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The Structure and Stereochemistry of Phytolaccagenic Acid

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Abstract—The structure and stereochemistry of phytolaccagenic acid, a new triterpenoid sapogenin from *Phytolacca americana* L. has been further characterized as 3β,23-dihydroxy-30-carbomethoxy-olean-12-en-28-oic acid (**I**).

Phytolaccagenic acid[1] (**I**), $C_{31}H_{48}O_6$, mp 309-311°, a new triterpene isolated from *Phytolacca americana* L. (Phytolaccaceae) was earlier proposed to be monomethylester of esculentic acid (**II**), which was initially isolated from *P. esculenta* VAN HOUTTE in this Institute.[2] This paper reports our detailed work of the chemical evidences related to phytolaccagenic acid which support the proposed structure (**I**), and data of some derivatives are given in the Experimental because we have not given a full detail of the results in the previous communications.

Complete esterification with diazomethane gave a substance which was identical with esculentic acid dimethylester (**III**) and saponification with KOH yielded esculentic acid (**II**) easily. Phytolaccagenic acid should therefore be either the C-28 ester (**IV**) or C-30 ester (**I**) of esculentic acid. Since the former can be hydrolyzed only under relatively drastic conditions,[3] saponification of **III** with KOH under mild conditions should effect partial hydrolysis of the C-30 ester group, leaving the C-28 ester group intact. On saponification with 10% MeOH-KOH for 8 hr, as a matter of fact, **III** furnished a monomethylester (**IV**), which was different from **I**. In the high mass region, **IV** showed a strong peak at *m/e* 456 corresponding to the loss of $COOCH_3+H$ from molecular ion, whereas **I** has a relatively intense peak at *m/e* 470 corresponding to the loss of COOH+H, as in the case of monomethyl esters of jaligonic acid.[4] Moreover, the bromolactone (**VII**) of **I** was

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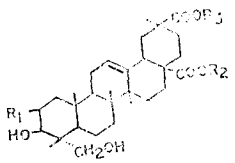
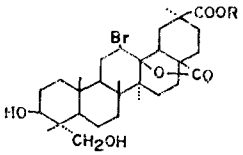
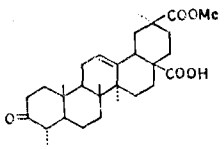
identical with the methylester of bromolactone (**VIII**) derived from **II** and CrO_3 oxidation of **I** yielded the product which was identified as the norterpene[5] (**IX**) derived from phytolaccagenin (**V**) by acid treatment. Thus phytolaccagenic acid (**I**) is 30-methylesculentic acid.







I; $R_1=R_2=H$, $R_3=Me$
II; $R_1=R_2=R_3=H$
III; $R_1=H$, $R_2=R_3=Me$
IV; $R_1=R_3=H$, $R_2=Me$
V; $R_1=OH$, $R_2=H$, $R_3=Me$
VI; $R_1=OH$, $R_2=R_3=H$

VII; R=Me
VIII; R=H

IX

It is appropriate to review at this stage the present state of knowledge regarding the triterpene constitutents of *P. americana*. The pentacyclic triterpene constitutents differ from each other in the degree of oxidation at positions 2, 3, 23, 28, and 30. Such oxidative modifications are generally assumed to result from secondary reactions which occur after the formation of the parent substance, β-amyrin. It would appear that in this particular plant, if reductive processes were not important, secondary oxidation might proceed by successive attacks at the following positions and in the order, 28, 30, 23, 2β.

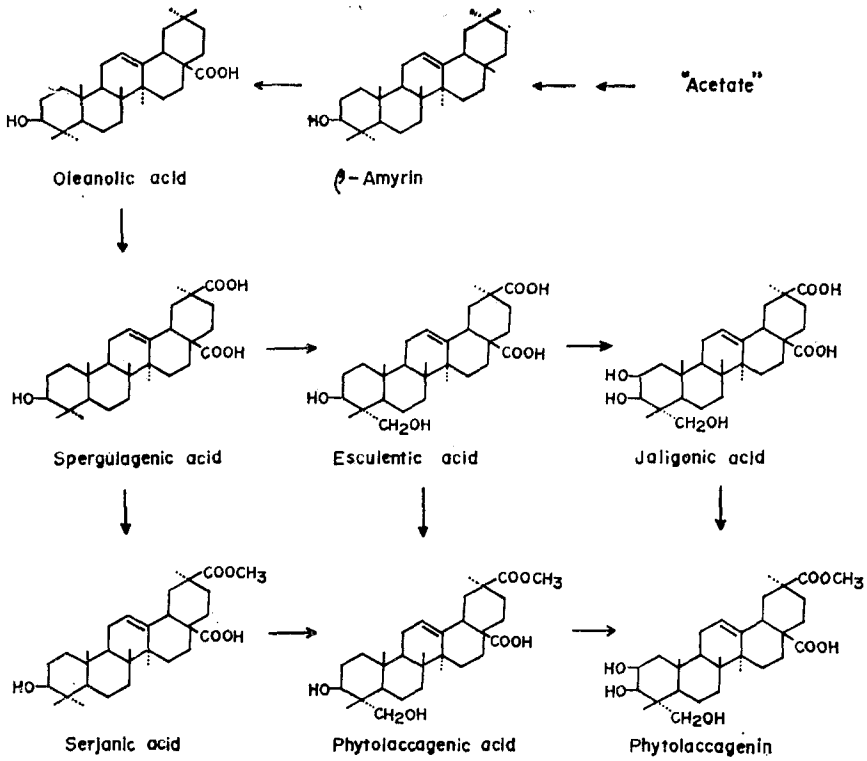
Although oleanolic acid and spergulagenic acid and their derivatives have not been detected in the roots of this plant,* such plausible genesis of the oleanane constituents is shown in Scheme 1.

EXPERIMENTAL**

Isolation of phytolaccagenic acid (**I**)—The methanolic extractive of the dried roots was

* However, Burke and Le Quesne[6] postulated the presence of oleanolic acid in the roots of this plant. Free spergulagenic acid has not been obtained from the nature, but its methyl ester, serjanic acid has been reported as aglycone in the saponin from the sister plant, *P. rivinoides*[7] and *P. octandra*.[8]

** The melting points were taken on a Mitamura-Riken apparatus and are uncorrected. The UV spectra were obtained in EtOH on a Shimadzu Model MPS-50L recording spectrophotometer and the IR spectra were determined in KBr pellets on a JASCO Model IR-S spectrophotmeter. We are grateful to Dr. D.Y. Han, College of Pharmacy, Jung Ang University, for the measurements of the mass spectra.



Scheme 1-Genesis of triterpenes in *P. americana*.

hydrolyzed by refluxing in dioxane-HCl(6 : 1) for 5 hr. The aglycone fraction precipitated by addition of water was chromatographed on silica gel and elution with MeOH-$CHCl_3$ (1 : 200 to 1 : 20), in addition to the known triterpenoids such as jaligonic acid(**VI**), mp 318—320°, phytolaccagenin(**V**), mp 317—319°, esculentic acid(**II**), mp>360°, and **IX**, mp 229—230°, gave **I**, which was crystallized from MeOH as needles, mp 309—311°, $[\alpha]_D$ +86.5°(c=0.4 in EtOH), UV λ_{EtOH}^{max} 204nm(logε, 3.79), IR 3400(OH), 1730(ester), 1700 (acid), 825 cm^{-1} (trisubstituted double bond), MS (m/e); 516(M^+, 0.5), 498(M-H_2O, 1.1), 480(M-2H_2O, 4.8), 470[M-(COOH+H), 5.6], 468(498-HCHO, 2), 456[M-($COOCH_3$+H), 1.2], 292 (RDA fragment a, 38.9), 246[a-(COOH+H), 76.6], 233 [a-(C•COOH+2H), 13.5], 232 [a-($COOCH_3$+H), 19.5], 223 (RDA fragment b, 7.7). 219(11.4), 215(17.5), 187[a-(COOH+$COOCH_3$+H), 100], 186[a-(COOH+H)-($COOCH_3$+H), 54.1] and 173[232-(C•COOH+2H), 34.4].

Anal. Calcd for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.19; H, 9.38.

Methylester(III)—— A solution of **I**(50mg) in MeOH (20ml) was treated with ethereal diazomethane in the usual manner. The crude product was purified by preparative TLC (Silica gel G, $CHCl_3$-MeOH-NH_4OH-H_2O=20 : 4 : 1 : 3) and crystallized from MeOH to give needles of **III**, mp 151—153°, $[\alpha]_D$ +86.2° (c=0.23 in EtOH), IR 1730 and 1710 cm^{-1} (two esters), which was identical with an authentic specimen prepared from **II** in a similar way.

Anal. Calcd for $C_{32}H_{50}O_6$: C, 72.42; H, 9.50. Found: C, 72.61; H, 9.56.

Hydrolysis of methyl ester(III)——A sample of **I**(50 mg) was heated under reflux in methanolic KOH solution (10%, 20ml), much water was added and unreacted ester was extracted with ether. The aqueous solution was acidified with HCl, extracted with ether, and the ether solution was washed, dried, and evaporated. The crude product was crystallized from MeOH to give needles of **IV**, mp 306—307°, $[\alpha]_D$+85.1° (c=0.2 in EtOH), IR 1740(ester), 1710cm^{-1} (acid), MS (m/e); 516(M^+, 0.9), 498(1.3), 480(1.3), 470(0.9), 468(1.6), 456(3.6), 292(33.3), 246(40.8), 233(31.7), 232(38), 223(6.5), 219(14.7), 215(10), 187(100), 186(26.5), 173(29.3). TLC behaviour and IR and MS spectra were very different from those of **I**.

Anal. Calcd for $C_{31}H_{48}O_6$:C, 72.06; H, 9.36. Found: C, 72.39; H, 9.40.

Hydrolysis of phytolaccagenic acid (I)——Phytolaccagenic acid (**I**) was hydrolyzed quantitatively under the same conditions as described above. The product was identified as **II**, by IR and co-TLC with an authentic specimen.

Bromolactone (VII) of phytolaccagenic acid (I)——To a solution of **I** (20mg) and NaOAc(300mg) in HOAc(10ml) was added dropwise a solution of bromine in HOAc (3%, 2ml). It was kept at room temperature for 2hr and working up in the usual way afforded **VII**, which was crystallized from MeOH as needles, mp 238°, IR 1770 (γ-lactone) and 1725cm^{-1}(ester).

Methylation of **VIII**, mp 231—233°, IR 1767(γ-lactone), 1747(ester), 1718cm^{-1}(acid), prepared from **II** under the same conditions as described above afforded a substance, identical with **VII** by mmp, IR, and co-TLC.

CrO_3 oxidation of phytolaccagenic acid (I)——Using a slight excess of the standard aqueous sulfuric acid solution of CrO_3,[9] 50mg of **I** in 10ml of acetone were oxidized for 15 min at 5°. The mixture was worked up in the usual manner and after recrystallization from MeOH yielded needles, mp 229—230°, identified as **IX** by mmp, IR and co-TLC with an authentic sample prepared from phytolaccagenin(**V**) as described below.

Preparation of norterpene (IX) from phytolaccagenin(V)——A solution of **V**(100mg) in 5% HCl-EtOH(50ml) was refluxed for 5 hr and distilled. The residue was chromatographed on silica gel and elution with $CHCl_3$-MeOH (1 : 100) gave **IX**, mp 229—230°.

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