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# PHYTOLACCINIC ACID, A NEW TRITERPENE FROM *PHYTOLACCA AMERICANA*

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**Abstract**—The saponin from the berries of *Phytolacca americana* yielded one known and two new aglycones. One of the new aglycones was identified as desmethylphytolaccagenin **2**, and the other as 3β, 23-dihydroxyolean-12-ene-28, 30-dioic acid-30-methyl ester **3**.

Recently, the triterpenoidal glycosides in the berries of *Phytolacca dodecandra*, "Endod", have attracted considerable attention for their strong molluscicidal activity.[1] This knowledge prompted us to examine the berries of a closely related domestic species, *Phytolacca americana*.†

The fresh berry juice yielded a crude saponin mixture (yield: 0·6% from fresh berries), which showed moderate molluscicidal activity.‡ Acid hydrolysis of the crude glycosides afforded a mixture of triterpenes from which three crystalline compounds, **1**, **2** and **3**, were obtained after chromatographic separation. Oleanolic acid, the major aglycone in the *P. dodecandra* saponins,[3] was detected only in a trace amount.

Compound **1**, the major aglycone, was identified as phytolaccagenin which had been isolated from the root portion of *P. americana* by Stout, Malofsky and Stout.[4] The second aglycone **2**, m.p. 315–325°, was the free acid of phytolaccagenin, and its identity was established by the comparison with the alkaline hydrolysis product of **1**, and with phytolaccagenin methyl ester **4** after methylation. Since the treatment of **1** under the condition used for the hydrolysis of the glycoside resulted in the recovery of the starting material, **2** is not an artifact formed during the acid hydrolysis.

Compound **3**, named phytolaccinic acid, was recrystallized from ethyl acetate as colorless needles, m.p. 295–299°, $[\alpha]_D - 66{\cdot}5°$, $C_{31}H_{48}O_6$ ($M^+$ *m/e* 516). The infrared spectrum showed absorptions at 3400 (OH), 1730 (ester C=O), 1700 (COOH) and 1220 (ester) $cm^{-1}$. The presence of a free carboxyl group was further verified by titration and by converting **3** with $CH_2N_2$ to a methyl ester **5** m.p. 128–130°, $C_{32}H_{50}O_6$ ($M^+$ *m/e* 530). The 60 MHz PMR spectrum of **3** in pyridine-$d_5$ shows a carbomethoxy signal at δ 3·68, a vinylic proton at δ 5·68 and five tertiary methyl groups at δ 1·25 (3H), 1·23 (3H, 1·05 (6H) and 1·13 (3H). The carboxyl and carbomethoxy group account for four of the six oxygens present in **3**, and if an oleanene skeleton is assumed for **3**, the remaining two oxygens should exist as hydroxyl functions.

In the mass spectrum of **3**, major peaks are seen at *m/e* 292 (the D/E ring fragment of retro Diels-Alder cleavage), 246 (292-COOH-H) and 187 (246-$COOCH_3$). The spectrum is essentially identical with that of phytolaccagenin **1**, which also shows the prominent peaks at *m/e* 292, 246, and 187. Therefore, it was assumed that **3** has the same functional arrangement on the D/E ring as that in **1**. The structure of phytolaccagenin is unequivocally established as the C-30 methyl ester of 2β, 3β, 23-trihydroxyolean - 12 - ene - 28,30 - dioic acid by X-ray crystallography.[4] Comparison of the PMR of **3** with that of phytolaccagenin showed two of the angular methyl signals shifted considerably upfield compared to those of phytolaccagenin, indicating the absence of the 2β-hydroxyl group which is in a 1,3-diaxial relationship to both 24- and 25-methyl groups.

On treatment with cupric sulfate in acetone at room temperature, **5** easily afforded an isopropylidene derivative **6** m.p. 294–298°, $C_{35}H_{54}O_6$ ($M^+$ *m/e* 570). Assuming the presence of a 3β-hydroxyl group as in almost all triterpenes, this easy acetonide formation limits the other hydroxyl group to either the 2α, 2β, or 23 positions. The 24 hydroxyl group forms an acetonide with the 3β-hydroxyl group only under drastic conditions.[5,6] The 2β position was already ruled out on the basis of the PMR data. The fact that only five methyl groups are observed in the PMR spectrum favors a 23-hydroxyl group over a 2α-hydroxyl group.

†Burke and LeQuesne reported oleanolic acid acetate in the seeds but the absence of triterpenes in the hydrolysate of the berry juice.[2]

‡The test has been done in collaboration with Dr. H. W. Bond, Department of Medicinal Chemistry, University of Rhode Island, and the results will be published elsewhere.

Comparison of methyl group chemical shifts ($\delta$ in pyridine-$d_5$)

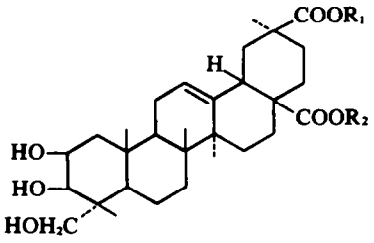
| | C-CH$_3$ | | | | | COOCH$_3$ |
|---|---|---|---|---|---|---|
| | 24 | 25 | 26 | 27 | 29 | |
| Phytolaccagenin (1) | 1·35 | 1·57 | 1·08 | 1·23 | 1·27 | 3·67 |
| Phytolaccagenin methyl ester (4) | 1·38 | 1·63 | 0·93 | 1·20 | 1·22 | 3·67<br>3·68 |
| Phytolaccinic acid (3) | 1·05 | 1·13 | 1·05 | 1·23 | 1·25 | 3·68 |
| | 1·04 | 1·08 | 0·89 | 1·22 | 1·22 | 3·62<br>3·63 |

Moreover, although the $\alpha$-hydrogens of the hydroxyl groups in 3 appear as undefined multiplets centered at $\delta$ 3·35 and 4·2 in the PMR, the integration implicated three $\alpha$-hydrogens rather than two. On this basis, the structure of 3$\beta$, 23 - dihydroxyolean - 12 - ene - 28,30 - dioic acid - 30 - methyl ester was assigned for phytolaccinic acid 3.

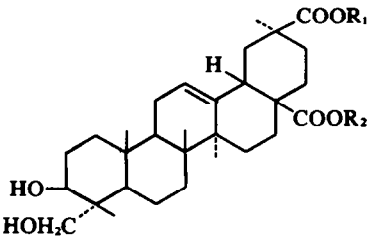
To correlate 3 with a known triterpene, an attempt was made to remove the 23-hydroxyl group selectively by tosylation and $LiAlH_4$ reduction. Although the $LiAlH_4$ reduction of a 3$\beta$ - hydroxy - 23-tosylate in the triterpene system is known to proceed in a complex manner due to the neighboring group participation, one of the products was expected to be the normal reduction product.[5,6] Partial tosylation of the methyl ester 5 resulted in a mixture of the ditosylate, two undetermined monotosylates and the starting material. The two monotosylates were individually reduced with $LiAlH_4$ in THF and the products were examined by TLC. The less polar tosylate 7 afforded, among others, a product which is identical with the $LiAlH_4$ reduction product of queretaroic acid methyl ester[7] 8, 9 on TLC in $R_f$ value and coloration using different systems.*

The berries of *P. dodecandra* contain oleanolic acid as the major aglycone and a lesser amount of bayogenin.[1c,3] Very recently, Howard has reported serjanic acid 10 as an aglycone in the saponin from the berries of an Australian Phytolacca, *P. octandra*.[8] The structure variation in the aglycones in these very closely related species is of considerable interest.

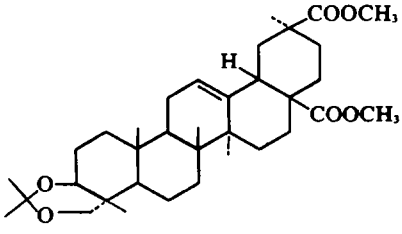
*Because a very small amount (< 1 mg) of queretaroic acid methyl ester was available, we were unable to use the conventional identification methods (mixed m.p., IR, etc.)



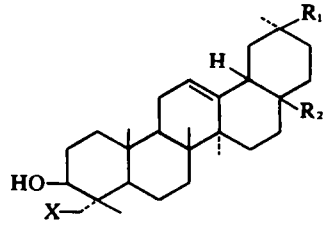

1: $R_1 = CH_3$, $R_2 = H$
2: $R_1$, $R_2 = H$
4: $R_1$, $R_2 = CH_3$




3: $R_1 = CH_3$, $R_2 = H$
5: $R_1$, $R_2 = CH_3$




6




7: X = OTS, $R_1$, $R_2 = COOCH_3$
8: X = H, $R_1 = CH_2OH$, $R_2 = COOCH_3$
9: X = H, $R_1$, $R_2 = CH_2OH$
10: X = H, $R_1 = COOCH_3$, $R_2 = COOH$

## EXPERIMENTAL

M.ps were determined using a Kofler hot stage and are uncorrected. Optical rotation measurements were carried out with a Rudolph polarimeter using a 1 dm tube in the specified solvent. Infrared spectra (IR) were recorded on a Perkin-Elmer Model 457 grating spectrometer using KBr micropellets. Proton magnetic resonance (PMR) spectra were recorded using a JEOL 60 MHz spectrometer with tetramethylsilane as the internal standard. Mass spectra (MS) were recorded on a DuPont 490B and 491 instrument. Microanalyses were conducted by MicroAnalysis, Inc., Wilmington, Delaware, U.S.A. Ripe berries of *Phytolacca americana* were collected in Narragansett, Rhode Island, during the autumn of 1972 and kept frozen for use. The plant was authenticated and the voucher specimen is deposited in the herbarium of the Department of Pharmacognosy, University of Rhode Island.

### *Extraction of crude glycosides from berries*

Sixty grams of frozen ripe *P. americana* berries were mixed with 60 ml of water and ground in a Waring blender just long enough to break the berry skins without grinding up the seeds. The frothy mixture was then filtered through a pad of Celite. The final filtrate volume after washing was 120 ml. The clear filtrate was thoroughly extracted with 240 ml (6 × 40 ml) of water saturated n-butanol. The butanol extract was then filtered through dry filter paper, and was evaporated to dryness under a reduced pressure resulting in a slightly yellowish powder (393 mg).

The powder showed typical saponin properties (foaming, hemolysis) and was separated into more than 16 components on TLC (Silica gel HF 254; $CHCl_3$/MeOH/$H_2O$ 65:25:4). Most of the spots seemed to be triterpenoidal and visualized as pink-purple spots by the acetic anhydride-sulfuric acid reagent.

### *Acid hydrolysis of crude glycoside mixture*

Two hundred mg of the crude glycoside was dissolved in a mixture of 2N $H_2SO_4$ (6 ml) and 95% EtOH (6 ml). The mixture was refluxed on a steam bath for 6 h. The ethanol was then removed under a stream of nitrogen on a steam bath. The resulting precipitate was then filtered and washed thoroughly with $H_2O$. The precipitate (71 mg) was then chromatographed on silica gel (12 g) using as eluting solvent $CHCl_3$/MeOH (79:1). This allowed the separation of, in the order of elution, phytolaccinic acid **3** (33·5 mg), phytolaccagenin **1** (56·7 mg) and desmethylphytolaccagenin **2** (5·4 mg).

### *Phytolaccagenin* **1**

Crystallized from ethyl acetate as colorless prisms, m.p. 284–294° (dec., effervescence), IR: $\nu$ $cm^{-1}$ 3360 (OH), 1728 (C=O). MS (major peaks): *m/e* 532($M^+$), 292, 246, and 187. The sample which was recrystallized from the same solvent (acetone) as the reference material[4] showed an identical IR spectrum and no depression in melting point on admixture.

### *Desmethylphytolaccagenin* **2**

Crystallized from MeOH as prisms, m.p. 315–325° (dec.), IR: $\nu$ $cm^{-1}$ 3400 (OH), 1690 (COOH), MS: *m/e* 518 ($M^+$), 278, 232, and 187. The sample obtained by drastic alkaline hydrolysis of phytolaccagenin[4] showed an identical IR spectrum and no depression in melting point on admixture.

### *Phytolaccinic acid* **3**

Phytolaccinic acid **3** was recrystallized from EtOAc as fine needles, m.p. 295–299°. This material was then recrystallized from dioxan as needles m.p. 300–303° (effervescence), $[\alpha]_D^{25°}$ −66·5° (C = 1·0, MeOH); IR: $cm^{-1}$ 3400 (OH), 1730 (CO) and 1220 (ester); MS: *m/e* 516 ($M^+$), 292, 246, and 187. PMR: $\delta$ (in pyridine-$d_5$) 5·68 (s, 1H), 4·2 (m, 2H), 3·68 (s, 3H), 1·25 (s, 3H), 1·23 (s, 3H), 1·13 (s, 3H), 1·05 (s, 6H) (Found: C, 72·13; H, 9·46. $C_{31}H_{48}O_6$ requires: C, 72·05; H, 9·36%).

### *Phytolaccinic acid methyl ester* **5**

Phytolaccinic acid **3** (30 mg) was methylated with $CH_2N_2$ in a mixture of MeOH and $Et_2O$. The product (30·2 mg) was recrystallized from EtOAc as rosettes of fine needles, m.p. 128–130°, IR: $\nu_{max}$ $cm^{-1}$ 3540, 3380 (OH) 1710, 1730 (C=O), 1228 (ester). PMR: $\delta$ (in pyridine-$d_5$) 5·53 (s, 1H) 3·63 (s, 3H), 3·62 (s, 3H), 1·22 (s, 6H), 1·04 (s, 3H), 1·08 (s, 3H), and 0·89 (s, 3H). (Found: C, 72·16; H, 9·42. $C_{32}H_{50}O_6$ requires: C, 72·42; H, 9·50%).

### *Methyl phytolaccinate acetonide* **6**

Methyl phytolaccinate **5** (50 mg) was dissolved in acetone dried over anhydrous $CuSO_4$. Fifteen mg of anhydrous $CuSO_4$ was added as a catalyst to the solution. The reaction mixture was allowed to stand at room temperature for 5 h. After filtration, the acetone was removed under a reduced pressure leaving the product (45 mg). Recrystallization from acetone gave fine needles, m.p. 294–298°, IR: $\nu_{max}$ $cm^{-1}$ 1725 (C=O) (no OH absorption seen). MS: *m/e* 570 ($M^+$) 510, 306, 246, and 187. (Found: C, 73·64; H, 9·82. $C_{35}H_{54}O_6$ requires: C, 73·65; H, 9·54%).

### *Tosylation of methyl phytolaccinate* **5**

Methyl phytolaccinate (100 mg) was dissolved in 2 ml of dry pyridine. Tosyl chloride (42 mg, 1·2 moles) was then added under ice-cooling. The mixture was kept at room temperature for 24 h. At the end of the reaction period, three products were formed as observed on TLC. The mixture was poured over 15 g of crushed ice and then extracted with ether. After the usual workup, the products were separated by using preparative TLC (Silica gel HF 254, $Et_2O/CH_2Cl_2$ 1:9). The bands were scraped off, eluted with ether and evaporated. PMR spectra were taken of the three compounds. The results indicated that the one with the highest $R_f$ value is the ditosylate, but insufficient material was available to allow a determination of which product was the desired monotosylate. Therefore, the two monotosylates were independently reduced without further purification and characterization.

### *$LiAlH_4$ reduction of methyl phytolaccinate tosylates*

The tosylated methyl phytolaccinates were separately reduced as follows: To a suspension $LiAlH_4$ (100 mg) in 16 ml of tetrahydrofuran, a solution of the tosylated compound in tetrahydrofuran (4 ml) was added dropwise under reflux. The mixture was then refluxed 10 h. Residual reagent was destroyed with EtOAc and the mixture was acidified with a dilute HCl solution. The products from the three tosylates were compared on TLC with a sample of queretaroatriol **9** prepared by the reduction of methyl queretaroic acid.[7] (See below.)

The product from the least polar tosylate **7** showed, besides two others, a spot corresponding to that of **9**. The compound corresponding to this spot was then isolated by preparative TLC (Silica gel HF 254, benzene/EtOH 9:1, double development).

*Queretaroatriol* **9** *from methyl queretaroic acid* **8**

Approximately 1 mg of queretaroic acid methyl ester **8** was dissolved in 1 ml of dry tetrahydrofuran and reduced with $LiAlH_4$ (10 mg). After workup in the same manner as the above experiment, the product was compared with the product derived from phytolaccinic acid using several TLC systems. In each case, they gave identical $R_f$ values and colorations with two spraying reagents.

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