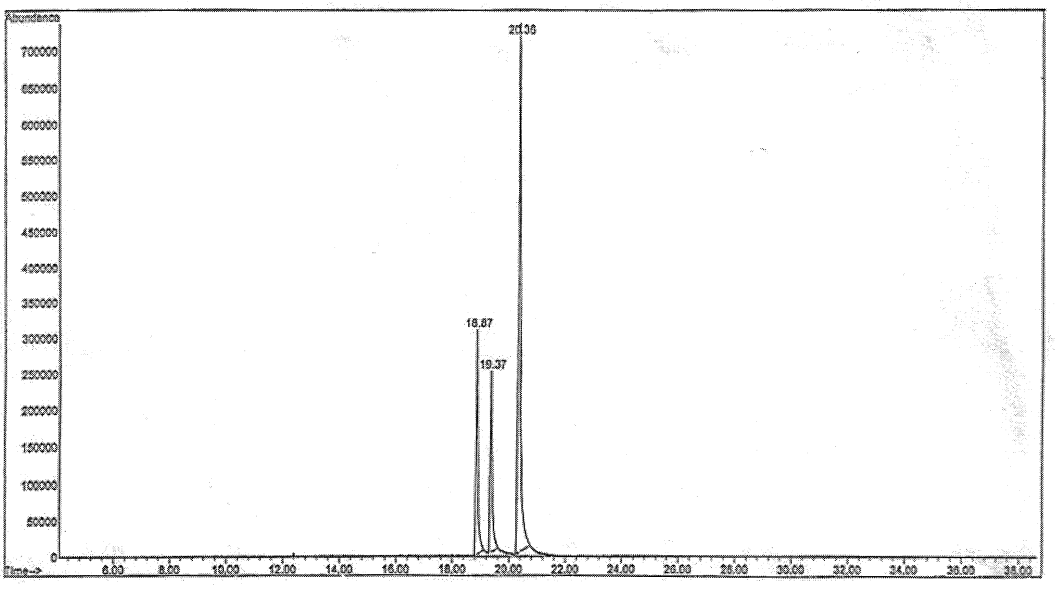


Figure 1. Gas chromatogram of the isolated sterols from palm oil distillate on Hewlett Packard HP5 capillary column (30 m x 250 m x 0.25mm id); column temperature: 140 ˚C; injection temperature: 280 ˚C. Retention time: 18.87; 22-dihydrobrassicasterol, 19.37; stigmasterol and 20.36; sitosterol.

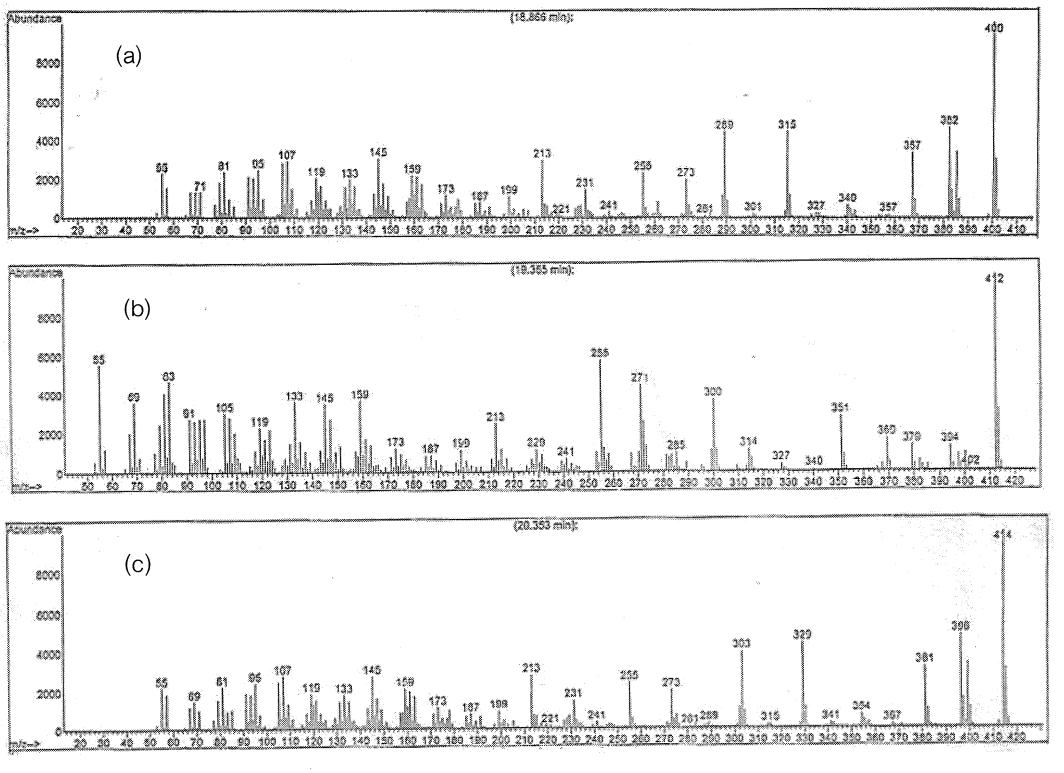
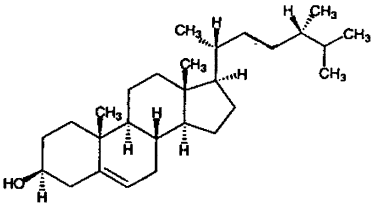
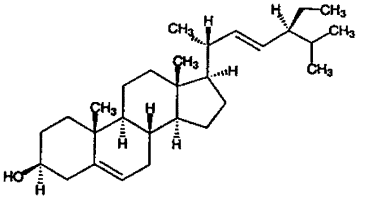
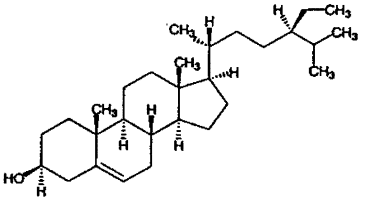


Figure 2. Mass spectra of (a) 22-dihydrobrassicasterol, (b) stigmasterol and (c) sitosterol. The spectra were measured on an oven temperature 255 ˚C at 70 eV.

Table 1. Chemical constituents of sterol from palm oil distillate.

Rt [a](min)	Compound Name & Structure	EIMS-m/z(%)[b]
18.87	Ergost-5-en-3β-ol [22-dihydrobrassicasterol], $C_{28}H_{48}O$ 	400 [M+](100), 315(44), 289(44), 273(20), 255 (24)
19.37	(22E)-stigmasta-5,22-dien-3β-ol [Stigmasterol], $C_{29}H_{48}O$ 	412 [M+](100), 300(36), 271(44), 255(56), 83(46)
20.36	Stigmast-5-en-3β-ol [Sitosterol], $C_{29}H_{50}O$ 	414 [M+](100), 396(48), 329(44), 303(40), 273 (23), 255(24)

[a] Retention time

[b] Electron impact ionization mass spectra (EIMS), [M+] = Molecular ion, major fragments and relative intensities of ions were shown in parentheses

(m/z) appear at 329, 303, 273, 255. The important fragmentation pattern of the sterols are fragmentation of sterol rings and fragmentation of the sterol side chain [14].

3.1 Mass-spectral Fragmentation of the Sterol Rings

3.1.1 Fragmentation of Δ^5-sterols with Saturated Side Chain

Two fragmentation patterns, which are most apparent only in some free 3β-hydroxy-Δ^5-sterols with a saturated side chain, involve rather complex cleavages across the A- and B-rings. These fragmentations result in losses of either 85 a.m.u. or 111 a.m.u., respectively. The most favored mechanisms, based upon studies with deuterium-labeled species of cholesterol, require the breaking of the C-1/C-10, C-5/C-10 and either the C-5/C-6 or the C-7/C-8 bonds, with hydrogen transfer from C-6 [14]. As a result, 22-dihydrobrassicasterol and sitosterol which are methyl and ethyl groups substituent in saturated side chain respectively, have a fragmentation patterns as indicated in Table 1.

3.2 Mass-spectral Fragmentations of Sterol Side Chain

3.2.1 Fragmentation of Saturated Side Chain

The saturated side chain of a free 4-desmethylsterol will be lost to give an [M-

SC]$^+$ ion at m/z 273 comprising the ring carbons. Further loss of ROH can then yield the [M-SC-ROH]$^+$ ion at m/z 255 which is a characteristic feature. With 22-dihydrobrassicasterol and sitosterol this ion is seen at m/z 273 and 255.

3.2.2 Fragmentation of Unsaturated Side Chains: Fragmentation of Δ^{22}- side chain

Two fragmentations are seen with Δ^{22}-side chain which were elucidated by using deuterium labeled sterols. Breaking the C-20/C-22 bond is accompanied by the transfer of the 17α-H and produces an ion at m/z 300 for a free 4-desmethylsterol monoene.

Alternatively cleavage of the C-17/C-20 bond involves transfer of two hydrogens (17α-H and 14α-H) and migration of the C-18 methyl group to C-17. The [M-SC-2H]$^+$ ion produced from a free monoene 4-desmethylsterol is at m/z 271. The [M-SC-ROH]$^+$ ion at m/z 255 is also usually strong in the spectrum of a $\Delta^{5,22}$-sterol [14]. Both of these ions are seen with in stigmasterol.

From hydrogenation reaction, it is pointed that the reaction was reduced the ratio of isopropanol: sterol from 7:1 ratio to 2.5:1 ratio (a 2.8 fold reduce) and decreased in the hydrogenation cycle time from 7 hours to 1 hour. Moreover, the pathway was decreased in the catalyst usage from 5%w/w to 2 %w/w. The product from hydrogenation reaction of sterols, can be proved by GC-MS (Figure 3-4). This product is 5α-ergostan-3α-ol (epiergostanol, 13.7%) and 23(Z)-ethylcholestanol (stigmastanol, 86.27%) by retention time at 19.02 and 20.54 min, respectively. These structures are shown in Table 2.

Epiergostanol and stigmastanol were characterized by intense peak at m/z 215. Usually this fragmentation is easily observed by the appearance of an ion which results from a further loss of the 3β-hydroxy function (i.e. [M-SC-42-ROH]$^+$, where: R=H for free sterol). The production of this [M-SC-42-ROH]$^+$ ion will permit the number of double bonds in the B- or C-rings to be deduced. Thus, with a stanol there is an abundant ion at m/z 215 (sometime base peak) [14]. Base on the mass spectra, 22-dihydrobrassicasterol was converted to epiergostanol, while the stigmasterol and sitosterol were

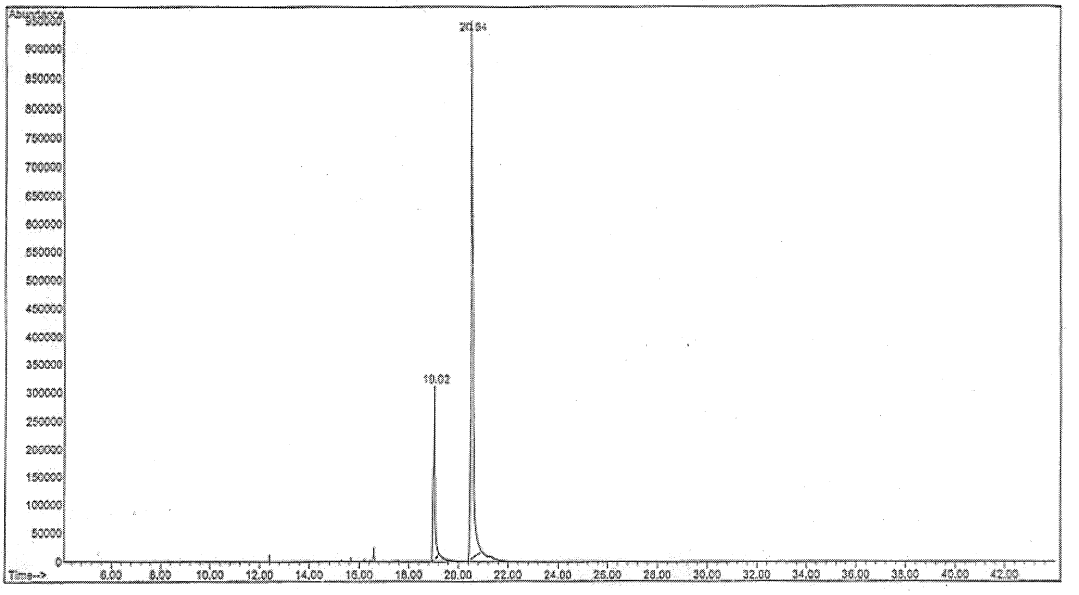


Figure 3. Gas chromatogram of the stanol from hydrogenation reaction of sterol on Hewlett Packard HP5 capillary column (30 m x 250 m x 0.25mm id); column temperature: 140 °C; injection temperature: 280 °C. Retention time: 19.02; epiergostanol and 20.54; stigmastanol.

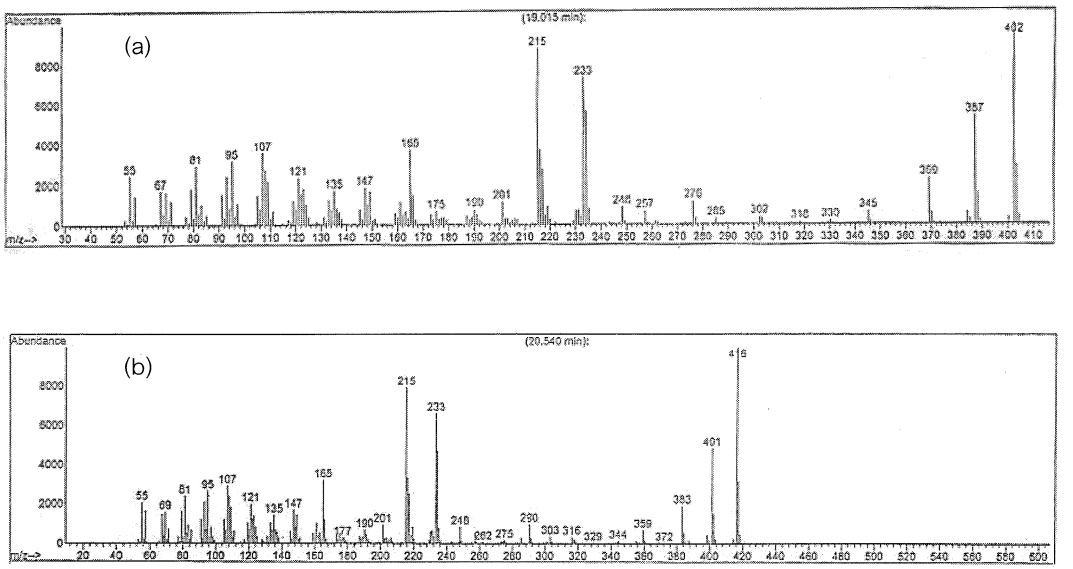
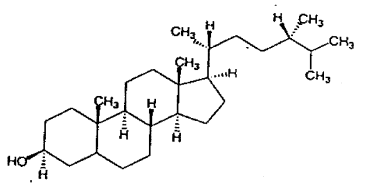
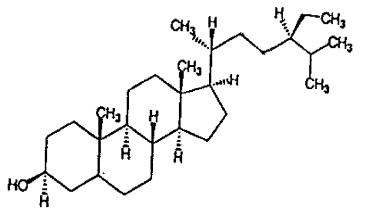


Figure 4. Mass spectra of (a) epiergostanol and (b) stigmastanol. The spectra were measured on an oven temperature 255 °C at 70 eV.

Table 2. Chemical constituents of the stanol from hydrogenation reaction of sterol.

Rt [a](min)	Compound Name & Structure	EIMS-m/z(%)[b]
19.02	5α-Ergostan-3α-ol [Epiergostanol], $C_{28}H_{50}O$	402 [M^+](100), 387(58), 215(88)
		
20.54	23(Z)-Ethylcholestanol [Stigmastanol], $C_{29}H_{52}O$	416 [M^+](100), 401(48), 233(64), 215(78)
		

[a] Retention time

[b] Electron impact ionization mass spectra (EIMS), [M^+] = Molecular ion, major fragments and relative intensities of ions were shown in parentheses.

converted to stigmastanol.

4. CONCLUSION

This research has been intended to synthesize stanol from sterol in palm oil distillate. The three sterols from palm oil distillate by GC-MS have revealed, in addition to 22-dihydrobrassicasterol (13.7%), stigmasterol (6.27%) and sitosterol (80%). The yield was 82%. Then the sterols are converted to stanols successfully via a hydrogenation process. These major stanols are epiergostanol and stigmastanol; yield is 99.97%.

ACKNOWLEDGEMENT

A special thank to the Pamola Co., Ltd., Kratumbaen, Samut Sakorn, Thailand for providing the palm oil distillate to this research project.

REFERENCES

[1] Tai E.S., and Tan C.E., Lipid lowering therapy: what have we learnt and what do we need to know?, *Med. Progress*, 2001; **28 (1):** 5-8.

[2] Gotto A.Jr., *Lipid disorders*, Pennsylvania: Handbooks in Health Care Co, 1999.

[3] Peterson D.W., Effect of soyabean sterols in the diet on plasma and liver cholesterol in chicks, *Proc. Soc. Exp.*, 1951; **78**: 143.

[4] Young J., Functional food market still to grow, *Food Ingr. Anal.*, 1997; **19:** 43.

[5] Hallikainen M.A., Sarkkinen E.S., and Unsitupa M.I.J., Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner, *J. Nutr.*, 2000; **130(4):** 767-776.

[6] Miettine T.A., and Gylling H., Regulation of cholesterol metabolism by dietary plant sterols, *Curr. Opin. Lipidol.*, 1999; **10**: 9-14.

[7] Moghadasian M.H., Pharmacological properties of plant sterols *in vivo* and *in vitro* observations, *Life Sci.*, 2000; **67 (6):** 605-615.

[8] Nguyen T.T., The cholesterol-lowering action of stanol esters, *J. Nutr.*, 1999; **129(12):** 2109-2112.

[9] Nguyen T.T., Dale L.C., von Bergmann K., and Croghan I.T., Cholesterol-lowering effect of stanol ester in a US population of mildly hypercholesterolemic men and women: a randomized controlled trial, *Mayo Clin. Proc.*, 1999; **74(12):** 1198-1206.

[10] Norman L., Dutta P., Lia A., and Andersson H., Soy sterol esters and b-sitostanol ester as inhibitors of cholesterol absorption in human small bowel, *Am. J. Clin. Nutr.*, 2000; **71(4):** 908-913.

[11] Sugano M., Morioka H., and Ikeda I., Lipid-lowering activity of phytostanols in rat, *Atherosclerosis*, 1976; **24:** 301-305.

[12] Augustine R.L., and Reardon Jr.E.J., The palladium catalyzed hydrogenation of cholesterol, *Org. Prep. Proced. Int.*, 1969; **1:** 107-109.

[13] Onwudike O.C., Oil palm; in Nwokolo E., and Smartt J., eds., *Food and seed from legumes and oilseeds*, London: Chapman & Hall, 1996: 318-329.

[14] Goad L.J., and Akihisa T., *Analysis of sterols*, London: Chapman & Hall, 1997.