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Extraction and Determination of Phytosterols from Corn Oil Foots

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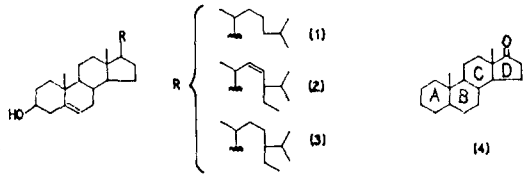
Abstract□By saponification and extraction of corn oil foots abandoned as waste during oil refining, a mixture of phytosterols was obtained, and its major components were determined as β-sitosterol, campesterol and stigmasterol by gas chromatographic analysis. The mixture is very cheap and regarded as an excellent substrate for direct fermentation of C-17 keto steroid intermediate for various steroid pharmaceuticals.

Keywords□Corn oil foots, insaponifiable, phytosterol, β-sitosterol, stigmasterol, campesterol.

Steroids are ubiquitous members of biological organic compounds that have the perhydro-1,2-cyclopentenophenanthrene ring system as a common stuructural feature. The vast diversity of the members of steroid family depends on the variation in the side chains, as well as nuclear substitution, degree of unsaturation and a lot of synthetic derivatives are used as phramaceuticals[1].

As natural source of steroid intermediate, diosgenin became more scarce and expensive[2], selective degradation of C-17 side chain of sterols such as cholesterol (**1**), stigmasterol (**2**), β-sitosterol (**3**) by *Mycobacterium* sp. has become more attractive for the various steroid compounds[3,4].



Proceeding to the our research for the microbial conversion of sterols to the C-17 keto steroid (**4**), we found that corn oil foots abandoned as waste during corn oil refining had a lot of phytosterols, of which the major component was β-sitosterol.

In this study, we have obtained the insaponifiable by saponification of fatty acids with alcoholic KOH and extraction with organic solvents from corn oil foots. From this insaponifiable, the colorless crystalline precipitate of phytosterols was obtained by recrystallization from ethanol, and each component of which was determined by gas chromatography.

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EXPERIMENTAL

Materials

Corn oil foots was purchased from Jung Nam Food Ind., Inc., (Seoul, Korea) and steroid standards of β-sitosterol, stigmasterol, campesterol were obtained from Sigma (St. Louis, Mo, U.S.A.). The properties of corn oil foots used for the experiment were as follow: Acid Value, 106.7; Saponification Value, 196.2; Iodine Value, 118.7; Water Contents, 42%. The organic solvents for the saponification and extraction were obtained from Oriental Chemical Inc., (Seoul, Korea) and all other reagents of analytical grade were obtained from Merck (Darmstadt, F.R.G.).

Saponification

30g of corn oil foots and 150 m*l* of ethanolic KOH (1 N)were placed to the 1 *l* round bottomed flask equipped with reflex condenser. After the reaction mixture was stirred for 2 hours at 70°C, it was cooled to room temperature and diluted with 100 m*l* of water and extracted with 100 m*l* of dichloromethane, 4 times. The combined organic layer was washed with 100 m*l* of 0.1 N NaOH aq. solution and 100 m*l* of water, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to give dark brown residue. This insaponifiable was placed to the 50 m*l* of round bottomed flask with condenser containing 20 m*l* of ethanol, and heated to 80°C with good stirring until it was dissolved completely. After cooling to room temperature, the crystalline precipitate was filtered and dried under vacuum.

For the GC analysis, the sterols were further

Table I. Gas chromatographic conditions

Column	Crosslinked phenylmethyl silicone coated 25 m capillary column
Carrier gas	He
Flow rate	40 ml/min.
Oven temp.	270°C
Sample size	3% $CHCl_3$ solution 0.5 *l*
Detector	TCD (Thermal Conductivity Detector)

Table II. The amounts of insaponifiables and their sterol contents by recrystallization in ethanol from 30 g of foots

Run	Insaponifiable (g)	Yield (%)	Sterol cont. (g)
1	2.12	7.0	1.59 (75%)
2	2.06	6.7	1.77 (86%)
3	1.78	5.9	1.39 (78%)

purified by silicagel column chromatography (ether/*n*-hexane = 1/5; mesh size 230-400; Merck).

Determination of Sterols by Gas Chromatography

Gas chromatograph (Hewlett Parckad Model No 5890) was used for the determination of each component of sterols obtained from corn oil foots. The chromatographic condition is described in Table I.

RESULTS AND DISCUSSION

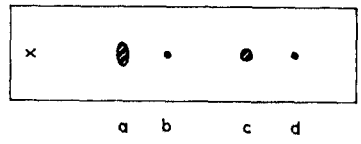
Sterols from corn oli foots

By saponification and extraction, 1.78-2.12g of yellow sticky solid i.e., insaponifiable was obtained from 30 g of corn oil foots. From this, the mixed sterols were isolated by recrystallization in ethanol. The results were summarized in Table II.

This insaponifiable was monitored by thin layer chromatography (Merck, Kieselgel 60F-254; ehter/*n*-hexane = 1/5; Indicator PMA), which showed the major fraction (a) was mixed sterols and the other fractions (b,c,d) were supposed to be hydrocarbons (Fig. 1).

Quantitative analysis of sterols by gas chromatograph

Sterols which were isolated and purified through the procedures of recrystallization and liquid column chromatography were quantitatively analyzed by gas chromatograph. The result is described in Table III and Fig. 2.



Rf value : a=0.4, b=0.5, c=0.8, d=0.9

Fig. 1. TLC chromatogram of the insaponifiable.

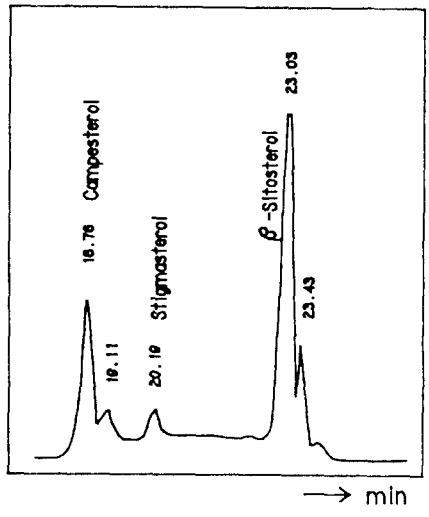


Fig .2. GC chromatogram of sterol.

Table III. Percentage of composition and retention times of the sterols

Sterol	Retention time	%
Campesterol	18.76 min.	19
Stigmasterol	20.19 min.	4
β-sitosterol	23.03 min.	58

The yield of the insaponifiable from corn oil fooots is 5.9 to 7.0%, and it contains 75 to 86% of mixed sterols, of which major components are 58% of β-sitosterol, 19% of campesterol, and 4% of stigmasterol, while crude corn oli is known to contain 0.8 to 2.9% of insaponifiable and 0.8 to 1.5% of mixed sterols[5].

Owing to the high concentrations of the same class of sterols, especially β-sitosterol, this mixed sterols are regarded as an excellent substrate of direct fermentation for the production of C-17 keto steroid that is the useful intermediate of steroid hormones[6]. Now, we are proceeding to convert this mixed sterols by using the *Mycobacterium* sp. to C-17 keto steroid.

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