# TRITERPENES FROM THE BERRIES OF *PHYTOLACCA AMERICANA*

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ABSTRACT.—From the hydrolysate of the pokeberry saponin acinosolic acid methyl ester (**1**) (2β,3β-dihydroxyolean-12-ene-28, 30-dicarboxylic acid 30-methylester) was isolated. In addition, four known compounds were isolated: phytolaccagenin (**2**), jaligonic acid (**3**), phytolaccagenic acid (**4**), and esculentic acid (**5**). Acinosolic acid (**9**) was detected in a trace amount.

*Phytolacca americana* L. (Phytolaccaceae) is a vigorous shrub-like perennial. The young shoots may be gathered in the spring before the red color appears and eaten as a cooked vegetable which resembles asparagus in flavor (1–4). The crimson juice from the long racemes of purple berries has been reputed to be useful to color food and wine (1, 5). However, it was reported that the berries are toxic to cattle, poultry and man. It has been found that ingestion of the berries produced severe gastrointestinal disturbances accompanied by weakened respiration and pulse (6) and caused mitosis of lymphoid cells (7). Abortion in cows and spasms, purging, convulsions and finally death caused by paralysis of the respiratory organs have been described as a result of pokeberry toxicity (8). Reduction of growth rate in turkey poults, ataxia, inability to walk and death by the administration of the berries have also been reported (9).

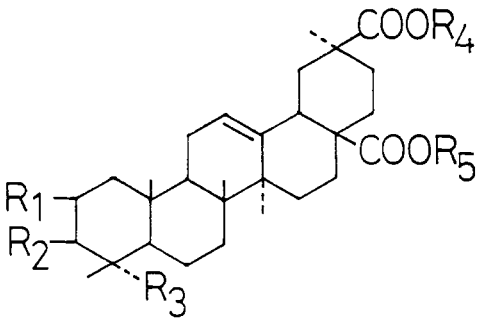
On the other hand, in various parts of the world, the roots of this plant have long been used in folk medicine for treating edema and rheumatism (4, 5, 10, 11). Recently, several anti-inflammatory saponins were isolated from the roots and the structures were determined (12, 13). Presence of the saponin in berry juice has also been known and Johnson and Shimizu (14) reported the isolation of phytolaccagenin (**2**) (15), jaligonic acid (**3**) (16) and phytolaccagenic acid (**4**) (17) (=phytolaccinic acid (14)) from the acid hydrolysate of the juice saponin.

In the course of our study on triterpenoids of *Phytolacca* plants, a new minor aglycone, tentatively designated pokeberrygenin (**1**), was isolated in addition to three aglycones mentioned above and esculentic acid (**5**) (18).

Pokeberrygenin (**1**), $C_{31}H_{48}O_6$, mp 208–209°, $(\alpha)^{25}D+79.2°$ gave a pink coloration in a Liebermann-Burchard test and a positive tetranitromethane color test. The infrared spectrum showed absorptions at 3320 $cm^{-1}$(OH), 1720(ester), 1700(acid) and 825(double bond). The methylester (**6**), $C_{32}H_{50}O_6$, mp 210°, $(\alpha)^{25}D+111.4°$, was oxidized by $HIO_4$ and one mole of reagent was consumed. This result indicated the existence of an α-glycol unit in **1**. The nmr spectrum of the methylacetate (**7**), $C_{36}H_{52}O_8$, mp 115–118°, $(\alpha)^{25}D+89.8°$, showed six tertiary methyl signals (δ0.75–1.20), two acetyl signals (δ2.02 and 2.03), two methylester signals (δ3.59 and 3.70), a doublet (δ4.64, 1H, $J=4$Hz, H–3), and a multiplet (δ5.36, 2H, H–2 and H–12).

The width at half-height of the signal at δ5.36 is a ca 8Hz; the coupling constant between H–2 and H–3 is 4Hz, being similar to those exhibited by dimethyl jaligonate triacetate (**8**) (16). These results indicated the presence of 2β,3β-diol in the compound (19).

Pokeberrygenin (**1**) was brominated to give a bromo-γ-lactone, $C_{31}H_{47}O_6Br$, mp 294°, $(\alpha)^{25}D+73.2°$, and easily hydrolyzed with alkali hydrolysis under mild




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | OH | OH | Me | Me | H |
| **2** | OH | OH | $CH_2OH$ | Me | H |
| **3** | OH | OH | $CH_2OH$ | H | H |
| **4** | H | OH | $CH_2OH$ | Me | H |
| **5** | H | OH | $CH_2OH$ | H | H |
| **6** | OH | OH | Me | Me | Me |
| **7** | OAc | OAc | Me | Me | Me |
| **8** | OAc | OAc | $CH_2OAc$ | Me | Me |
| **9** | OH | OH | Me | H | H |
| **10** | OH | OH | $CH_2OH$ | Me | Me |
| **11** | $-O-C(Me)_2-O-$ | | $CH_2OH$ | Me | Me |
| **12** | $-O-C(Me)_2-O-$ | | CHO | Me | Me |

conditions to give a new triterpenoid, acinosolic acid (**9**), $C_{30}H_{46}O_6$, mp 358–359°, $(\alpha)^{25}D+117.9°$. The mass spectrum of **1** was very similar to that of phytolaccagenic acid (**4**) (20). The intensity of the peak at *m/e* 470, which was formed by the loss of COOH+H from the molecular ion of *m/e* 516, was much higher than that of the peak at *m/e* 460 which was formed by the loss of $COOCH_3+H$. Moreover, the intensity of the peak at *m/e* 246 corresponding to the loss of COOH+H from *RDA* fragment ion of *m/e* 292 was also higher than that of the peak at *m/e* 232 corresponding to the loss of $COOCH_3+H$. These results indicated the presence of a carbomethoxyl group at C-20 of pokeberrygenin (**11**). In addition, observed values in ppm (0.75, 0.91, 1.06, 1.14 x 2 and 1.20) of the chemical shifts for the tertiary methyl groups of the methylacetate (**7**) were in excellent agreement with the values (0.73, 0.91, 1.06, 1.15 x 2 and 1.20 for Me-26, 23, 24, 27, 29 and 25, respectively) calculated for the 30-carbomethoxy structure, based on the data presented by Cheung and Williamson (21), Tursch, *et al.* (22) and Woo and Kang (11). Therefore, the structure of pokeberrygenin (**1**) was established as 2β,3β-dihydroxy-olean-12-ene-28, 30-dioic acid 30-methylester. In order to correlate **1** with a known triterpene, reductive elimination of the 23-OH group in phytolaccagenin (**2**) was carried out. On treatment with acetone in the presence of HCl, dimethyljaligonate (**10**) afforded 2β,3β-acetonide, $C_{35}H_{54}O_7$, mp 209–210°. The acetonide (**11**) was oxidized with $CrO_3$-pyridine to yield a dehydroderivative (**12**), $C_{35}H_{52}O_7$, mp 141–144°, which was converted into acinosolic acid (**9**) by a modified Huang-Minlon reduction followed by acid hydrolysis.

In 1975, Glombitza, *et al.* (23) described the presence of acinosolic acid in the berries of *P. acinosa*. However, they isolated the substance only as a dimethyldiacetate, and no description of free acinosolic acid and its derivatives was given. Therefore, this is the first reported occurrence of pokeberrygenin (**1**) in nature.

Although free acinosolic acid (**9**) has not been isolated, tlc examination showed its presence in the juice in a trace amount. These two triterpenes have not been detected in the other parts of the plant.

Examination of *Phytolacca esculenta* berries also resulted in the isolation of **1** and only trace amounts of **9** were detected by tlc.

## EXPERIMENTAL[1]

ISOLATION.—Ripe berries of *Phytolacca americana* were mixed with an equal volume of water and macerated, and the juice was filtered through several thicknesses of cheesecloth and extracted with n-butanol. The butanol extract was filtered and then concentrated to dryness under reduced pressure.

The residue was refluxed for 3 hr with 4% HCl in methanol. The solvent was removed *in vacuo* and water was added. The precipitate was filtered, dissolved in a saturated $NaHCO_3$ solution and then filtered; the filtrate was acidified with dilute-HCl. The resulting precipitate was then chromatographed on silica gel and eluted with benzene-dioxane-acetic acid (90:25:4). This allowed the separation, in the order of elution, of pokeberrygenin (**1**); phytolaccagenic acid (**4**), mp 309–310°; phytolaccagenin (**2**), mp 317–320°; esculentic acid (**5**), mp>360°; and jaligonic acid (**3**), mp 317–318°. The known compounds were identified by direct comparison with authentic samples.

POKEBERRYGENIN (**1**).—The compound (**1**) was crystallized from methanol as a colorless amorphous powder, mp 208–209°, $(\alpha)^{25}D$+79.2° (C=0.29, MeOH); uv λ max (EtOH) nm: 205 (log ε 3.80); ir ν max $cm^{-1}$: 3320 $cm^{-1}$ (OH), 1720 (ester), 1700 (acid), 825 (double bond); ms *m/e* (%): 516 ($M^+$, 1.3), 470 [$M^+$–(COOH+H), 9.2], 292 [D/E ring of RDA, 60.8], 246 (292–(COOH+H), 84.3], 232 [292–($COOCH_3$+H), 31.4], 223 (A/B ring of RDA, 34.1), 187 [292–(COOH+$COOCH_3$+H), 100], 173 [232–(C.COOH+2H), 37.3].

Anal. Calcd for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. found C, 72.10; H, 9.38.

METHYLATION OF POKEBERRYGENIN (**1**).—A sample of **1** was methylated with ethereal $CH_2N_2$. The product (**6**) was crystallized from methanol as needles, mp 210°, $(\alpha)^{25}D$+111.4° (C=0.2, MeOH); ir ν max $cm^{-1}$: 1719 (ester); ms *m/e* (%): 530 ($M^+$, 4.0), 512 (3.3), 470 (28.6), 306 (95.2), 246 (100), 233 (27.2), 223 (22.1), 187 (95.2), 173 (33.3). Anal. Calcd for $C_{32}H_{50}O_6$: C, 72.42; H, 9.50. found C, 72.40; H, 9.52.

$HIO_4$ OXIDATION OF METHYLPOKEBERRYGENIN (**6**).—An ethanol solution of **6** (53 mg) was treated with 0.05M-$HIO_4$ (5 ml), the volume was made up to 25 ml with ethanol, and the solution was kept in the dark room at room temperature. Aliquot portions (5 ml each) were removed after 0.5, 1, 2 and 24 hr. The excess of $HIO_4$ was estimated using 0.01N-$Na_3AsO_3$ solution. The compound (**6**) consumed 0.810, 0.965, 0.989, and 0.989 mole of oxidant, respectively.

ACETYLATION OF METHYLPOKEBERRYGENIN (**6**).—A sample (20 mg) of **6** was heated with $Ac_2O$ (0.5 ml) and pyridine (0.5 ml) for 2 hr. The reaction mixture was poured over ice and extracted with ether. The ether extract was chromatographed on silica gel, eluted with $CHCl_3$ and crystallized from MeOH–$H_2O$ as an amorphous powder, mp 115–118°, $(\alpha)^{25}D$+89.8° (C=0.58, MeOH).

BROMOLACTONE OF POKEBERRYGENIN (**1**).—To a solution of **1** (10 mg) and NaAc (40 mg) in HAc (1 ml) was added dropwise a solution of bromine in HAc (3%, 0.5 ml). The mixture was kept at room temperature for 2 hr and then poured into water containing $Na_2S_2O_3$ (100 mg) to discharge any excess bromine. The resulting mixture was extracted with ether and crystallized from chloroform-methanol as needles, mp 294°, $(\alpha)^{25}D$+73.2° (c=0.05, MeOH); ir ν max $cm^{-1}$: 1760 (γ-lactone), 1720 (ester).

SAPONIFICATION OF POKEBERRYGENIN (**1**).—A sample (10 mg) of **1** was refluxed with 10% KOH–MeOH (10 ml) for 8 hr. After the usual work-up, the product was crystallized from methanol as needles, mp 358–359°, $(\alpha)^{25}D$+117.9° (C=0.17, MeOH); uv λ max (EtOH) nm: 205 (log ε 3.70); ir ν max $cm^{-1}$: 3400 (OH), 1680 (acid), 820 (double bond).

ISOPROPYLIDENE DIMETHYLJALIGONATE (**11**).—Dimethyljaligonate (**10**, 500 mg) was dissolved in dry acetone (50 ml) and treated with three drops of HCl and kept at room temperature overnight. The reaction mixture was added to crushed ice and the precipitate was filtered, chromatographed on silica gel, eluted with benzene-ether (4:1) and crystallized from methanol

[1]Mps were taken on a Mitamura-Riken apparatus and are uncorrected. Ir spectra were recorded in KBr pellets on a JASCO model-IR-S spectrophotometer. Nmr spectra were recorded in $CDCl_3$ using a Perkin-Elmer 90 MHz spectrometer with TMS as an internal standard. Mass spectra were run on an AEI MS 1073 spectrometer.

as needles (340 mg), mp 209–210°, $[\alpha]^{25}D+121.7°$ (C=0.36, MeOH); nmr δ: 0.71–1.12 (5 x Me), 1.32, 1.48 (2 x Me for isopropyl), 3.36 (2H, s, $CH_2O$–), 3.58, 3.69 (2 x MeO), 4.15 (1H, d, $J$= 10 Hz, H-3), 4.40 (1H, m, H-2), 5.36 (1H, m, H-12).

$CrO_3$ OXIDATION OF THE ISOPROPYLIDENE DIMETHYLJALOGONATE (**11**).—A sample (0.5 g) of of **11** in pyridine (10 ml) was added to $CrO_3$-pyridine complex ($CrO_3$ 1 g + pyridine 10 ml) and allowed to stand at room temperature for 4 hr. The reaction mixture was poured into crushed ice and filtered. The precipitate was chromatographed on silica gel and eluted with benzene-ether (4:1) to yield **12**, crystallized from methanol as a colorless amorphous powder (350 mg), mp 141–144°, $[\alpha]^{25}D+108.5°$ (c=0.51, $CHCl_3$); uv λ max (EtOH) nm: 206, 290 (log ε 3.92, 1.43); nmr δ: 0.73–1.23 (5 x Me), 1.32, 1.51 (2 x Me for isopropyl), 3.59, 3.70 (2 x MeO), 4.18 (1H, d, $J$=7 Hz, H-3), 4.46 (1H, m, H-2), 5.42 (1H, m, H-12), 9.41 (1H, s, CHO).

HUANG-MINLON REDUCTION OF THE 23-DEHYDROISOPROPYLIDENE-DIMETHYLJALIGONATE (**12**) FOLLOWED BY ACID HYDROLYSIS.—A sample (0.7 g) of **12** was dissolved in ethyleneglycol (30 ml) and 85% hydrazine hydrate (10 ml) and refluxed for 1 hr. After cooling, 3.5 g of NaOH was added to the mixture, which was heated without condenser until the temperature reached 195°, when refluxing was continued for 3 hr. The mixture was cooled and acidified. The precipitate formed was filtered and refluxed at 80° for 1.5 hr with 80% HAc (30 ml).

Pouring into ice gave solids which were chromatographed on silica gel, and eluted with benzene-dioxane-acetic acid (90:25:4) to give **9** (70 mg), which proved to be identical by ir, mmp, co-tlc with an authentic sample of **9** obtained by alkaline hydrolysis of **1** as above.

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