[Chem. Pharm. Bull. 26(2) 520—525 (1978)]

UDC 547.918.02.05 : 581.192

Studies on the Constituents of Phytolaccaceous Plants. I.[1] On the Structures of Phytolaccasaponin B, E and G from the Roots of *Phytolacca americana* L.

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(Received June 28, 1977)

Phytolaccasaponin B, E and G, the major saponins of *Phytolacca americana* L. (Phytolaccaceae), have been isolated. The structures of phytolaccasaponin B, E and G have been established to be phytolaccagenin 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-xylopyranoside]-28-*O*-β-D-glucopyranoside (**4**), phytolaccagenin 3-*O*-β-D-glucopyranosyl(1→4)-β-D-xylopyranoside (**5**) and phytolaccagenin 3-*O*-β-D-xylopyranoside (**6**), respectively.

Phytolaccatoxin reported by Stout, *et al.* was assumed to be a mixture of more than three saponins, but the main saponin was identified as **5** by thin-layer chromatography.

Keywords——*Phytolacca americana* L.; Phytolaccaceae; phytolaccasaponins; phytolaccatoxin; NMR; column chromatography

Three kinds of phytolaccaceous plants, namely *Phytolacca americana* L., *P. esculenta* van Houtte and *P. japonica* Makino, are known to grow wild in our country. Among them, the roots of *P. esculenta* van Houtte have been used as a crude drug against edema and rheumatism in the Far East, including China, Korea and Japan.

Woo, *et al.* reported the isolation and structure elucidation of an anti-inflammatory triterpenoid, named jaligonic acid[3] (=2β, 3β, 23-trihydroxy-olean-12-ene-28, 30-dioic acid) (**1**), a mixture of sterols (α-spinasterol and Δ^7-stigmasterol)[4] and a glucoside mixture of α-spinasterol and Δ^7-stigmastenol[5] from the roots of *P. esculenta* van Houtte.

In 1949, Jenkins, *et al.*[6] reported the isolation of the toxic principle of Pokeroot, the root of *P. americana* L., and Stout, *et al.*[7] also obtained the same substance for which they proposed the name phytolaccatoxin and they established the chemical structure of the aglycone named phytolaccagenin(**2**) (=2β, 3β, 23-trihydroxy-olean-12-ene-28, 30-dioic acid 30-methyl ester) by X-ray analysis. The roots of *P. americana* L. are said to be reputed in Korean medicine to alleviate rheumatism. They were found rich in saponins with strong anti-inflammatory activity.[8] Burke, *et al.*[9] found the presence of **2** and oleanolic acid in the petroleum ether extract of the roots by thin-layer chromatography (TLC), while Johnson and Shimizu[10] examined the berries of *P. americana* L. and they succeeded in identification of three sapogenins as **1**, **2** (major sapogenin) and phytolaccinic acid (=3β, 23-dihydroxy-olean-12-ene-28, 30-dioic acid 30-methyl ester) (**3**). Woo[11a] investigated triterpenoids and steroids in detail

1) This work was presented in part at the 21st Annual meeting of The Japanese Society of Pharmacognosy, Chiba, October, 1975.
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3) W.S. Woo, *J. Pharm. Soc. Korea*, **16**, 99 (1971); *idem*, *Lloydia*, **36**, 326 (1973).
4) W.S. Woo and S.S. Kang, *J. Pharm. Soc. Korea*, **17**, 152 (1973).
5) W.S. Woo and S.S. Kang, *J. Pharm. Soc. Korea*, **17**, 161 (1973).
6) Z.F. Ahmed, C.Z. Zufall, and G.L. Jenkins, *J. Am. Pharm. Assoc.*, **38**, 443 (1949).
7) G.H. Stout, B.M. Malofsky, and V.F. Stout, *J. Am. Chem. Soc.*, **86**, 957 (1964).
8) W.S. Woo, K.H. Shin, and S.S. Kang, *Kor. J. Pharmacog.*, **7**, 47 (1976).
9) D.E. Burke and P.W. Le Quesne, *Phytochemistry*, **10**, 3319 (1971).
10) A. Johnson and Y. Shimizu, *Tetrahedron*, **30**, 2033 (1974).
11) *a*) W.S. Woo, *Phytochemistry*, **13**, 2887 (1974); *b*) W.S. Woo and S.S. Kang, *J. Pharm. Soc. Korea*, **18**, 231 (1974); *c*) W.S. Woo, *Phytochemistry*, **14**, 1885 (1975).

to describe the presence of **1**, **2**, α-spinasterol, Δ^7-stigmasterol, α-spinasteryl-D-glucoside, Δ^7-stigmasteryl-D-glucoside, α-spinasteryl-D-(6-*O*-palmityl)-glucoside and Δ^7-stigmastenyl-D-(6-*O*-palmityl)-glucoside from the ether extract of the roots and a genin mixture consisted of **1**, **2** (major component), phytolaccagenic acid[11b] (=phytolaccinic acid, **3**), esculentic acid[11c] (=desmethylphytolaccagenic acid) and a compound suggested to be 3-oxo-30-carbomethoxy-23-norolean-12-en-28-oic acid from the total methanol soluble saponins.

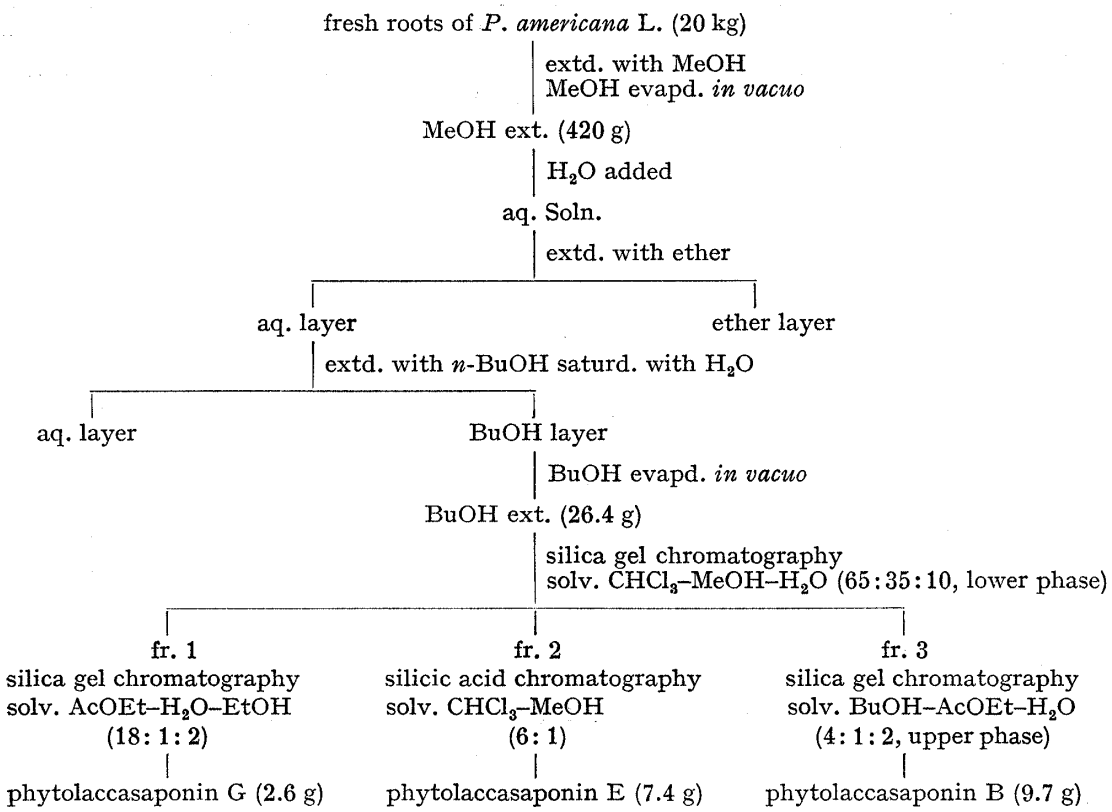


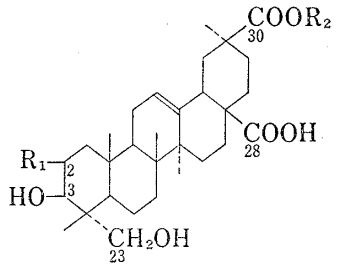
Chart 1

TABLE I

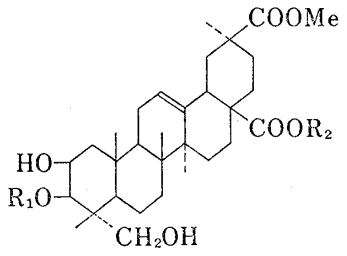
Phytolaccasaponins	Properties	mp (°C)	$[\alpha]_D$ (*c*, solv., °C)	Formula	IR (KBr, cm^{-1})	NMR $\delta_{TMS}^{C_5D_5N}$ (ppm)
B (**4**)	Colorless needles (iso-PrOH–AcOEt–$CHCl_3$–H_2O)	218—220	+38.31° (0.93, EtOH, 29)	$C_{48}H_{76}O_{21}\cdot 3H_2O$	3400 (OH) 1750 (COOR)	1.02 (3H, s, CH_3) 1.10—1.25 (3H×2, s, CH_3) 1.32 (3H, s, CH_3) 1.45 (3H, s, CH_3) 3.60 (3H, s, OCH_3) 5.55 (1H, m, olefinic H)
E (**5**)	A white powder (iso-PrOH–H_2O)	(257—258)	+51.26° (0.99, EtOH, 21)	$C_{42}H_{66}O_{16}$	3400 (OH) 1730 (COOR) 1710 (COOH)	1.10 (3H, s, CH_3) 1.22 (3H, s, CH_3) 1.25 (3H, s, CH_3) 1.32 (3H, s, CH_3) 1.55 (3H, s, CH_3) 3.75 (3H, s, OCH_3) 5.65 (1H, m, olefinic H)
G (**6**)	Colorless needles (MeOH–H_2O)	266—269	+67.27° (1.44, EtOH, 26)	$C_{36}H_{56}O_{11}\cdot H_2O$	3400 (OH) 1730 (COOR) 1690 (COOH)	1.12 (3H, s, CH_3) 1.25 (3H, s, CH_3) 1.30 (3H, s, CH_3) 1.38 (3H, s, CH_3) 1.62 (3H, s, CH_3) 3.75 (3H, s, OCH_3) 5.65 (1H, m, olefinic H)

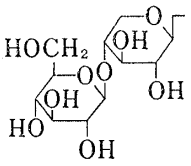
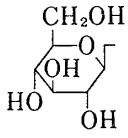
Recently, Woo, *et al.*[12] reported that the structure of phytolaccoside B, one of the major saponins isolated from the roots of *P. americana* L., was elucidated as 3-*O*-*β*-D-glucopyranosyljaligonic acid 30-methyl ester(3-*O*-*β*-D-glucopyranosylphytolaccagenin). This paper describes the structural elucidation of three major saponins, phytolaccasaponin B(**4**), E(**5**) and G(**6**), isolated from the roots of *P. americana* L.

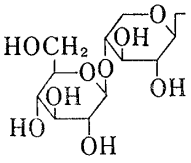
Three new triterpenoidal glycosides were obtained from the methanolic extract of the fresh roots of *P. americana* L. as shown in Chart 1. TLC of the butanol extract revealed the presence of more than eight saponins and each spot was named phytolaccasaponin A—G in order of decreasing polarity. The general properties of **4**, **5** and **6** are given in Table I. Their infrared (IR) and nuclear magnetic resonance (NMR) spectra show the presence of one esteric *O*-methyl group in each glycoside.



1 : R_1=OH, R_2=H
2 : R_1=OH, R_2=CH_3
3 : R_1=H , R_2=CH_3



4 : R_1=  , R_2= 

5 : R_1=  , R_2=H

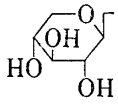
6 : R_1=  , R_2=H

Chart 2

On acid hydrolysis, **4**, **5** and **6** gave phytolaccagenin(**2**) as a common aglycone. The monosaccharides in each hydrolysate were isolated and identified as follows: **4** (D-xylose, D-glucose); **5** (D-xylose, D-glucose); **6** (D-xylose).

Acetylation of **4**, **5** and **6** with acetic anhydride and pyridine afforded undecaacetate of **4**, heptaacetate of **5** and tetraacetate of **6**, respectively. IR spectra of each acetate show the presence of hydroxyl group which resists to the acetylation.

On methylation by the Hakomori's method, **5** gave an octa-*O*-methylate methyl ester which was proved to be fully *O*-methylated derivative by IR spectrum. Methanolysis of per-*O*-methylate of **5** afforded an aglycone, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,3-di-*O*-methylxylopyranoside. The aglycone, $C_{34}H_{54}O_7$, was suggested to be a di-*O*-methylphytolaccagenin methyl ester by IR and NMR spectra and the location of the free hydroxyl group was proved as follows. On acetylation by the usual manner, the aglycone afforded a monoacetyl derivative, $C_{36}H_{56}O_8$, which does not show any hydroxyl absorption

12) W.S. Woo and S.S. Kang, *Phytochemistry*, **15**, 1315 (1976).

band in IR spectrum. The NMR spectrum of the monoacetyl derivative shows the presence of one acetyl group(δ=2.15), four *O*-methyl groups(δ=3.24—3.60) and one proton (δ=5.00, 1H, d, J=4 Hz) corresponding to a methine proton located on a carbon bearing an *O*-acetoxyl group. Based on the NMR analysis, the location of a hydroxyl group of the aglycone was established to be C-3. Furthermore, each coupling constant(7 Hz) of two anomeric protons of per-*O*-methylate of **5** in NMR spectrum indicated that the configurations of both D-xylose and D-glucose are β. Consequently, the structure of phytolaccasaponin E was elucidated to be phytolaccagenin 3-*O*-β-D-glucopyranosyl(1→4)-β-D-xylopyranoside(**5**).

The authentic sample of phytolaccatoxin kindly given us from Dr. Stout was found by TLC to be a mixture of more than three saponins, but the main spot of phytolaccatoxin on TLC(plate: Kieselgel H; *Rf* 0.12(solvent: $CHCl_3$–MeOH–AcOEt–H_2O=2: 2: 4: 1 lower phase), *Rf* 0.35(solvent: $CHCl_3$–MeOH–H_2O=7: 3: 1 lower phase)) was identified as **5** by direct comparisons.

The structure of **6** was deduced as follows. On methylation with diazomethane, **6** gave a methyl ester, which shows the presence of two esteric *O*-methyl groups by NMR spectrum. Furthermore, methyl ester of **6** was acetylated by the usual method to afford a tetraacetate, which shows the presence of one hydroxyl group, two esteric *O*-methyl groups and four *O*-acetyl groups by IR and NMR spectra. Based on the foregoing experiments, **6** was assumed to be a monoxyloside of phytolaccagenin and the presence of esteric linkage of xylose with the aglycone was excluded. In order to compare the prosapogenin of **5** with **6**, the former was hydrolyzed with 0.2N H_2SO_4-dioxane to afford phytolaccagenin 3-*O*-β-D-xylopyranoside(**6**), which was identified with phytolaccasaponin G by direct comparisons.

Finally, the structure of **4** was elucidated as follows. On methylation by the Hakomori's method, **4** gave a per-*O*-methyl derivative which shows the presence of thirteen *O*-methyl groups and two anomeric protons(δ=3.46, 1H, d, J=6 Hz; δ=3.55, 1H, d, J=6 Hz) by NMR spectrum. Per-*O*-methylate of **4** was methanolyzed with 2N HCl to afford methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,3-di-*O*-methylxylopyranoside. On the other hand, the reduction of per-*O*-methylate of **4** with lithium aluminium hydride followed by acidification with 2% H_2SO_4 gave 2,3,4,6-tetra-*O*-methylglucopyranose, 2,3,4,6-tetra-*O*-methylsorbitol and a reduced prosapogenin. Furthermore, the hydrolysis of **4** with 0.5% KOH in ethanol gave **5**. The configuration of the esteric glucose was deduced to be β from the difference of molecular optical rotation($\Delta[M]_D$ −26°) between phytolaccasaponin B($[M]_D$ +398°) and phytolaccasaponin E($[M]_D$ +424°).[13] Based on the foregoing experiments, phytolaccasaponin B was deduced to be phytolaccagenin 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-xylopyranoside]-28-*O*-β-D-glucopyranoside(**4**).

The chemical and biological studies on the glycosides of *P. americana* L. and the related plants are now being in progress.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto OR-50 automatic polarimeter. IR spectra were obtained with a Hitachi Model EPI-2 and NMR spectra were taken at 90 MHz with a Hitachi Model R-22 High Resolution NMR spectrometer and chemical shifts are given in δ(ppm) scale with tetramethyl silane as the internal standard. GLC was run on a Shimazu GC-6A with flame ionization detector. Paper partition chromatography (PPC) was conducted on Tôyô Roshi No. 51 using the following solvent system a) *n*-BuOH–AcOH–H_2O (4: 1: 5 upper phase), b) phenol saturated with water. Detection was made by aniline hydrogen phthalate as the spray reagent. TLC was performed on Kieselgel H (Merck) by using the following solvent system: a) $CHCl_3$–MeOH–H_2O=65: 35: 10 lower phase, b) hexane–acetone=1: 1, c) benzene–acetone=1: 1, d) $CHCl_3$–MeOH=8: 1. Detection was made by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation of Phytolaccasaponins——Fresh roots of *Phytolacca americana* L. (20 kg) was crushed and treated as shown in Chart 1. The BuOH extractives were submitted to column chromatography

13) Methyl α-D-glucopyranoside $[M]_D$=+307°, methyl β-D-glucopyranoside $[M]_D$=−63°.

over silica gel with $CHCl_3$–MeOH–H_2O (65: 35: 10, lower phase) to separate into three fractions (Fr. 1, 2 and 3). Fr. 1 was purified by rechromatography on silica gel with AcOEt–H_2O–EtOH (18: 1: 2) to give **6**. Fr. 2 and Fr. 3 were submitted to column chromatography on silicic acid with $CHCl_3$–MeOH (6: 1) for Fr. 2 and on silica gel with BuOH–AcOEt–H_2O (4: 1: 2 upper phase) for Fr. 3 to afford **5** from the former and **4** from the latter.

Properties of 4, 5 and 6——The general properties of phytolaccasaponins are listed in Table I. **4**: *Anal.* Calcd. for $C_{48}H_{76}O_{21}\cdot 3H_2O$: C, 55.26; H, 7.92. Found: C, 54.98; H, 7.75. **5**: *Anal.* Calcd. for $C_{42}H_{66}O_{16}$: C, 60.10; H, 8.05. Found: C, 60.13; H, 8.33. **6**: *Anal.* Calcd. for $C_{36}H_{56}O_{11}\cdot H_2O$: C, 63.33; H, 8.56. Found: C, 63.50; H, 8.47.

Hydrolysis of 4, 5 and 6——Each phytolaccasaponin was refluxed with 2 N H_2SO_4–dioxane (4 N H_2SO_4–dioxane=1: 1 v/v) on a water bath for 1 hr. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extractive was washed with water, dried on anhyd. Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel with hexane–acetone (4: 1) to afford colorless prisms from ethyl acetate, mp 312—317° (dec.). *Anal.* Calcd. for $C_{31}H_{48}O_7$: C, 69.98; H, 9.08. Found: C, 69.50; H, 9.34. The aglycone was identified with authentic phytolaccagenin kindly given us from Dr. Stout by comparing TLC (solvent b, *Rf* 0.49) and IR spectra.

The aqueous layer was neutralized with Amberlite IR-4B and evaporated *in vacuo* to dryness. Each residue was examined by PPC and GLC. PPC: solvent a) *Rf* 0.27 (glucose), 0.37 (xylose); solvent b) *Rf* 0.38 (glucose), 0.47 (xylose). GLC (column: 5% SE-52 on chromosorb W glass column 3 mm×2 m, column temp.: 160°, injection temp.: 240°, carrier gas: N_2 flow 0.6 kg/cm², samples: TMS derivatives) t_R (min) 8.0, 11.3 (xylose), 28.6, 35.6 (glucose).

Furthermore, the residue originated from **4** was chromatographed over cellulose powder using $CHCl_3$–MeOH–H_2O=7: 3: 1 (lower phase) as the developing solvent to afford glucose and xylose, which were measured optical rotations. Glucose: $[\alpha]_D^{28}$ +49.0° (c=0.66, H_2O) (D-glucose lit.[14] $[\alpha]_D$ +52.7°); xylose: $[\alpha]_D^{28}$ +14.7° (c=0.30, H_2O) (D-xylose lit.[14] $[\alpha]_D$ +18.8°).

Acetylation of 4, 5 and 6——Each saponin (100 mg) was dissolved in pyridine (2 ml) and Ac_2O (2 ml), and the solution was allowed to stand for 24 hr at room temperature. The reaction mixture was worked up as usual and the product was purified by recrystallization. Undecaacetate of **4**: colorless needles from EtOH, mp 160—161°, $[\alpha]_D^{21}$ +27.27° (c=0.99, $CHCl_3$), *Anal.* Calcd. for $C_{70}H_{98}O_{32}$: C, 56.51; H, 6.91. Found: C, 56.51; H, 6.56. IR ν_{max}^{Nujol} cm⁻¹: 3560 (OH), 1750 (COOR). NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 0.91 (3H, s, CH_3), 1.05—1.12 (3H×2, s, CH_3), 1.25 (3H, s, CH_3), 1.90—2.20 (3H×11, $OCOCH_3$), 3.70 (3H, s, $COOCH_3$), 5.35 (1H, m, olefinic H). Heptaacetate of **5**: a white powder from aq. MeOH, (mp 168—170°), $[\alpha]_D^{21}$ +40.0° (c=1.00, $CHCl_3$), *Anal.* Calcd. for $C_{56}H_{80}O_{23}$: C, 59.99; H, 7.19. Found: C, 59.51; H, 7.01. IR ν_{max}^{Nujol} cm⁻¹: 3550 (OH), 1740 (COOR). NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 0.90 (3H, s, CH_3), 1.10—1.20 (3H×2, s, CH_3), 1.28 (3H, s, CH_3), 1.90—2.20 (3H×7, $OCOCH_3$), 3.70 (3H, s, $COOCH_3$), 5.35 (1H, m, olefinic H). Tetraacetate of **6**: a white powder from aq. MeOH, (mp 138—140°), $[\alpha]_D^{23}$ +33.0° (c=1.00, EtOH). IR ν_{max}^{Nujol} cm⁻¹: 3400 (OH), 1710 (COOR). NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.30 (3H, s, CH_3), 2.08—2.10 (3H×3, s, $OCOCH_3$), 2.15 (3H, s, $OCOCH_3$), 3.85 (3H, s, $COOCH_3$), 5.40 (1H, m, olefinic H).

Methylation of 4 and 5 by the Hakomori's Method——NaH (650 mg) was stirred with dimethylsulfoxide (DMSO 12 ml) at 70° for 1 hr under N_2 gas flow. To this reagent **4** or **5** (800 mg) in DMSO (6 ml) was added. The mixture was stirred for 20 min at room temperature under N_2 gas flow. CH_3I (6 ml) was added to the solution and the reaction mixture was stirred at room temperature for 6 hr. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and evaporated to afford a syrup (600 mg). The residue was chromatographed on 30 g of silica gel using hexane–acetone=10: 1 to afford a homogeneous per-*O*-methylate of **4** and **5**, respectively. Per-*O*-methylate of **4**: a white powder from aq. MeOH, (mp 96—98°), IR ν_{max}^{Nujol} cm⁻¹: OH (nil.), 1740 (COOR), NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.28 (3H, s, CH_3), 3.38 (3H×3, s, OCH_3), 3.47 (3H, s, OCH_3), 3.56 (3H, s, OCH_3), 3.60 (3H, s, OCH_3), 3.63 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.78 (3H×6, s, OCH_3), 4.46 (1H, d, J=7 Hz, anomeric H), 4.55 (1H, d, J=7 Hz, anomeric H), 5.50 (2H, m, olefinic H and anomeric H). Per-*O*-methylate of **5**: a white powder from aq. MeOH, (mp 76—77°), $[\alpha]_D^{21}$ +3.82° (c=1.31, EtOH), IR ν_{max}^{Nujol} cm⁻¹: OH (nil.), 1735 (COOR), NMR (in C_5D_5N) δ: 0.95 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.22 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.40 (3H, s, CH_3), 3.33 (3H, s, OCH_3), 3.40 (3H×2, s, OCH_3), 3.48 (3H, s, OCH_3), 3.60 (3H, s, OCH_3), 3.68 (3H×4, s, OCH_3), 3.72 (3H, s, OCH_3), 4.65 (1H×2, d, J=7 Hz, anomeric H), 5.60 (1H, m, olefinic H).

Methanolysis of Per-*O*-methylate of 5——Per-*O*-methylate of **5** (80 mg) was refluxed with 2 N HCl in MeOH (6 ml) for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* to give a syrup which was chromatographed on silica gel using hexane–acetone (8: 1) to give a di-*O*-methylphytolaccagenin methyl ester, a white powder from aq. MeOH, (mp

14) J. Stanek, M. Cerny, J. Kocourek, and J. Pacak, "The Monosaccharides," Academic press, New York and London, 1963, p. 83.

92—93°), $[\alpha]_D^{31}$ +4.04° (c=1.73, EtOH), IR ν_{max}^{Nujol} cm^{-1}: 3440 (OH), 1750 (COOR), NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 0.85 (3H, s, CH_3), 1.15 (3H×2, s, CH_3), 1.18 (3H, s, CH_3), 3.33 (3H×2, s, OCH_3), 3.60 (3H, s, $COOCH_3$), 3.70 (3H, s, $COOCH_3$), 5.40 (1H, m, olefinic H). Di-*O*-methylphytolaccagenin methyl ester was acetylated in the same way as in **4**, **5** and **6** to give a white powder from aq. MeOH, (mp 57—59°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 1750 (COOR), NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.23 (3H×2, s, CH_3), 1.32 (3H, s, CH_3), 2.15 (3H, s, $OCOCH_3$), 3.24 (3H, s, OCH_3), 3.27 (3H, s, OCH_3), 3.30 (3H, s, $COOCH_3$), 3.60 (3H, s, $COOCH_3$), 5.00 (1H, d, J=4 Hz, $>C<^{OCOCH_3}_{H}$), 5.35 (1H, m, olefinic H).

The *O*-methylated monosaccharides separated by chromatography were identified by GLC (column: 5% NPGS on chromosorb W, glass column 3 mm×2 m, column temp: 145°, injection temp.: 228°, carrier gas N_2 flow 1.0 kg/cm^2) t_R (min) 13.3, 18.7 (methyl 2,3,4,6-tetra-*O*-methylglucopyranoside); 15.9, 18.9 (methyl 2,3-di-*O*-methylxylopyranoside). TLC (solvent c): *Rf* 0.56, 0.59 (methyl 2,3,4,6-tetra-*O*-methylglucopyranoside), 0.32, 0.43 (methyl 2,3-di-*O*-methylxylopyranoside).

Methanolysis of Per-*O*-methylate of 4——Per-*O*-methylate of **4** (75 mg) was refluxed with 2 N HCl in MeOH (6 ml) for 1 hr. The reaction mixture was treated by the same procedure as described above, and methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,3-di-*O*-methylxylopyranoside were identified by GLC.

Reductive Cleavage of Per-*O*-methylate of 4 with $LiAlH_4$——Per-*O*-methylate of **4** (50 mg) in absolute ether was reduced with $LiAlH_4$ (110 mg) under refluxing for 2 hr. The reaction mixture was treated with water under ice cooling to decompose the excess $LiAlH_4$ and then acidified with 2% H_2SO_4 to dissolve the precipitates. The solution was extracted with ether, washed with water, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using hexane–acetone (4: 1) as a solvent, followed by reprecipitation from aq. MeOH, affording a white powder, (mp 67—68°), IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), NMR (in $CDCl_3$) δ: 0.90 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.28 (3H×3, s, CH_3), 3.33 (3H×2, s, OCH_3), 3.42 (3H, s, OCH_3), 3.55 (3H×2, s, OCH_3), 3.65 (3H×3, s, OCH_3), 5.05 (1H, m, olefinic H).

The aqueous layer was extracted with $CHCl_3$, washed with water, dried over Na_2SO_4 and evaporated. The residue was examined by GLC (column: 5% NPGS on chromosorb W glass column, 3 mm×2 m, column temp.: 150°, injection temp.: 210°, carrier gas: N_2 flow 1.0 kg/cm^2, samples: TMS derivatives) t_R (min) 4.8, 6.5 (2,3,4,6-tetra-*O*-methylglucose), 9.2 (2,3,4,6-tetra-*O*-methylsorbitol).

Hydrolysis of 4 with Ethanolic 0.5% KOH——**4** was refluxed with ethanolic 0.5% KOH under N_2 flow for 1 hr. The reactant was diluted with water and the EtOH was removed *in vacuo*. The aqueous residue was neutralized with dilute HCl to pH 6.0 and extracted with BuOH. The BuOH solution was evaporated *in vacuo* and the residue was purified by chromatography on silica gel using $CHCl_3$–MeOH–H_2O (65: 35: 10, lower phase) to afford a prosapogenin which was identified as phytolaccasaponin E by direct comparisons. *Anal.* Calcd. for $C_{42}H_{66}O_{16}$: C, 60.10; H, 8.05. Found: C, 60.62; H, 8.36.

Methylation of 6 with CH_2N_2——To a solution of **6** (300 mg) in MeOH was added excess CH_2N_2 in ether and the mixture was allowed to stand for 1 hr. The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (10:1). Methyl ester of **6** was obtained as a white powder by reprecipitation from aq. MeOH, (mp 138—140°), IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1720 (COOR), NMR (in C_5D_5N) δ: 0.90 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.28 (3H×2, s, CH_3), 1.55 (3H, s, CH_3), 3.70 (3H, s, $COOCH_3$), 3.75 (3H, s, $COOCH_3$), 5.10 (1H, m, olefinic H).

Acetylation of Methyl Ester of 6——A solution of methyl ester of **6** in pyridine and Ac_2O was allowed to stand for 24 hr at room temperature. The reactant was worked up as usual and the product was purified by recrystallization from hexane–acetone to afford colorless needles, mp 231—233°, *Anal.* Calcd. for $C_{45}H_{66}O_{15}$: C, 63.00; H, 7.80. Found: C, 63.37; H, 8.06. IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1750 (COOR). NMR (in $CDCl_3$) δ: 0.75 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.28 (3H, s, CH_3), 2.00—2.18 (3H×4, s, $OCOCH_3$), 3.65 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.75 (1H, d, J=7 Hz, anomeric H), 5.10 (1H, m, olefinic H).

Partial Hydrolysis of 5——**5** (700 mg) was refluxed with 0.2 N H_2SO_4–dioxane=1: 1 v/v) for 2 hr. After cooling, the reaction mixture was extracted with BuOH saturated with water. The BuOH solution was washed with water and then evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH (8: 1) to give a prosapogenin, colorless needles from aq. MeOH, mp 268—270°, which was identified as **6** by mixed mp, TLC (solvent d, *Rf* 0.45) and by comparing IR spectra.

Acknowledgement The authors express their gratitude to Dr. G.H. Stout, University of Washington, and Dr. Y. Shimizu, University of Rhode Island for their kind supply of the authentic samples. Thanks are also due to the members of Analytical Laboratory of this School for elemental analysis and for the measurement of IR and NMR spectra.