

3-Oxo-30-carbomethoxy-24-norolean-12-en-28-oic Acid, a Nortriterpene Artifact from *Phytolacca americana*

Ki Sook Yang, Won Sick Woo* and Sam Sik Kang

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Abstract—The constitution of genin A—a new triterpenoid isolated from the roots of *Phytolacca americana* L. has been shown to be 3-oxo-30-carbomethoxy-24-norolean-12-en-28-oic acid. This compound is not a genuine saponin but an artifact produced during hydrolysis of the *Phytolacca* saponins.

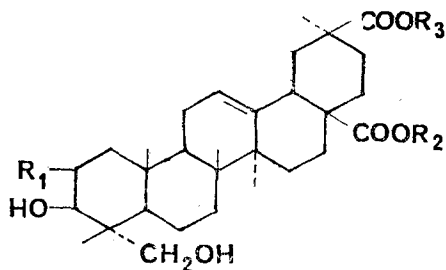
In the previous communications ^{1,2)}, it has been reported that on hydrolysis in dioxane-hydrochloric acid the crude saponin fraction obtained from the roots of *Phytolacca americana* L. yield a mixture of five saponin, esculentic acid(**Ia**), phytolaccagenic acid(**Ib**), jaligonic acid(**Ic**), phytolaccagenin(**Id**), and an unknown compound, which was designated as genin A. The present paper deals with the complete structure and stereochemistry of genin A.

Genin A(**IIa**), C₃₀H₄₄O₅ · 1/2 H₂O (M⁺ m/e 484), mp 229~230°, [α]_D +110.75° (c=0.4 in EtOH), [α]_D +108.1° (c=0.17 in CHCl₃), gave a positive Liebermann-Burchard test (pink→violet→blue) and showed in its IR spectrum peaks at 3490 cm⁻¹ (OH), 1710 cm⁻¹ (CO), and 840 and 825 cm⁻¹ (trisubstituted double bond). Genin A(**IIa**) was, however, recovered unchanged after attempted acetylation and benzylation. Genin A(**IIa**) showed in its UV spectrum a characteristic carbonyl absorption at 285 nm (log ϵ , 1.49)³⁾ besides a strong absorption at 204 nm (log ϵ , 3.80) due to trisubstituted double bond⁴⁾. The presence of one carbonyl function was demonstrated by the formation of a 2,4-dinitrophenylhydrazone(**IIb**), mp 190~191°, $\lambda_{\max}^{\text{EtOH}}$ 366 nm (log ϵ , 4.32 in EtOH); 435 nm

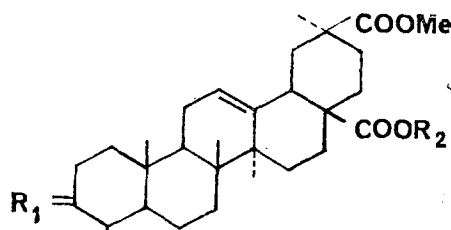
From the Natural Products Research Institute, Seoul National University, Seoul, Korea.

* To whom communications regarding this paper should be addressed.

(log ϵ , 4.06 in EtOH-NaOH), which regenerated **IIa** on treatment described by Demaecker and Martin⁵). Since **IIa** was not a reducing substance it was concluded that **IIa** was a ketone, not an aldehyde. The UV absorption spectra of **IIa** and **IIb** were also in agreement with this view^{3,6} and **IIa** could be formulated as a saturated 3-keto-compound³). The positive Zimmermann test also demonstrated the existence of the keto group at C-3⁷.

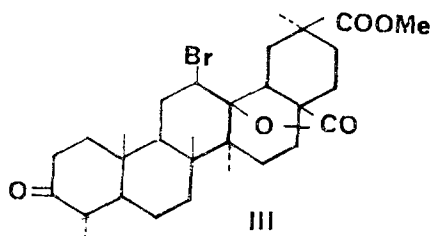


	R ₁	R ₂	R ₃
a	H	H	H
b	H	H	Me
c	OH	H	H
d	OH	H	Me
e	H	Me	Me
f	OH	Me	Me

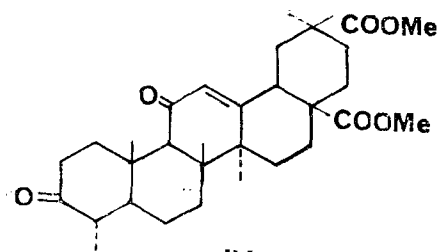


II

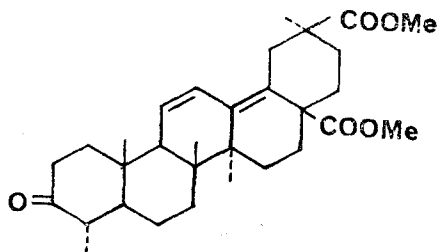
	R ₁	R ₂
a	=O	H
b	=N-DNPH	H
c	=O	Me



III



IV



V

The existence of an ester group and a carboxyl group in **IIa** was suggested by peaks at 1720 and 1700 cm^{-1} in the IR spectrum of **IIb** and by peaks at 1720 and 1550 cm^{-1} in that of potassium salt of **IIa**. The presence of one carbomethoxyl group was confirmed by micro-Zeisel method and one free carboxyl group by titration and by the preparation of a methyl ester **IIc**, mp 190°, $[\alpha]_D +128.6^\circ$ ($c=0.35$ in EtOH), which showed no band for free OH group in its IR spectrum.

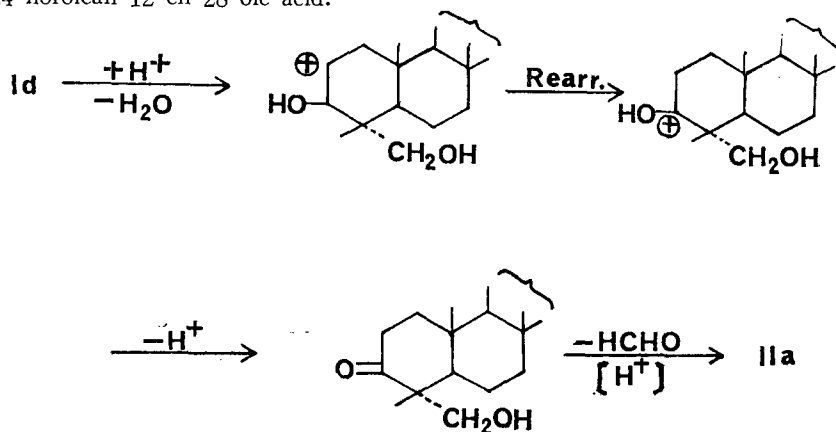
On treatment with $\text{Br}_2\text{-HOAc}$ **IIa** formed a bromolactone(**III**), mp 273~275°, having a γ -lactone peak at 1767 cm^{-1} in its IR spectrum together with peaks at 1730 cm^{-1} due to methylester group and at 1712 cm^{-1} due to α -methyl ketone group⁸⁾, and giving negative tetranitromethane color reaction. Easy lactonisation is characteristic of carboxylic acids of the β -amyirin series having a γ,δ -unsaturated acid⁹⁾ and it was accordingly inferred that **IIa** is unsaturated at the 12-position as in oleanolic acid. With tetranitromethane **IIc** gave yellow color and nearly one mole of perbenzoic acid was consumed. Oxidation of **IIc** with $\text{CrO}_3\text{-HOAc}$ afforded an α,β -unsaturated ketone(**IV**) mp 275~276°, which showed a UV absorption maximum at 253 nm ($\log \epsilon$, 4.26)¹⁰⁾, and SeO_2 oxidation yielded a pale yellow dehydroproduct(**V**) mp 198~202°, having in its UV spectrum the triple absorption maxima at 242, 251, and 260nm ($\log \epsilon$, 4.03, 4.06, and 3.90, respectively), typical of the 11 : 12, 13 : 18 dienes of the oleanane series¹¹⁾. These results established the presence of a double bond at 12 : 13 position and thus it was concluded that the free carboxyl group was located at C-17. The color reaction, the specific rotation, the chemical reactions, and the presence of one carbomethoxyl group in **IIa** having 30 carbon atoms support the idea that **IIa** appears to be a norterpene belonging to the group of oleanane series.

The mass spectra of **IIa** and **IIc** showed typical retro-Diels-Alder fragmentation patterns expected for olean-12-enes and a striking similarity in the ratio and an identity of position of the peaks up to m/e 310 compared to those of phytolaccagenic acid(**Ib**) and phytolaccagenin(**Id**) and their methylesters **Ie** and **If**, respectively^{2,12,13)}. The mass spectral analysis clearly shows the C-20 position of a carbomethoxyl group and the lack of a methyl group at C-4 (or C-10).

Phytolaccagenin(**Id**), on treatment under the conditions identical with those applied to saponin hydrolysis, gave **IIa** and formaldehyde which was characterized both as the dimedone derivative and by the chromotropic acid color test. The fact with regard to formaldehyde elimination reveals the presence of only one methyl group at C-4. Genin

A(**IIa**) was stable to acids and no significant shift of optical rotation was observed in changing from ethanol to chloroform as a solvent. These evidences indicate that the methyl group at C-4 is equatorial (C-23) rather than axial (C-24)⁸. As for the reaction mechanism concerned in the conversion of **Id** into **IIa** on acid treatment, an acid catalysed dehydration involving elimination of the less stable axial OH at C-2¹⁴, followed by a reversed aldol-type condensation as in methyl hederagenate⁷ in inferred as shown in Scheme 1.

The NMR spectrum of **IIc** in CDCl_3 showed tertiary methyl signals at δ 0.80(3H) and 1.13 (9H), a doublet centered at 1.00 (3H, $J=6\text{Hz}$) assignable to protons of the 23-secondary methyl group attached to C-4, and two singlets at 3.90 and 3.71(3H each) due to the methyl protons of two esters. Recent data¹⁵ of the influence of substitution on the methyl frequencies in the NMR spectrum of Δ^{12} -oleanene derivatives were applied and it was found that the observed values in Hz of the chemical shifts for C-methyl groups of **IIc** were in excellent agreement with the calculated ones (Table I). Consequently, the structure and stereochemistry of genin A is established as 3-oxo-30-carbomethoxy-24-norolean-12-en-28-oic acid.



Scheme 1—Acid catalysed conversion of phytolaccagenin to genin A.

Table I—The chemical shifts for C-methyl groups of genin A methyl ester.

	Me-23	Me-25	Me-26	Me-27	Me-29
Methylhederagenate ¹⁶	60	67	48	67	54
30-COOCH ₃ ¹⁵	0	0	-1	0	13
Calculated	60	67	47	67	67
Observed	60	68	48	68	68

Frequencies are given in Hz relative to TMS measured in CDCl_3 at 60 MHz.

EXPERIMENTAL*

Isolation of Sapogenins — The methanolic extractive of the dried roots was hydrolyzed by refluxing in dioxane-HCl(6:1) for 5hr. The aglycone fraction precipitated by addition of water was chromatographed on silica gel and elution with MeOH-CHCl₃ (1:200 to 1:20) gave 5 components, which have been designated as genin A-E in order of increasing polarity on TLC (Silica gel G, MeOH-CHCl₃-7% HOAc=1:5:1).

Genin A(**IIa**) was crystallized from MeOH as needles, mp 229~230°, $[\alpha]_D^{25} +110.75^\circ$ (c=0.4 in EtOH); +108.1° (c=0.17 in CHCl₃), $\lambda_{\max}^{\text{EtOH}}$ 204nm (log ϵ , 3.80); 285nm (log ϵ , 1.49), IR 3490cm⁻¹(OH), 840 and 825cm⁻¹ (double bond), MS (m/e) 484(M⁺, 1.4), 438[M-(COOH+H), 13.2], 424[M-(COOCH₃+H), 3.4], 378[M-(COOH+H)-(COOCH₃+H), 3.9], 292(RDA fragment a, 19.0), 246[a-(COOH+H), 56.6], 232[a-(COOCH₃+H), 19.0], 191(RDA fragment b, 9.5), 187[a-(COOH+COOCH₃+H), 100], 186[a-(COOH+H)-(COOCH₃+H), 53.2].

Anal. Calcd for C₃₀H₄₄O₅·½H₂O: C, 72.98; H, 9.18; CH₃O, 6.29. Found: C, 72.88; H, 9.33; CH₃O, 7.50.

Genin B, mp 309~311°, genin C, mp 317~319°, genin D, mp>360°, genin E, mp 318~320°, were identified as **Ib**, **Id**, **Ia**, and **Ic**, respectively, by direct comparison with authentic specimens.

2,4-Dinitrophenylhydrazone(IIb) of Genin A(IIa) — A solution of **IIa** (80mg) and 2,4-dinitrophenylhydrazine (34mg) in 1% H₂SO₄-EtOH (25ml) was refluxed for 2 hr. Dilution with water yielded an amorphous substance which was recrystallized from EtOH, mp 190~191°, $\lambda_{\max}^{\text{EtOH}}$ 366nm (log ϵ , 4.32 in EtOH); 435nm (log ϵ , 4.06 in 0.25 N-NaOH-EtOH), IR 1720cm⁻¹(ester), 1700cm⁻¹(acid), and 1505 and 1332cm⁻¹(NO₂).

To a solution of **IIb** (30mg) in acetone(100 ml) were added 5 ml of c-HCl. The mixture was refluxed for 45 min, cooled, and then a solution of SnCl₂(1g) in c-HCl (4 ml) and 15 ml of water were added. The mixture was heated again to reflux in an atmosphere of nitrogen for 45 min. After the acetone was distilled off under reduced pressure at room temperature, the residue was extracted with ether. The ether layer was washed

* 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 spectrophotometer. The NMR spectra were recorded in CDCl₃ on a JEOL Model JEM-3H-60 instrument with TMS as internal standard. The authors are grateful to Dr. D.Y. Han, College of Pharmacy, Jung Ang University, for the measurements of the mass spectra.

with water and evaporated. The recovered ketone was proved to be identical by mixed mp and co-TLC with an authentic sample of **IIa**.

Methylester(IIc) — This was prepared in the usual manner by treatment of ethereal diazomethane. The crude product was crystallized from MeOH as needles, mp 190°, $[\alpha]_D + 128.6^\circ$ ($c=0.35$ in EtOH), IR 1722 and 1710 cm^{-1} (two esters). MS m/e 498(M^+ , 5.1), 438[$M-(\text{COOCH}_3+H)$, 26.9], 378[$M-2(\text{COOCH}_3+H)$, 24.3], 306(RDA fragment a, 38.4), 246[$a-(\text{COOCH}_3+H)$, 44.8], 191(RDA fragment b, 10.2), 187[$a-(2\text{COOCH}_3+H)$, 100], 186[$a-2(\text{COOCH}_3+H)$, 66.6].

Anal. Calcd for $\text{C}_{31}\text{H}_{46}\text{O}_5$: C, 74.66; H, 9.30. Found: C, 74.88; H, 9.35.

Bromolactone(III) — To a solution of **IIa** (35mg) and NaOAc(300mg) in HOAc (10 ml) was added dropwise a solution of bromine in HOAc (3%, 2ml). It was kept at room temperature for 1 hr and the mixture then poured into water (50ml) containing $\text{Na}_2\text{S}_2\text{O}_3$ (1g). The precipitate was filtered, washed with water and dried. Crystallization from MeOH gave needles of **III**, mp 273~275°, IR 1767 cm^{-1} (γ -lactone), 1730 cm^{-1} (ester), and 1712 cm^{-1} (ketone).

Perbenzoic Acid Oxidation of the Methylester(IIc) — A solution of **IIc** (80mg) in chloroform was treated with 0.1N perbenzoic acid (5ml) in the dark at 0° for 10 days. The consumption of the per acid measured by titrating with 0.01 N- $\text{Na}_2\text{S}_2\text{O}_3$ in the usual way was 1.1 mole.

CrO₃ Oxidation of the Methylester(IIc) — To a boiling solution of **IIc**(70mg) in HOAc (20ml), CrO_3 (100mg) in 85% HOAc (10ml) was added dropwise over a period of 1 hr. Refluxing was continued further for an additional 90min and then the mixture was poured onto ice. The solids were filtered, chromatographed on silica gel (eluent, CHCl_3), and crystallized from MeOH to give **IV**, mp 275~276°, $\lambda_{\text{max}}^{\text{EtOH}}$ 253nm(log ϵ , 4.26).

SeO₂ Oxidation of the Methylester(IIc) — A solution of **IIc** (100mg) and freshly prepared SeO_2 (100mg) in HOAc (20ml) was refluxed for 5 hr. The solution was filtered and the filtrate was diluted with water and extracted with ether. The ether solution was washed with 10% NaHCO_3 , and then with water, and dried. Dehydroproduct was separated by preparative tlc (Silica gel G, MeOH- CHCl_3 -pet.ether-HOAc=3:20:70:1) to give pure **V**, which could not be crystallized, mp 198~202°, $\lambda_{\text{max}}^{\text{EtOH}}$ 242, 251, and 260nm (log ϵ , 4.03, 4.06, and 3.90, respectively).

Acid Treatment of Phytolaccagenin(Id) — A solution of **Id** (100mg) in 5% HCl-EtOH

(50ml) was refluxed for 5 hr and distilled. To the distillate dimedone (70mg) was added. After 30 min the mixture was concentrated to separate HCHO-dimedone, which was identified by mp and mixed mp 187~189°. A control experiment, without addition of terpenoid, yielded none of the compound. HCHO in the distillate was also detected by the chromotropic acid method. The residue was chromatographed on silica gel to give **IIa** mp 229~230°, identical with an authentic sample by mixed mp, IR, and co-TLC.

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