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Glucolipid from Phytolacca esculenta

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Abstract — An acylated steryl glucoside preparation has been isolated from *Phytolacca esculenta* VAN HOUTTE. Two components of the sterol moiety are α-spinasterol and Δ^7-stigmastenol. Fatty acids are palmitic, stearic and myristic acids, and are located on C-6 of the glucose portion. 6′-Palmityl-α-spinasteryl-β-D-glucoside is the main component.

The toxic plant *Phytolacca esculenta* has been previously reported to contain α-spinasterol, Δ^7-stigmastenol, and their glucosides.[1] Although it is well known that sterols in higher plants occur in at least four different forms such as free sterols, steryl esters, steryl glucosides, and acylated steryl glucosides,[2-18] and the occurrence of α-spinasteryl glucoside in various plants has been described in several reports,[19-24] the esterified from of α-spinasteryl glucoside was not mentioned in any of these publications. The present paper deals with the isolation and structure elucidation of acylated steryl glucoside from this plant.

The compound, $C_{51}H_{88}O_7 \cdot \frac{1}{2}H_2O$, mp 168—170°, $[\alpha]_D^{22.5} = -49.6°$ (c=0.125 in $CHCl_3$) gave positive Molish test. On treatment with the modified Liebermann-Burchard reagent[25] the compound reacted very rapidly and the change of color developed was quite similar to that for α-spinasteryl-D-glucoside from same plants.[1] Its ir absorption spectrum shows a broad OH peak at 3400cm^{-1}, ester peaks at 1740 and 1165 cm^{-1}, peaks in the region of 1000—1100 cm^{-1} due to glycosidic bond,[26,27] a peak at 965 cm^{-1} characteristic of a *trans*-disubstituted double bond,[28-31] peaks at 840, 825, and 790cm^{-1} indicating a trisubstituted double bond,[32,33] and a peak at 717 cm^{-1} due to methylene groups in long alkane chain.[34]

The mass spectrum shows two molecular ion peaks at *m/e* 812 and 814 and fragmentation peaks which are characteristic of α-spinasteryl-D-glucoside at *m/e* 574, 531, 462, 433, 412, 395, 379, 369, 351, 271 and 255 with less abundant peaks at *m/e* 576, 414, and 397, indicating presence of Δ^7-stigmastenol derivative.[1] Moreover the presence of

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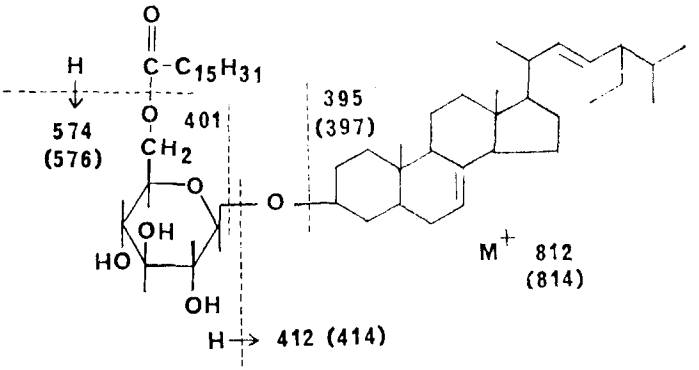
peak at *m/e* 401, which corresponds to the pyronium ion of acylated glucose moiety [35] implies that fatty acid in the compound under study is palmitic acid. Thus this compound is supposed to be a palmitate of α-spinasteryl and Δ^7-stigmastenyl glucoside mixture.



Alkali hydrolysis of this compound gave a glucoside mixture, mp 282—283° and a fatty acid mixture. The properties of this glucoside mixture were exactly same as those of

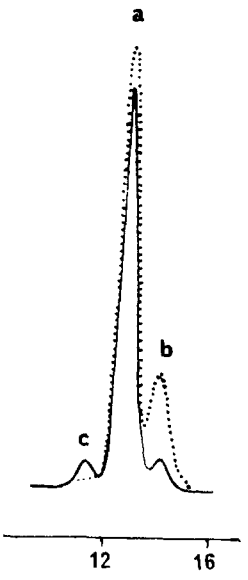


Fig. 1 — Glc of aglucones.——aglucone obtained by acid hydrolysis;·····by HIO_4-alkali degradation; a, α-spinasterol; b, Δ^7-stigmastenol; c, $\Delta^{8(14),22}$-and $\Delta^{14,22}$-stigmastadienol. 2% OV-17 on chromosorb W (60—80 mesh); column temp., 265°; injector temp., 240°; detector temp., 320°; N_2 flow, 40 ml/min.

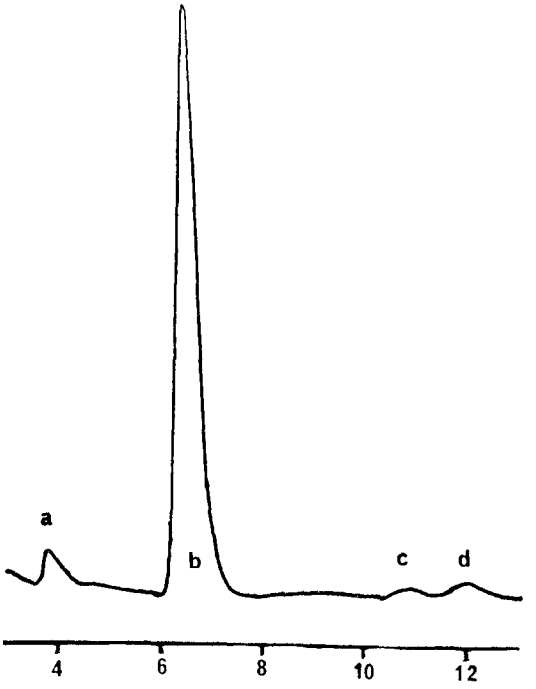


Fig. 2 — Glc of methyl esters of fatty acids derived from ASG. a, myristic; b, palmitic; c, unknown; d, stearic. 15% DEGS on Shimalite (60—80 mesh); column temp., 170°; injector temp., 235°; detector temp., 275°; N_2 flow, 40 ml/min.

glucoside mixture isolated from same plants.[1] As a matter of fact, this mixture on HIO_4-alkali degradation gave a mixture of α-spinasterol and Δ^7-stigmastenol (20 : 1) which were identified by comparison with authentic samples by glc (Fig. 1). D-Glucose was the sole sugar found in hydrolysate obtained on acid hydrolysis of this glucoside mixture.

As shown in Fig. 2, glc analysis of the methylester of the the fatty acid mixture derived from the compound by hydrolysis indicated that palmitic acid was the main fatty acid. A trace of myristic and stearic acids was also present.

Release of formic acid from the acylated glucoside by periodate oxidation established the presence of at least three vicinal unsubstituted hydroxyl groups, which could only be the C-2, C-3, and C-4 hydroxyl groups of the sugar portion. This indicates that the fatty acid is linked to C-6 of the sugar moiety.

On the basis of these results the acylated steryl glucoside preparation isolated from the roots of *P. esculenta* is heterogeneous in composition, containing α-spinasterol and Δ^7-stigmastenol as sterol moieties, and palmitic, stearic, and myristic acids as fatty acyl moieties.

EXPERIMENTAL*

Extraction and Separation of Acylated Steryl Glucoside (ASG) — The MeOH extracts of roots were extracted with $CHCl_3$. The $CHCl_3$ solution was extracted with alkali and then acid, washed with water, and evaporated. The residue from $CHCl_3$ solution was chromatographed over SiO_2. Elution with $CHCl_3$, followed by $CHCl_3$-MeOH-NH_4OH-H_2O (20:4:1:3) gave ASG, mp 168-170°, $[\alpha]_D^{22.5}$ =-49.6° (c=0.125 in $CHCl_3$) ir, 3400, 965, 840, 825, 790 and 717 cm^{-1}. This substance gave a single spot on tlc and the melting point was not altered substiantally by further recrystallizations.

Anal. Calcd for $C_{51}H_{88}O_7 \cdot \frac{1}{2}H_2O$: C, 74.50; H, 10.91. Found : C, 74.01 ; H, 11.09.

Alkali Hydrolysis of ASG — A sample of ASG (500 mg) was heated under reflux in 100 ml of 1 % methanolic NaOH for 1 hr. After removal of MeOH, water was added, extracted with Et_2O. The Et_2O solution was washed with H_2O and dried. The residue

* The melting points were taken on a Mitamura-Riken apparatus and are uncorrected. The ir spectra were determined in KBr pellets on a JASCO Model IR-S spectrophotometer. Glc was carried out with a Shimadzu GC-4B fitted with FID was used. Authentic samples were subjected to glc for comparison purposes, and small quantities were added to the sample for peak enhancement studies.

from the Et_2O solution was crystallized from MeOH to give crude steryl glucoside (SG) mp 272-274°. Acetylation of SG with acetic anhydride-pyridine followed by recrystallization from MeOH gave acetate, mp 174-175°. Hydrolysis of the acetate with 5% methanolic NaOH regenerated pure SG, mp 282-283°, ir 3400, 965, 880 (β-glucosidic bond), 840, 825, and 795 cm^{-1}, Ms *m/e*; 576 and 574 (M^+), 559, 531, 462, 433, 414, 412, 397, 395 (base peak), 379, 369, 351, 271, and 255.

Analysis of Fatty Acids — After separation of SG as above, alkaline aqueous layer was acidified and extracted with Et_2O. The Et_2O solution was washed with water and dried, and the residue was methylated with diazomethane. It methyl ester was analyzed by glc and identified as mixted fatty acid esters, as shown in Fig. 2.

Acid Hydrolysis of SG — SG (20 mg) was hydrolyzed with boiling alcoholic H_2SO_4 (0.5%) for 2 hr. Sterols were taken up in Et_2O and analyzed by glc and tlc and identified as mixture of sterols, α-spinasterol, Δ^7-stigmastenol, and $\Delta^{8(14),22}$-and $\Delta^{14,22}$-stigmastadienols as artifacts[36] (Fig. 1).

Analysis of Sugar — After separation of sterols as above, water layer was refluxed with acid to hydrolyze ethyl glucoside formed during acid hydrolysis in EtOH solution. After neutralizing with $Ba(OH)_2$, the aqueous solution was concentrated. Only D-glucose was detected by tlc, developer; MeOH-$CHCl_3$-NH_4OH-$(CH_3)_2CO$=5 : 2 : 3 : 2 (Rf=0.23), $CHCl_3$-MeOH=3 : 2 (Rf=0.5).

Degradation of Glucoside with HIO_4-alkali — To the solution of glucoside (30 mg) in pyridine (20 ml), 1 ml of 10% $NaIO_4$ was added. The mixture was allowed to stand at room temperature over night, diluted with water and extracted with Et_2O. The Et_2O solution was washed with water, dried over Na_2SO_4 and evaporated to give a residue. The intermediate was refluxed with 50ml of 3% EtOH-KOH for 1hr. The mixture was diluted with water and acidified. The ppt formed was chromatographed over SiO_2. Elution with $CHCl_3$ gave a aglucone mixture, mp 164-165°, which was analyzed on glc and tlc, and identified as α-spinasterol and Δ^7-stigmastenol only as shown in Fig. 1.

HIO_4 Oxidation of ASG : Formation of HCOOH. — To the solution of ASG (10mg) in dioxane (1 ml), 5 drops of 5% HIO_4 was added. After standing overnight, 1 ml of ethylenglycol was added to a portion of the reaction mixture. After 10 min, 0.5 ml of 10% $HgCl_2$ and 0.5 ml of acetate buffer solution, containing 1 ml of glacial acetic acid and

l g of sodium acetate in 100 ml of water, were added. The mixture was brought to dryness in the oven at 105°. Addition of a drop of water and a drop of 0.1N-ammonia to it produced intense black color. [37]

The Liebermann-Burchard Reaction — This reaction was carried out as descrived previously [1].

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