# Sterol Lipids Isolated from Pea Seeds (*Pisum sativum*)

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**ABSTRACT**

Four classes of sterol lipids—free sterols, sterol esters, sterylglycosides, and acylsterylglycosides—were isolated from pea seeds, identified, and examined for fatty acid and sterol composition as well as sugar constituents. β-Sitosterol was the principal free sterol. The sterol esters were mainly composed of linoleic acid and β-sitosterol, sterylglycosides of glucose and β-sitosterol, and acylsterylglycosides of palmitic acid, glucose, and β-sitosterol.

Sterol lipids are now known to be widely distributed in the plant kingdom, existing in four classes: free-form, fatty-acid ester, glycoside, and esterified glycoside. The present paper describes isolation, identification and chemical composition of free sterols (S), sterol esters (SE), sterylglycosides (SG) and acylsterylglycosides (ASG), all of which have been isolated from seeds of pea (*Pisum sativum*).

Few detailed studies of pea lipids have been carried out up to now. Haydar and Hadziyev (1) recently referred to the lipid components of pea in the study on oxidation of lipids, but the analysis of the lipids themselves was not delineated, at least as far as the sterol lipids are concerned.

## MATERIALS AND METHODS

### Extraction of Total Lipid

Pea seeds, harvested at Hokkaido Prefecture in 1971, were ground into powder of 28 mesh and extracted three times with 4 volumes of chloroform:methanol (2:1) according to the procedure of Folch et al. (2). The combined extract was washed with water, dehydrated with sodium sulfate, and condensed under reduced pressure to obtain total lipid. Yield was approximately 2.1% of the powdery material. The lipid was subjected to silicic acid column chromatography by sequential elution with chloroform, acetone, and methanol according to the method of Rouser et al. (3). Crude neutral lipid was eluted with chloroform, crude glycolipid with acetone, and crude phospholipid with methanol. Yields for the total lipid of the crude lipid fractions were 45.9, 9.5, and 44.6%, respectively.

### Fractionation of Sterol Lipids

The crude neutral lipid was dissolved in a small volume of chloroform and subjected to preparative silica gel G, thin-layer chromatography with a solvent of

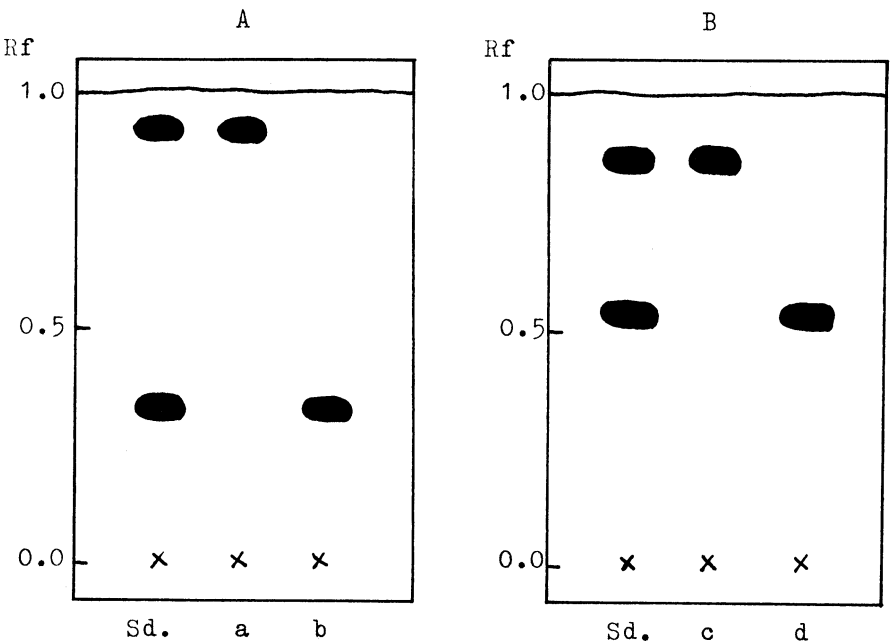


**Fig. 1. Thin-layer chromatogram of sterol lipids isolated from pea. Coated by silica gel G, developed by petroleum ether: diethyl ether: acetic acid (80:20:1) for *A* and by chloroform: methanol (95:12) for *B*, and detected by 50% sulfuric acid or anthrone reagent. A) *Sd.* = mixture of standard SE and S; *a* = Sterolester from pea; *b* = Free sterol from pea. B) *Sd.* = mixture of standard ASG and SG; *c* = ASG from pea; *d* = SG from pea.**

hexane:diethyl ether:acetic acid (80:20:1) in order to isolate SE and S. SE and S each comprised about 15% of the crude neutral lipid. The crude glycolipid was subjected to silicic acid column chromatography by a stepwise elution with a solvent of chloroform-acetone, following the techniques of Vorbeck and Marinetti (4) and Ito and Fujino (5). The eluates with chloroform-acetone, 9:1 and 7:3, contained ASG and SG, respectively. Having been contaminated with a small amount of glycerolipids and cerebroside, both fractions were rechromatographed repeatedly and subjected to preparative thin-layer chromatography with chloroform:methanol (95:12). ASG and SG purified were thus isolated. ASG and SG amounted to 12 and 14% of the crude glycolipid, respectively.

#### Infrared Spectroscopy of Sterol Lipid

Infrared spectra were taken on an infrared spectrophotometer (IR-G type, Nippon Bunko Kogyo Co. Ltd., Tokyo), using 300 mg. of KBr pellets for each 3.0 mg. sterol lipids.

#### Degradation of Sterol Lipids

SE was incubated with 0.4N KOH in methanol for 4 hr. at 37°C. After saponification, the mixture was cooled and added to chloroform and water. The resulting chloroform layer contained the sterol components. The water-methanol layer was acidified to pH 1 to 2 with conc. HCl, extracted with hexane, and refluxed with 5% HCl in methanol at 100° C. for 4 hr. Hexane extract of the methanolysate yielded fatty acid methyl esters.

SG was refluxed with 5% HCl in methanol for 4 hr. at 100° C., cooled, and extracted with hexane. The hexane layer contained sterols. The residual

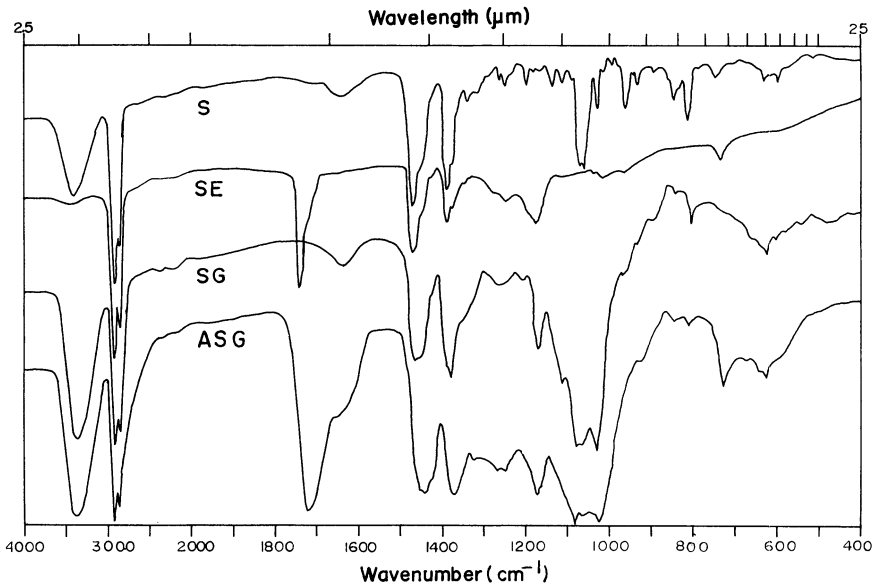


**Fig. 2. Infrared spectra (in KBr) of sterol lipids isolated from pea.**

TABLE I. FATTY ACID COMPOSITION OF PEA STEROL LIPIDS

| Fatty Acid | SE | ASG |
|---|---|---|
| | % | % |
| 14:0 | 3.3 | 3.6 |
| 14:1 | 6.2 | 1.0 |
| 16:0 | 14.8 | 39.3 |
| 16:1 | 6.6 | 5.9 |
| 18:0 | 18.1 | 9.4 |
| 18:1 | 10.9 | 18.6 |
| 18:2 | 21.3 | 19.9 |
| 18:3 | 1.0 | trace[1] |
| 20:0 | 15.0 | --- |
| 20:1 | 2.6 | --- |

[1]Less than 0.5%.

TABLE II. STEROL COMPOSITION OF PEA STEROL LIPIDS

| Sterol | Retention Relative to β-Sitosterol | S | SE | SG | ASG |
|---|---|---|---|---|---|
| | | % | % | % | % |
| Campesterol | 0.78 | 11.7 | 11.4 | 6.5 | 6.1 |
| Stigmasterol | 0.87 | 11.7 | 11.9 | 6.9 | 29.9 |
| β-Sitosterol | 1.00 | 77.6 | 77.7 | 86.6 | 64.0 |

methanol layer was fractionated on an ion-exchange column using Amberlite IR-4B ($OH^-$) to obtain purified methyl glycosides.

ASG was saponified and divided into chloroform and water-methanol fractions, from the latter of which the fatty acid methyl esters were prepared, as described in the case of SE. From the chloroform fraction, sterols and methyl glycosides were separately prepared, as in the case of SG.

#### Paper Chromatography

Methyl glycosides prepared from ASG and SG were subjected to paper chromatography. Samples were applied to Toyo filter paper No. 51, developed upwards with *n*-butanol:pyridine:water (6:4:3) for 15 hr. at room temperature, and located with $AgNO_3$-NaOH reagent.

#### Gas-Liquid Chromatography

Fatty-acid methyl esters were analyzed by gas-liquid chromatography as they were, and the sterols and the methyl glycosides were chromatographed after trimethylsilylation (6). The analysis was performed with a Hitachi gas chromatograph (Model 063, Hitachi Seisakusho Co. Ltd., Tokyo) equipped with a hydrogen flame ionization detector. A glass column (0.3 × 200 cm.) was packed with 20% diethylene glycol succinate polyester on Chromosorb W for fatty acid methyl esters, and with 5% SE-30 on Chromosorb W for trimethylsilylether derivatives of sterols and methyl glycosides. Carrier gas was $N_2$, and column temperatures were 180°, 170°, and 230° C., respectively. Peaks revealed in the chart were identified by comparing the relative retention times with those of the

standard specimens. (Unsaturated acid esters, as occasion demands, were hydrogenated and confirmed by agreement with the reference standards.)

### Reference Compounds

Palmitic and stearic acids were purchased from Tokyo Kasei Industrial Co., Tokyo, Japan. β-Sitosterol and stigmasterol were a gift from M. Katayama, Department of Agricultural Chemistry, Osaka Prefectural University, Osaka Japan. Glucose was procured from Kanto Chemical Co., Tokyo, Japan.

## RESULTS

### Thin-Layer Chromatogram of Pea Sterol Lipids

Thin-layer chromatograms of sterol lipids isolated from pea are shown in Fig. 1. Sterol lipids gave a single spot on the plate and the $R_f$ value agreed well with those of the standards of SE, S, ASG, and SG.

### Infrared Spectra of Pea Sterol Lipids

Infrared spectra of sterol lipids isolated from pea are shown in Fig. 2. An absorption for OH group is seen at 3,440 $cm.^{-1}$ in the spectra of S, SG, and ASG, but not in that of SE. Strong absorptions at 2,940 and 2,860 $cm.^{-1}$ are based on $CH_2$; absorptions at 1,467, 1,445, 1,385, and 1,370 $cm.^{-1}$ are based on methyl and methylene groups. A strong absorption at 1,730 $cm.^{-1}$ present in the spectra of SE and ASG is attributed to C=O. A broad absorption in the region of 1,125 to 1,000

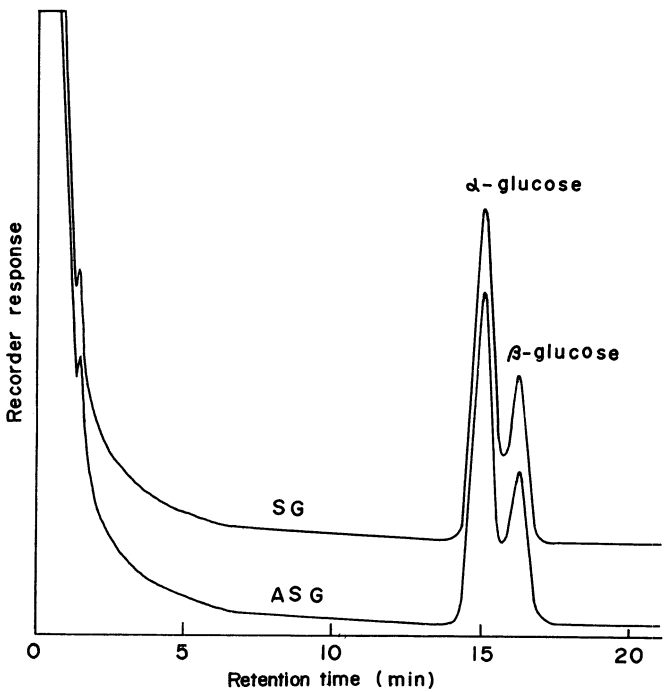


**Fig. 3. Gas-liquid chromatogram of trimethylsilyl ether derivatives of methyl glycosides obtained from pea sterol lipids.**

$cm.^{-1}$, characteristic of sugar, is depicted in the spectra of SG and ASG. The infrared spectra of the said SG and ASG were in good agreement with those of SG and ASG isolated from potato tuber (7), soybean (8), rice grain (9), and alfalfa (10).

### Fatty Acid Composition of Pea Sterol Lipids

Fatty acid composition of SE and ASG in pea, having been analyzed by gas-liquid chromatography, is shown in Table I. Ten peaks were found in SE, and eight in ASG. Predominant were linoleic, stearic, arachidic, palmitic, and oleic acids in SE; and palmitic, linoleic and oleic acids in ASG.

### Sterol Composition of Pea Sterol Lipids

Table II shows sterol composition of sterol lipids S, SE, SG, and ASG in pea. The gas-liquid chromatographic analysis gave three peaks, which were identified as campesterol, stigmasterol, and β-sitosterol. The main component sterol was β-sitosterol in every case.

### Sugar Composition of Pea Sterol Lipids

Methyl glycosides prepared from SG and ASG of pea were analyzed by paper chromatography, which tentatively indicated that the component sugar was D-glucose in both lipids. When trimethylsilyl ether derivatives of the methyl glycosides were applied to gas-liquid chromatography, two peaks corresponding to α-methyl and β-methyl glucoside were detected and no other peaks were found in the chromatogram (Fig. 3).

## DISCUSSION

The present paper has demonstrated isolation, identification, and constituent analysis of four sterol lipid classes of pea: S, SE, SG, and ASG. The purity of lipids was proved by thin-layer chromatography and infrared spectroscopy. The main sterol in free sterol fraction was β-sitosterol. The principal components of SE were linoleic acid and β-sitosterol, implying that the representative molecular species of SE would be β-sitosterol linoleate. The major components of SG were glucose and β-sitosterol, and those of ASG were palmitic acid, glucose, and β-sitosterol. According to the findings here and the general information of structure of sterol glycosides in the literature (11), the essential molecular species of SG and ASG will be characterized as β-D-glucopyranosyl-(1→3′)-β-sitosterol (or β-sitosterol glucoside) and 6-O-palmitoyl-β-D-glucopyranosyl-(1→3′)-β-sitosterol (or β-sitosterol 6-palmitoylglucoside), respectively.

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