

Saponins from the Callus Mass of *Phytolacca americana*

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Abstract □ Strong anti-inflammatory saponins *Phytolacca americana* (Phytolaccaceae) were obtained from callus mass derived from the stems and also from that derived from the roots of cultivated *Phytolacca americana* (which were designated as PAS and PAR, respectively). The callus were grown on Linsmair and Skoog's agar medium supplemented with 1ppm of 2,4-dichlorophenoxyacetic acid. Phytolaccoside B and D were obtained from PAS and phytolaccoside A and B from PAR. The thin layer chromatograms of the crude saponins from PAS and PAR were similar to those of original plants. PAS contained phytolaccoside B as a major component while phytolaccoside E was a major saponin in original plants.

Keywords □ Phytolaccaceae, the callus mass of *Phytolacca americana*, triterpenoid saponins, phytolaccoside A, B and D.

The roots of *Phytolacca americana* have been used in the treatment edema and rheumatism as a folk medicine. Anti-inflammatory saponins have recently been isolated from this plant¹⁾ and their structures were elucidated.^{2,3)}

The saponins produced in the callus mass derived from the stems and roots of this plant were investigated in order to apply the callus method for the production of anti-inflammatory saponins of *Phytolacca americana*.

The callus mass derived from the stems and from the roots were designated as PAS and PAR respectively. Phytolaccoside B and D were obtained from PAS and phytolaccoside A and B from PAR.

EXPERIMENTAL METHODS

Tissue culture of Phytolacca americana

The root and stem of cultivated *Phytolacca americana* were transferred on to Linsmair and Skoog's agar medium supplemented with 1ppm of 2,4-dichlorophenoxyacetic acid (2,4-D). The callus have been subcultured on the same medium at about 25° in the light and at four to five weeks intervals.

Isolation of saponins from the callus mass

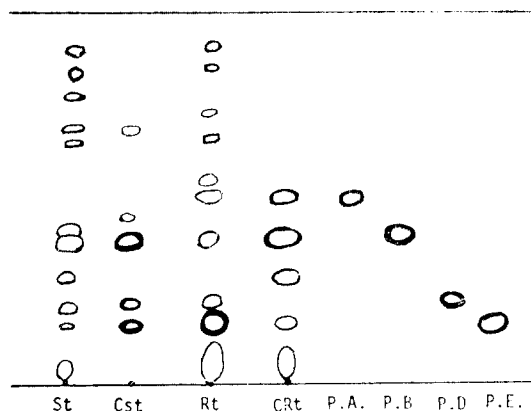


Fig. 1: Chromatogram of fraction I.
Developer; MeOH-CHCl₃-NH₄OH-H₂O=30:
60:3:4

St; stem of *Phytolacca americana*
CSt; callus mass from St
Rt; root of *Phytolacca americana*
CRt; callus mass from Rt
P.A; Phytolaccoside A
P.B; Phytolaccoside B
P.D; Phytolaccoside D
P.E; Phytolaccoside E

The saponin mixture obtained from the MeOH extract of PAS and PAR harvested as described previously⁴⁾ was chromatographed on a silica gel column. Elution with CHCl_3 -MeOH (7:1-gradient) gave saponin I and V from PAS and VIII and XI from PAR, respectively.

Phytolaccoside B (I)

mp. 252~6°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3340 (OH), 1725 (ester), 1000~1100 (glycoside)

Phytolaccoside D (V)

mp. 220~2°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1730 (ester), 1703 (acid), 1000~1100 (glycoside).

Phytolaccoside A (VIII)

mp. 273~4°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420 (OH), 1710 (ester), 1700 (acid), 1000~1100 (glycoside).

Phytolaccoside B (XI)

mp. 254~6°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3340 (OH), 1724 (ester), 1000~1100 (glycoside).

Acid hydrolysis of phytolaccoside B (I)

I was hydrolyzed by refluxing with 5% H_2SO_4 in 50% dioxane for 3hr. After addition of water the resulting ppt was filtered and washed with water, identified as phytolaccagenin by co-TLC with an authentic sample. The aqueous layer was neutralized with BaCO_3 and filtered. The filtrate was concentrated under reduced pressure. The residue was found to be D-xylose by TLC (cellulose plate, pyridine-EtOAc-HOAc- H_2O =36:36:7:21, hydrogen phthalate).

Methylation of I

I was dissolved in MeOH and treated with ethereal CH_2N_2 to give a methylester (II), mp. 182~6°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1725, 1150 (ester).

Acetylation of I

To the I Ac_2O and pyridine were added and heated in W.B. for 3hr. The reaction mixture was poured onto crushed ice and filtered.

The precipitate was chromatographed on silica gel eluted with CHCl_3 -MeOH=9:1 to give the pentaacetate (III), mp. 154~6°, NMR(CDCl_3)

δ 0.75~1.25ppm (5 X Me), 2.40~2.13 (5 X acetyl), 3.69 (3H, s, MeOCO), 4.48 (1H, d, J=7Hz, anomeric H) and tetraacetate (IV), mp. 170~2°.

Acid hydrolysis of phytolaccoside D (V)

A sample of V was hydrolyzed by 5% H_2SO_4 —50% dioxane under similar conditions as above gave phytolaccagenic acid. D-xylose and D-glucose were detected in the aqueous layer by TLC.

Partial hydrolysis of V

A sample of V in 0.025 N-HCl—dioxane was refluxed for 1hr. After addition of water and filtration, chromatography on silica gel(CHCl_3 -MeOH=5:1) of the resulting ppt yielded phytolaccagenic acid, phytolaccoside A and unchanged phytolaccoside D.

Methylation of V

Methylation of V with CH_2N_2 under similar conditions as above gave methylester (VI) which was chromatographed over silica gel, eluted with ethylacetate saturated with water to give an amorphous powder, mp. 210~3°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730 (ester).

Acetylation of V

Acetylation of V with acetic anhydride and pyridine under similar conditions as above gave an acetate (VII) which was purified by chromatography using CHCl_3 -MeOH (98:1), mp. 139~142°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1735, 1225 (acetate), NMR(CDCl_3) δ 0.77~1.27ppm (5 X Me), 2.02~2.12 (7 X acetyl), 3.69 (3H, s, MeOCO), 4.43 (1H, d, J=7Hz, anomeric H), 4.63 (1H, d, J=8Hz, anomeric H), 5.35 (1H, m, C_{12} -H).

Hydrolysis of phytolaccoside A (VIII)

A solution of VIII in 5% H_2SO_4 —50% dioxane was refluxed for 3hr. The reaction mixture was worked up as described above to yield phytolaccagenic acid, mp. 309~311°, which was identified by co-TLC with an authentic sample. D-xylose was detected in the aqueous layer by

TLC as above.

Methylation of VIII

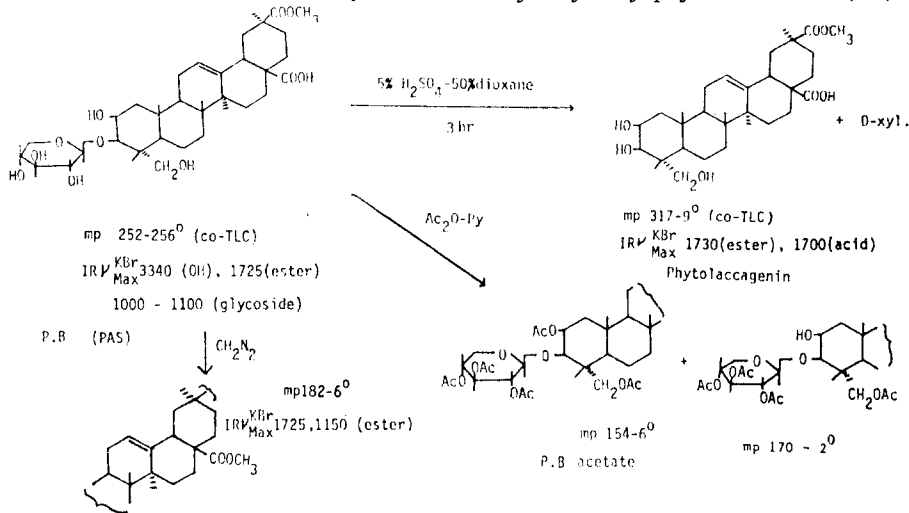
Methylation of VIII with CH_2N_2 under similar conditions as above gave methyl ester (IX), mp. $143\sim 4^\circ$, $\text{IR } \nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1730 (ester).

Acetylation of VIII

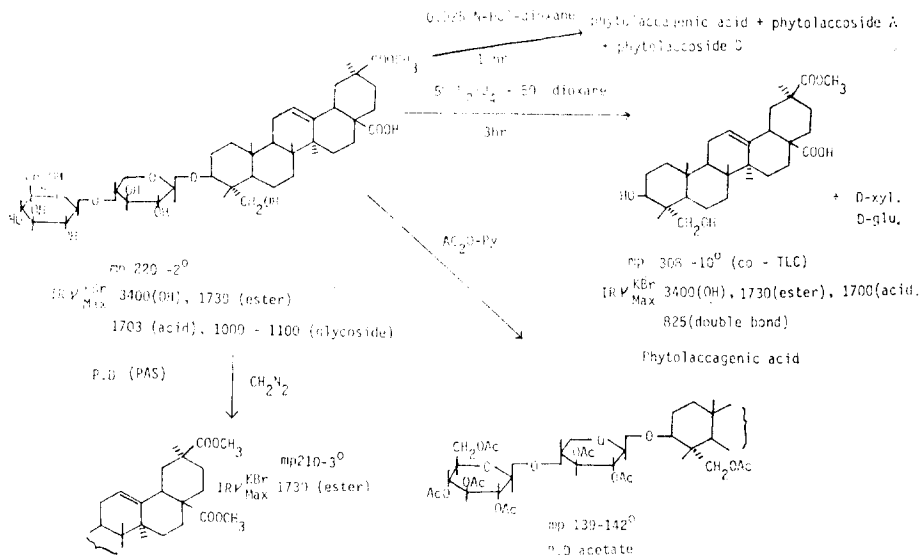
A sample of VIII was acetylated with acetic anhydride and pyridine in the usual way. The

reaction product was chromatographed over silica gel. Elution with CHCl_3 yielded the acetate (X), mp. $142\sim 5^\circ$, $\text{IR } \nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1735, 1220 (acetate), $\text{NMR } (\text{CDCl}_3) \delta$ 0.74~1.28ppm (5 X Me), 2.05~2.10 (4 X acetyl), 3.70 (3H, s, MeOCO), 4.47 (1H, d, $J=7\text{Hz}$, anomeric H), 5.35 (1H, m, $\text{C}_{12}\text{-H}$).

Hydrolysis of phytolaccoside B (XI)



Scheme 1: Acid hydrolysis, methylation and acetylation of phytolaccoside D (PAS).



Scheme 2: Acid hydrolysis, methylation and acetylation of phytolaccoside D (PAS).

A sample of XI was hydrolyzed by refluxing with 5% H_2SO_4 in 50% dioxane under similar conditions as above gave phytolaccagenin, mp. 317~9° and D-xylose.

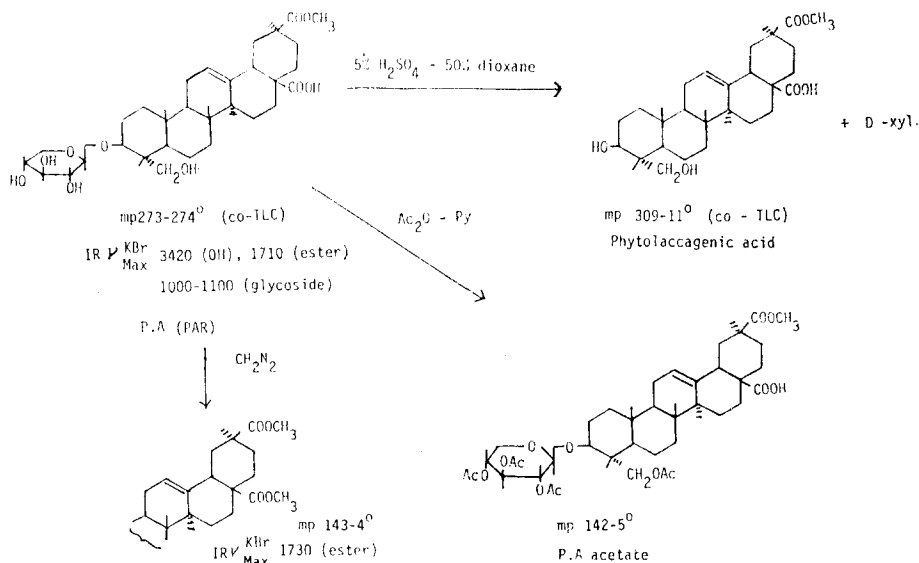
Methylation of XI

Methylation of XI with CH_2N_2 under similar conditions as above gave methylester (XII), mp. 180~4°, IR $\nu_{max}^{KBr} cm^{-1}$: 1725, 1150(ester), which was found to be the same compound as II by

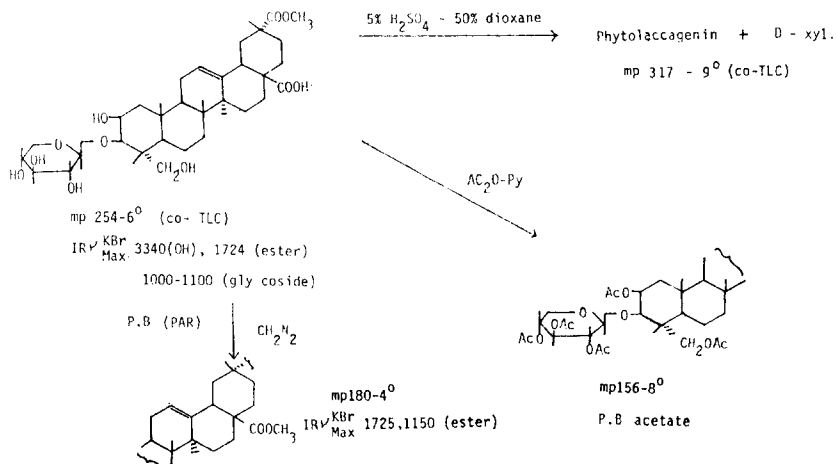
co-TLC with an authentic sample.

Acetylation of XI

A sample of XI was acetylated with acetic anhydride and pyridine in the usual way. The reaction product was worked up as described above to yield pentaacetate (XIII), mp. 156~8; IR $\nu_{max}^{KBr} cm^{-1}$: 1735, 1225 (acetate), NMR ($CDCl_3$) δ 0.82~1.17ppm (5 X Me), 2.01~2.18 (5 X acetyl), 3.69 (3 H, s, MeOCO), 4.46 (1H,



Scheme 3: Acid hydrolysis, methylation and acetylation of phytolaccoside A (PAR).



Scheme 4: Acid hydrolysis, methylation and acetylation of phytolaccoside B (PAR).

d, $J=7\text{Hz}$, anomeric H), 5.37(1H, m, C₁₂-H).

RESULTS AND DISCUSSION

Phytolaccoside B³¹ and D²¹ (from PAS)

Phytolaccoside B (I) mp. 252~6°, on acid hydrolysis, gave phytolaccagenin as the genin was identified by direct comparison with an authentic sample (co-TLC, mmp, IR). D-xylose was identified as the sugar component by TLC. Methylation with CH₂N₂ gave a methylester (II) mp. 180~4°, indicating that the sugar was not linked as an ester to the carboxyl function at C-17. Acetylation with acetic anhydride and pyridine on a steam bath afforded two compounds, a pentaacetate (III) mp. 154~6° and a tetraacetate mp. 170~2° (IV) which were identified by direct comparison with an authentic samples. When acetylated at 4°C, the major product was tetraacetate.

Saponification of tetraacetate with NH₄OH gave the original saponin, however a monoacetate was formed from pentaacetate under same conditions (Scheme 1). This result suggests that the more sterically hindered axial secondary alcohol group of the genin in this saponin is free and therefore the sugar is linked to the 3-β-OH group. The NMR spectrum of the pentaacetate showed signals for five acetoxy groups (2.04~2.13ppm). This result indicated the presence of only one D xylose unit in the saponin I. Also one anomeric proton was observed at 4.48ppm ($J=7\text{Hz}$), indicated the β-configuration of glycosidic linkage in this saponin.

Saponin I was identical with phytolaccoside B by direct comparison with an authentic sample (co-TLC, mmp). Thus the structure of phytolaccoside B was established as 3-O-β-D-xylopyranosyl phytolaccagenin.

Phytolaccoside D (V) mp. 220~2° gave D-

xylose, D-glucose and phytolaccagenic acid identified as the sugar components and genin by direct comparison with an authentic sample on acid hydrolysis (co-TLC, IR). Partial hydrolysis of phytolaccoside D cleaved 1 mole of glucose yielding phytolaccoside A, identified by direct comparison with an authentic sample. Methylation of V with CH₂N₂ gave the methylester (VI) mp. 210~13° and acetylation with acetic anhydride pyridine yielded an acetate (VII) mp. 139~142° (Scheme 2), showing seven acetoxy signals (2.02~2.12ppm) and two anomeric proton signals (4.43ppm, 1H, d, $J=7\text{Hz}$ and 4.63ppm, 1H, d, $J=8\text{Hz}$) in its NMR spectrum. The J values of the two anomeric protons indicated β-configurations for both the interglycosidic and the xylose-genin linkage. Saponin V was identified with phytolaccoside D by comparison with an authentic sample (co-TLC, mmp). Thus the structure of phytolaccoside D was established as 4'-O-β-D-glucopyranosyl phytolaccoside A.

Phytolaccoside A²¹ and B³¹ (from PAR)

Phytolaccoside A (VIII) mp. 273~4°, on acid hydrolysis, gave phytolaccagenic acid as the genin, identified by direct comparison with an authentic sample (co-TLC). D-xylose was identified as the sugar component by TLC. Formation of methylester (IX) mp. 143~4° with CH₂N₂ indicated that no sugar was present in ester linkage with the carboxyl function at C-17. Acetylation with acetic anhydride and pyridine yielded an acetate (X) mp. 142~5° (Scheme 3) whose NMR spectrum showed signals for four acetoxy groups (2.05~2.10ppm). This result indicated the presence of only one D-xylose unit in the saponin X. Also, NMR spectrum showed the presence of one anomeric proton signal (4.47ppm, 1 H, d, $J=7\text{Hz}$). Saponin X was identical with phytolaccoside A by comparison with an authentic sample (co-TLC,

mmp). Thus phytolaccoside A was given the structure 3-O- β -D-xylopyranosylphytolaccagenic acid.

Phytolaccoside B (XI) mp. 252~4°, on hydrolysis, methylation and acetylation of XI under similar conditions as I gave phytolaccagenin, D-xylose, methyl ester (XII) mp. 180~4° and pentaacetate (XIII) mp. 156~8° (Scheme 4). XI was identified with phytolaccoside B by direct comparison with an authentic sample (co-TLC, mmp).

The Thin layer chromatograms of the crude saponin from PAS and PAR (CHCl₃-MeOH-NH₄OH-H₂O=60:30:3:4) were similar to those of original plants. Phytolaccoside E was a major saponin in original plants but both of callus tissues contained phytolaccoside B as a major component (Fig. 1). Through the results we

obtained phytolaccoside B and D from PAS and A and B from PAR.

ACKNOWLEDGEMENT

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