NOTE

4-Desmethylsterol Composition of Steryl Glycoside in Oil Palm Leaf

Masakazu YAMAOKA*, Peesamai JENVANITPANJAKUL**, and Akio TANAKA*

* National Chemical Laboratory for Industry
(1-1, Higashi, Tsukuba-shi, 〒305)

** Thailand Institute of Scientific and Technological Research
(196 Phahonyothin Rd, Bangkok, Bangkok 10900, Thailand)

4-Desmethylsterols of steryl glycoside (SG) of oil palm leaves were separated and analyzed by GLC on both OV-1 (0.5 μm) and OV-17 (0.25 μm) capillary columns of the same size (25 m $\times$ 0.25 mm i. d.) under the same conditions. The extent of separation of each 4-desmethylsterol of SG was essentially the same on both columns. Campesterol, stigmasterol, sitosterol, 7-stigmastenol and avenasterol were found, in addition to a traces of cholesterol and brassicasterol. The compositions of these sterols by the OV-17 column were 4.8, 8.9, 84.2, 1.1, and 1.0%, respectively.

1 Introduction

Glycolipid composition of oil palm leaf was reported previously and the results were compared with that of crude palm oil, fiber and empty bunch¹⁾. As to the component of each glycolipid, fatty acid composition only was thus far analyzed.

Plant glycolipids such as steryl glycoside (SG), cerebroside²⁾, and digalactosyl diglyceride³⁾ were reported to have physiological effects. Among these glycolipids, aglycone of SG was related to the physiological effect, antiulcerogenic activity²⁾. Then, we planned to analyze sterol aglycone of SG in oil palm leaf. This paper describes about the 4-desmethylsterol composition of SG in oil palm leaf.

2 Experiment

2.1 Materials

SG was prepared from oil palm leaf by the method described in the previous work¹⁾.

TMS reagent, the mixture of hexamethyldisilazane, trimethylchlorosilane, and pyridine (2 : 1 : 10, vol/vol), was purchased from Gasukuro Kogyo Inc.

Stigmasterol and cholesterol were purchased

from Applied Science Laboratories, Inc. The standard sterol mixture containing brassicasterol, campesterol, stigmasterol, 7-ergosterol, sitosterol, isofucosterol, 7-stigmastenol, and avenasterol was prepared from the unsaponifiable matter of refined safflower oil purchased from Rinoru Oil and Fat Co.

Other chemicals of guaranteed grade were used without further purification except diethyl ether, which was distilled again after removal of peroxides by iron (II) sulfate.

2.2 Methods

2.2.1 Trimethylsilylation and mass spectrometry

TMS reagent (0.5 mL) was added to 1 mg of SG and heated at 50°C for 1 min. Diethyl ether (1 mL) was added after pyridine was removed from the reaction mixture.

Mass spectrum of SG TMS derivatives was recorded with JEOL D-300 mass spectrometer. Sample was directly injected. The accelerating voltage and ionization current were 70 eV and 100 μA , respectively. Ion source temperature was programmed from 50 to 350°C by 10°C per min.

2.2.2 Preparation of 4-desmethylsterol fraction of SG

4-Desmethylsterol fraction of SG was prepared

according to the modified method described in Yukagaku⁴⁾.

SG (99.3 mg) in 3 mL of 1 N alcoholic potassium hydroxide was refluxed for 1 h. After alkaline hydrolysis, the reaction mixture was shaken with 10 mL of hot water, 5 mL of water and 10 mL of diethyl ether. Aglycone of glycolipids from the lower layer was extracted with two 5 mL portions of diethyl ether. Upper layer and ether extracts were combined and washed with distilled water to remove excess potassium hydroxide. After evaporation of the solvent, 16.72 mg of the aglycone of glycolipids was obtained.

4, 4'-Dimethylsterol, 4-monomethylsterol and 4-desmethylsterol from the aglycone of SG was separated by TLC using stigmasteryl as authentic compound. For determination of each sterol group, the solution of aglycone of glycolipids in chloroform-methanol (1 : 2, vol/vol) was spotted on 10×20 cm plates, which were precoated with 0.25 mm silica gel 60 (Merck), and developed twice with hexane-diethyl ether (7 : 3, vol/vol) each for 1 h.

Spots were detected by spraying TLC plates with aqueous solution of 13% H₂SO₄ and 2% CuSO₄ followed by heating at 140°C for 10 min, or with aqueous 20% solution of perchloric acid followed by heating at 100°C for 10 min. 4-Desmethylsterol fraction was identified by comparing *R_f* value with that of stigmasteryl.

For preparation of each sterol group, 20×20 cm plates were used. 4-Desmethylsterol fraction was detected by iodine vapor, scraped off and extracted with chloroform-methanol (1 : 2, vol/vol). 4-Desmethylsterol fraction was further analyzed by GLC.

2.2.3 GLC

Shimadzu GC 8 A equipped with fused silica capillary column was used to determine 4-desmethylsterol composition of SG. Two types of the capillary column of the same size (25 m×0.25 mm i.d.), OV-1 (0.5 μm, OV-1 Bonded, Gasukuro Kogyo Inc.) and OV-17 (0.25 μm, Bonded MPS 50, Quadrex Corp.) were used separately at 260°C. The injector/detector temperature was 280°C. Flow rate of the carrier gas, nitrogen, was 1.7 mL/min. Split ratio was 1 : 6. Retention times and sterol contents were recorded with Shimadzu Chromatopac CR 1 B.

3 Results and Discussion

Mass spectrum of SG TMS derivatives showed mass fragments at *m/z* 191, 204, 217 and 451 characteristic to glycopyranoside TMS derivative. An intense peak at *m/z* 397 characteristic to sitosterol or 7-stigmasteryl (C₂₉H₄₈) was also shown. Other fragments shown as quite weak peaks at *m/z* 852, 864, and 866 were thought to be molecular ions of campesteryl, avenasteryl or stigmasteryl, and sitosteryl glycoside TMS derivatives, respectively.

The unsaponifiable matter of SG (7.92 mg) yielded 0.54 mg of 4-desmethylsterols. Judging from the staining reaction with perchloric acid, the amounts of 4-mono and 4, 4'-dimethyl sterols were quite small.

Kornfeldt and Croon⁵⁾ used OV-1 WCOT glass capillary column (50 m) to analyze sterols in vegetable oils. We used shorter OV-1 capillary column than theirs, as well as OV-17 column. Both OV-1 and OV-17 capillary columns were available for the separation of each 4-desmethylsterol of the standard mixture as shown in **Figs. -1** and **2**. OV-17 column showed, however, better separation of isofucosterol from sitosterol or 7-stigmasteryl under the analytical conditions employed. Separation of 4-desmethylsterol of SG in oil palm leaf was achieved on both columns. **Table-1** showed the 4-desmethylsterol composi-

From a through h in the figure denote brassicasterol, campesterol, stigmasteryl, 7-ergosterol, sitosterol, isofucosterol, 7-stigmasteryl, and avenasterol, respectively.

Fig. -1 Separation of 4-desmethylsterols of standard mixture by GLC equipped with OV-1 capillary column (OV-1 Bonded, 0.5 μm, 25 m×0.25 mm i.d.).

See the footnotes of Fig.-1 for a-h.

Fig.-2 Separation of 4-desmethylsterols of standard mixture by GLC equipped with OV-17 capillary column (Bonded MPS 50, 0.25 μ m, 25 m $\times$ 0.25 mm i. d.).

Table-1 4-Desmethylsterol composition of SG in oil palm leaf analyzed by GLC with OV-1 and OV-7 capillary column.

	OV-1	OV-17
Cholesterol	Tr.	Tr.
Brassicasterol	Tr.	Tr.
Campesterol	4.7	4.8
Stigmasterol	11.1	8.9
Sitosterol	78.5	84.2
7-Stigmastenol	4.3	1.1
Avenasterol	1.4	1.0

tion of SG. SG in oil palm leaf contains campesterol, stigmasterol, sitosterol, 7-stigmastenol and avenasterol, 4.8, 8.9, 84.2, 1.1 and 1.0%, respectively, on OV-17 column. SG in oil palm leaf contains more stigmasterol and sitosterol but less campesterol than SG in palm oil which was reported to contain cholesterol, campesterol, stigmasterol and sitosterol 2.1, 22.1, 5.3 and 70.5%, respectively⁶⁾.

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アブラヤシの葉のステロールグリコシドの 4-デスメチルステロール組成

山岡正和*¹・Peesamai JENVANITPANJAKUL*²・

田中章夫*¹

*¹ 化学技術研究所 (〒305 茨城県つくば市東1-1)

*² Thailand Institute of Scientific and Technological Research (196 Phahonyothin Rd, Bangkok, Bangkok 10900, Thailand)

アブラヤシの葉のステロールグリコシドの 4-デスメチルステロールを分離し、GLC で分析した。カラムは、内径 0.25 mm, 全長 25 m の OV-1 (膜厚 0.5 μ m) 及び OV-17 (膜厚 0.25 μ m) キャピラリーカラムを同じ分析条件で用いた。ステロールグリコシドの各 4-デスメチルステロールはどちらのカラムでもほぼ同様に分離し、微量のコレステロール、ブラシカステロールの他に、カンペステロール、スチグマステロール、シトステロール、7-スチグマステノール、アベナステロールの存在を認めた。OV-17 カラムでの分析結果によれば、それぞれの割合は 4.8, 8.9, 84.2, 1.1, 及び 1.0 % であった。