The Occurrence and Chemistry of
Phytolacca Triterpenoids*

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Phytolacca species grown in Korea are *Phytolacca esculenta*, *P. americana*, and *P. insularis*.[1] These plants are perennial shrub, roots of which have been used as an indigenous medicine against edema and rheumatism in the Far East Asia including China, Japan, and Korea, since ancient times. Several old dispensatories have described these plants as a diuretic agent.[2-4]

The older generation of physicians in U.S.A. also used these plants as a medicine. *P. americana* was official in the U.S.P. of 1880 and 1890. And it has been listed in the National Formulary and various dispensatories, in which we read that the plant has been recommended as an emetic, a purgative, a narcotics, and a gargle, and as a remedy for chronic rheumatism, granular conjunctivitis, ringworm, parasitic infections of the scalp, other skin diseases, syphilis, and cancer[5,6].

The young tender shoots, when properly prepared in spring, are edible[6,7], especially familiar to many Kentuckians, in fact, a nine-day poke festival is held annually at Harllan, Kentucky.[8] Europeans have used the purple juice of the berries extensively for coloring their wines[5].

In Ethiopia the berries of *P. dodecandra* have been extensively used as a potential molluscide for the control of bilhaziasis, a disease transmitted by aquatic snails[9].

The authors reported the anti-inflammatory action of sapogenins[10] as well as saponins[11] from *P. esculenta*.

We wish to describe the occurrence and chemistry of *Phytolacca* triterpenoids in this paper.

Occurrence—As *Phytolacca* plants are poisonous, several attempts were made to isolate the poisonous principle.

In 1949, Ahmed, Zufall, and Jenkins[12] of Purdue University reported the isolation of the toxic principle of *P. americana* and proposed the formula $C_{66}H_{90}O_{22} \cdot 2H_2O$, suggesting that it was a steroidal glycoside.

In 1964, Stout, Malofsky, and Stout[13] of University of Washington obtained the same material and named it phytolaccatoxin.* Hydrolysis of phytolaccatoxin gave glucose and xylose and a aglycone, phytolaccagenin, $C_{31}H_{48}O_7$, mp 317~318°, the structure of which was determined as **VIII** by the use of a light atom technique of x-ray diffraction analysis.

In 1971, Woo[10] isolated a new triterpene, $C_{30}H_{46}O_7$, mp 318~320°, possessing anti-inflammatory action from *P. esculenta* and named it jaligonic acid after Korean name "jaligong", its structure was elucidated as **VII** based on the chemical reactions and spectroscopic data[16].
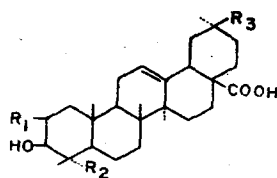
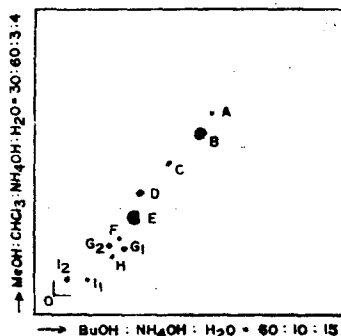|	R_1	R_2	R_3
I Oleanolic acid	H	CH_3	CH_3
II Spergulagenic acid	H	CH_3	COOH
III Serjanic acid	H	CH_3	COOMe
IV Esculentic acid	H	CH_2OH	COOH
V Phytolaccagenic acid	H	CH_2OH	COOMe
VI Bayogenin	OH	CH_2OH	CH_3
VII Jaligonic acid	OH	CH_2OH	COOH
VIII Phytolaccagenin	OH	CH_2OH	COOMe
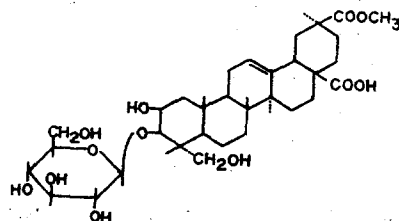
IX

Fig. 1—Diagram of thin layer chromatography of *Phytolacca* saponins.

The roots of *P. esculenta*, in addition to jaligonic acid, contain phytolaccagenin(30-methyl jaligonate)[17], esculentic acid[18](**IV**), $C_{30}H_{46}O_6$, mp>360°, and phytolaccagenic acid (30-methyl esculentate)[19] (**V**), $C_{31}H_{48}C_6$, mp 309~311°, in the free or combined state.

Tweive saponins were detected in MeOH extratcs (Fig. 1) and one of them, phytolaccoside B. mp 215~218°, $[\alpha]_D = +75.8°$ was isolated in pure state and its structure was recently determined as **IX**[20].

* Phytolaccotoxin, $C_{24}H_{30}O_6$, as a toxic substance of *P. esculenta*, reported by Nagai[14] once in 1890, is quite another kind of substance. Later, even the presence of this substance in the plant was, moreover, denied by Iwakawa[15].

In 1971, Burke and Le Quesne[21] of University of Michigan, from TLC result only, postulated the presence in the petrol. extract of roots of *P. americana* of phytolaccagenin including oleanolic acid (I), $C_{30}H_{48}O_3$, mp 303~306°, as free terpenoids, but no other triterpenoids were detected. Whereas, Woo[22] isolated from the roots of *P. americana* all of four triterpenoids (IV, V, VII, and VIII), which were identical substances isolated from *P. esculenta*, but oleanolic acid could not be detected. Although we worked with different solvents and used the materials obtained at different place and time, the discrepant results have not been yet explained clearly. But the seeds of this plant contain the considerable amounts of acetyl oleanolic acid, $C_{32}H_{50}O_4$, mp. 290~300°.

The terpenoid constituents of *P. insularis* are identical to those of *P. esculenta* and *P. americana*[23]. However, sapogenins of *P. rivinoides*[24] in Spain and *P. octandra*[25] in Australia were found to be serjanic acid(III), $C_{31}H_{48}O_5$, mp 285~287°. On the other hand, Oleanolic acid and bayogenin(VI), $C_{30}H_{48}O_5$, mp 325~330°, were found in the hydrolysis products of the crude active saponins of *P. dodecandra*[26], which contain oleanoglycotoxin-A(X)[27], lemmatoxin(XI)[28], and lemmatoxin-C(XII)*[29] in concentrations of apporoximately 18%, 16%, and 17%, respectively.

Table I shows triterpenoids detected in *Phytolacca* spp. From the viewpoint of chemotoxomy and biogenesis, it is worth noting that triterpenoids possessing 28, 30-dicarboxyl groups of β-amyrin series were found only in the genera *Phytolacca, Mollugo,* and *Serjanica*[30].
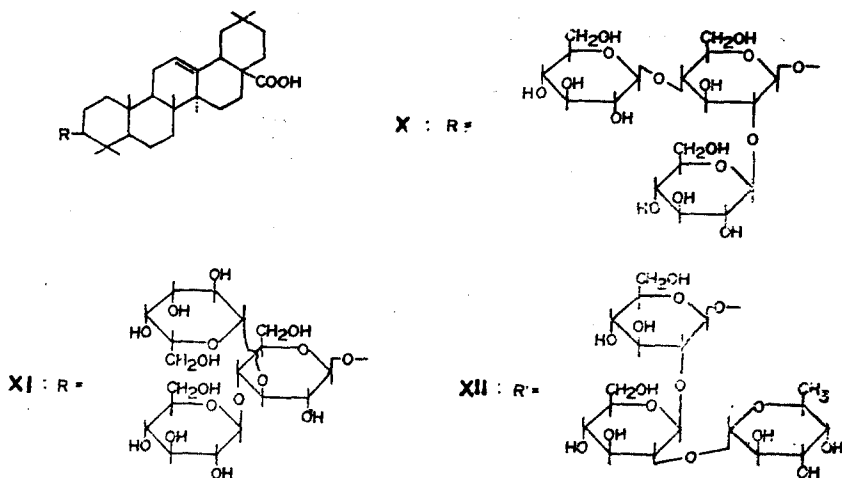
Jaligonic Acid[16]—Jaligonic acid(VII), $C_{30}H_{46}O_7$, mp 318~320°, $[\alpha]_D^{26}=+113.7°$ (c, 0.89 in MeOH), λ_{max}^{EtOH} 204 nm(log ε, 3.65), is largest in quantity among the terpenoids in free

* Lemmatoxin C is a mixture of two closely related compound, 70% (at least) is XII and the remaining component is the galactose containing species.[29]

Table I—Triterpenoids identified in *Phytolacca* spp.

Plant	I	II	III	IV	V	VI	VII	VIII	Reference
P. esculenta	—	—	—	+	+	—	+	+	(10, 17, 18)
P. americana	+	—	—	+	+	—	+	+	(13, 19, 21, 22)
P. insularis	—	—	—	+	+	—	+	+	(23)
P. rivinoides	—	—	+	—	—	—	—	—	(24)
P. octandra	—	—	+	—	—	—	—	—	(25)
P. dodecandra	+	—	—	—	—	+	—	—	(26~29)

state[17, 22]. It gives a pink coloration in the Liebermann-Burchard test and a pale yellow coloration in the tetranitromethane color test. Its IR absorption spectrum shows a broad OH^- peak at $3435 cm^{-1}$, a COOH peak at $1705 cm^{-1}$, and peaks at 1665 and $825 cm^{-1}$ indicating a trisubstituted double bond.

The compound is esterified when dissolved in MeOH and treated with ethereal diazomethane to give dimethyl ester(XIII), $C_{32}H_{50}O_7$, mp 213~215°, $[\alpha]_D^{25} = +117.0°$ (c, 0.94 in MeOH), UV λ_{max}^{EtOH} 204 nm (log ε, 3.66) and its IR spectrum shows two peaks at 1717 and $1735 cm^{-1}$ due to two ester groups. Acetylation of jaligonic acid with acetic anhydride-pyridine gives a triacetate(XIV), $C_{36}H_{52}O_{10}$, mp 224~226°, $[\alpha]_D^{25} = +106.0°$ (c, 0.95 in MeOH), λ_{max}^{EtOH} 204 nm (log ε, 3.72). This compound shows in its IR spectrum acetoxyl peaks at 1745, 1370, and $1235 cm^{-1}$, but does not absorb in the region corresponding to hydroxyl group.

It is known the formation of an anomalous acetate[31](XV) derived from katonic acid[32] (XVI) which has a C-29 carboxyl group, when treated with acetic anhydride in the presence of perchloric acid. However, on treatment under the same conditions, unlike katonic acid, jaligonic acid gives only the normal triacetate, since it has a C-30 carboxyl group. The triacetate is saponified on treatment with K_2CO_3 solution for 40 minutes, although these mild conditions are usually insufficient to saponify the conventional 3β-acetoxyl group of pentacyclic triterpenes[33].

In reaction with perbenzoic acid the equivalent of one double bond is consumed by the dimethyltriacetate(XVII), $C_{38}H_{56}O_{10}$, mp 115~120°, $[\alpha]_D^{20.5} = +182.5°$ (c, 0.115 in MeOH), IR cm^{-1}, 1725 (ester) and 1235 (acetate), obtained by methylation of the triacetate(XIV) or acetylation of the dimethylester(XIII).

Oxidation of the dimethyltriacetate with CrO_3-HOAc furnishes an α,β-unsaturated ketone (XVIII), $C_{38}H_{54}O_{11}$, which shows a UV absorption maximum at 250 nm (log ε, 4.24) and SeO_2-HOAc oxidation leads to a heteroannular diene(XIX), $C_{38}H_{54}O_{10}$, which shows three UV absorption maxima at 243, 251, and 260 nm (log ε, 4.18, 4.23, and 4.15, respectively), typical of the 11 : 12, 13 : 18 diene of the oleanane series[34]. On treatment with Br_2-HOAc jaligonic acid gives a monobromo-γ-lactone(XX), $C_{30}H_{45}O_7Br$, mp 235~238°, IR $1763 cm^{-1}$, (γ-lactone).

Dimethly ester(XIII) is quantitatively oxidized by HIO_4 within ca. 1hr and the resulting dialdehyde(XXI) is subjected to cyclizatien using piperidine and HOAc, and the product,
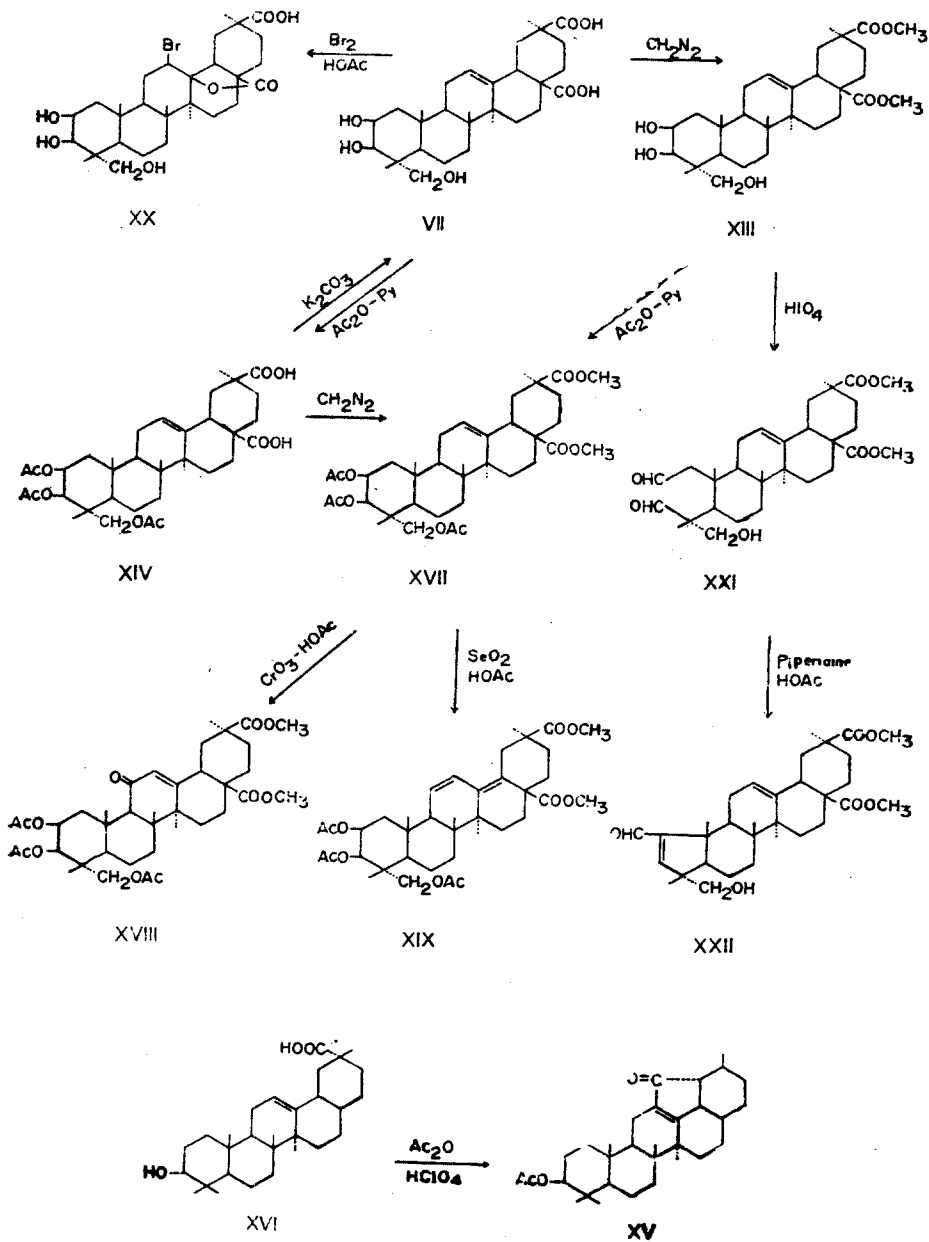
α,β-unsaturated aldehyde(**XXII**) exhibits a UV maximum at 235nm (log ϵ, 3.99). Fig. 2 shows oxidation rate of the dimethylester(**XIII**) by lead tetraacetate to prove the presence of *cis*-glycol OH in jaligonic acid(**VII**). As expected oxidation rate is very fast and rate constant 107×10^{-3} liter-mole^{-1}-sec^{-1}. This rate constant is about 3 times greater than those of medicagenic acid (31×10^{-3})[33] and $22\alpha,5\alpha$-spirostane $2\beta,3\beta$-diol (31.9×10^{-3})[35], both of them are $2\beta, 3\beta$ diols. The large rate constant of dimethyl jaligonate could not fully explained.

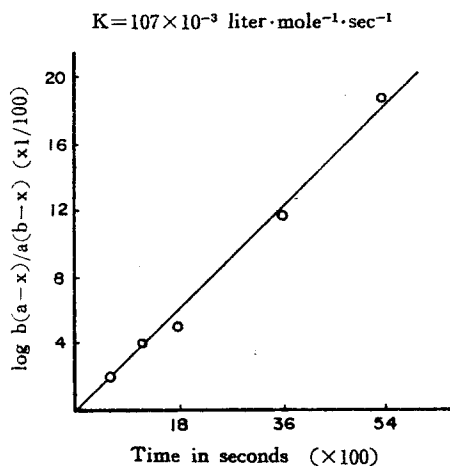$$K=107\times10^{-3}\ \text{liter·mole}^{-1}\text{·sec}^{-1}$$

Fig. 2—Lead tetraacetate oxidation of dimethyl jaligonate. a, initial concentration of Pb $(OAc)_4=2.72\times10^{-3}$ mole/L; b, initial concentration of sample$=2.00\times10^{-3}$ mole/L; x, amount reacted at time t.

When heated with Cu catalyst at 290°, jaligonic acid gives small but consistent amounts of HCHO, a typical reaction of the 3,23-diol system in the triterpene series[38].

The NMR spectrum of the dimethyl triacetate shows, as expected, five tertiary methyl signals at δ 0.74(3H), 1.03(3H), 1.12(3 H), 1.14(3 H), 1.22(3H) due to C-26, 24, 27, 29, and 25 methyls, respectively; three acetyl signals at 1.98(3H) and 2.03(6H); two methyl ester signals at 3.57(3H) and 3.68(3H); a multiplet centered at 5.32(2 H) due to H-2 and H-12; a doublet centered at 4.88(1H, $J=$ 4 Hz) due to H-3; and the ill-defined close AB system centered at 3.75 assignable to the two methylene protons of the 23-acetoxymethyl group attached to an asymmetric center, C-4.

The width at half-height of signals due to the H-2 is *ca.* 8Hz and the coupling constant between H-2 and H-3 is 4Hz, being in agreement with the assigned stereochemistry of the acetoxyl groups as $2\beta, 3\beta$ diol[39]. The chemical shift attributed to the protons on C-23 is within the range for an equatorial CH$_2$OAc[34].

Phytolaccagenin[17]—Phytolaccagenin(**VIII**), $C_{31}H_{48}O_7$, mp 317~319°, $[\alpha]_D^{29}=+114.7°$ (c, 0.83 in MeOH), IR cm^{-1}; 3430 (OH), 1732 (ester), 1698 (acid), 1660 and 824 (trisubstituted double bond), occurred, for the most part, in the combined state with sugar, though small in quantity in free state. In other words, major aglycone of total *Phytolacca* saponins is found to be phytolaccagenin[20,22].

It undergoes easy saponification with methanolic KOH to give jaligonic acid(**VII**). Methylation with diazomethane gives a substance which is identical with jaligonic acid dimethylester(**XIII**). The dimethylester on saponification with 10% methanolic KOH for 18 hr furnishes a 28-monomethyl ester(**XXIII**), $C_{31}H_{48}O_7$, mp 321~324°, $[\alpha]_D^{25}=+113.1°$ (c, 0.93

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* It seems that the additional substituents at C-4 play no role in the case of 2α, 3β diol, since the agreement in the lead tetraacetate oxidation of arjunolic acid (1.07×10^{-3}) and asiatic acid (1.6×10^{-3}) series[33] and the steroidal 2α, 3β-diol (1.63 to $1.83\times10^{-3})$[36,37] is excellent.

in MeOH), IR cm^{-1}; 1732 (ester), 1710 (acid) and on further saponification with KOH in ethylene glycol gives jaligonic acid(VII). Phytolaccagenin⁻ is synthesized by methylation of jaligonic acid with 3% methanolic HCl[16]. On treatment with acetone in the presence of HCl, dimethyl jaligonate(XIII) affords only $2\beta,3\beta$-acetonide(XXIV), $C_{35}H_{54}O_7$, mp 209~210°, IR cm^{-1}; 1370, 1383 (gem-CH$_3$).* The acetonide derivative is oxidized by CrO$_3$-pyridine to yield dehydro derivative(XXV), $C_{35}H_{52}O_7$, mp 123~125°, IR cm^{-1}; 1730, 1782 (gem-CH$_3$), no OH band, which is readily converted into dimethyl deoxyjaligonate(XXVI), $C_{32}H_{50}O_6$, mp 198~199°, by a modified Huang-Minlon⁻reaction of its hydrazone followed by acid hydrolysis and re-esterification[41]. This compound was once prepared from spergulagenic acid dimethylester(XXVII), $C_{32}H_{50}O_5$, m.p. 235~237°, by Djerassi and coworkers[33].

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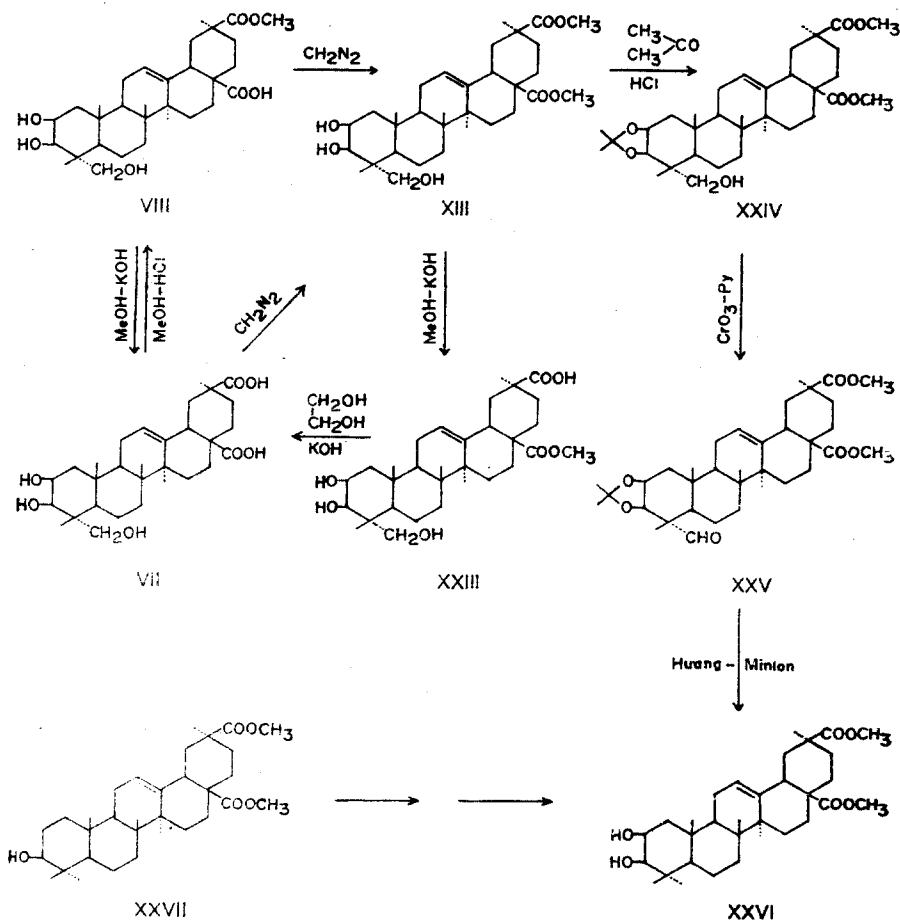
* Although it is possible to prepare two isomeric acetonides, all triterpenoids, but polygalacic acid[40] have been reported to form 2β, 3β-acetonide only.

On treatment with acid for long period gives a substance(XXVIII) having the molecular formula $C_{30}H_{44}O_5$, mp 229~230°, the structure of which was established as 3-oxo-30-carbomethoxy-24-norolean-12-en-28-oic acid[42]. This compound is stable to acids and no significant shift of optical rotation was observed in changing from ethanol to chloroform as a solvent, since the methyl group at C-4′ is equatorial[43]. As for the reaction mechanism concerned in the conversion of phytolaccagenin into this compound on acid treatment, an acid catalysed dehydration involving elimination of the less stable axial OH at C-2, followed by reversed aldol-type condensation as in methyl hederagonate, $C_{31}H_{48}O_4$, mp 213~215°[44], is inferred as. shown below.
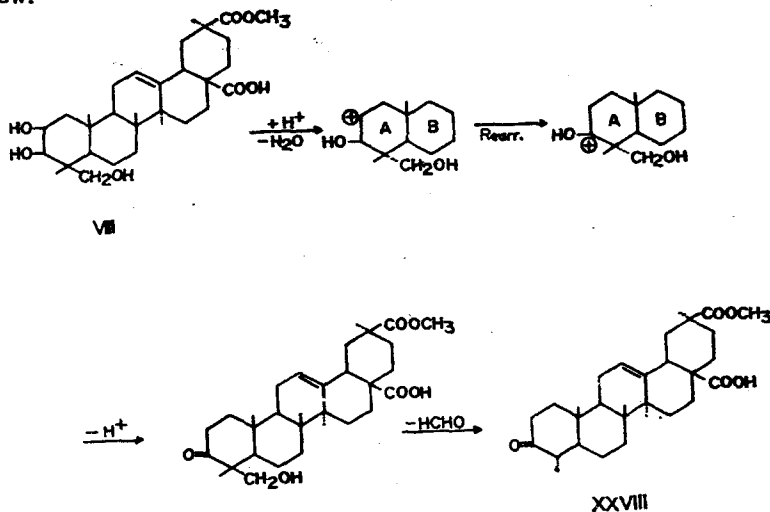
The NMR spectrum of methylester of this compound is in complete agreement with the structure and shows tertiary methyl signals at δ 0.80(3H) and 1.13(9H), a doublet centered at 1.00(3H, J=6 Hz) assignable to protons of the 23-secondary methyl group attached to C-4, two singlets at 3.60 and 3.71(3H each) due to the methyl protons of two esters, and a multiplet centered at 5.38(1H) due to H-12.

This compound has been initially isolated from the acid hydrolysis products of crude saponins and named genin A[22]. However, it is supposed not to be a genuine sapogenin but an artifact produced during hydrolysis of the saponins.

Table II shows physical constants of the derivatives of genin A.

Esculentic . Acid and 30-Monomethylester(Phytolaccagenic Acid)—Esculentic acid[18,45] (IV), $C_{30}H_{46}O_6$, mp >360°, was isolated from the roots of *P. esculenta*, and phytolaccagenic acid [19,46](V), $C_{31}H_{48}O_6$, mp 309~311°, from the roots of *P. americana* in this Institute. Since esculentic acid differs from jaligonic acid only in the lack of a hydroxyl group at C-2. it undergoes all of chemical reactions as jaligonic acid but HIO_4 oxidation. CrO_3 oxidation of phytolaccagenic acid yields the norterpene(XXVIII) and removal of the 23-hydroxyl group by tosylation and $LiAlH_4$ reduction gives quaratarol(XXIX)[24,30,47,49], $C_{30}H_{50}O_3$, mp

Table II—Physisical constants on genin A derivatives.

Name	Formula	Mp	UV nm(log ε) (in EtOH)	IR cm^{-1} (KBr)	$[\alpha]_D$ (in EtOH)
Genin A	$C_{30}H_{44}O_5$	229~230°	204(3.80) 285(1.49)	1710(CO) 825(CH=C)	+110.75° +108.10° **
2,4-Dinitrophenyl hydrazone	$C_{36}H_{48}O_3N_4$	190~191°	366(4.32) 435(4.06)*	1720(ester) 1700(acid) 1505(NO$_2$) 1332	
Bromolactone	$C_{30}H_{43}O_5Br$	273~275°		1767(γ-lactone) 1730(ester) 1712(ketone)	
Methylester	$C_{31}H_{46}O_5$	190°		1710(ester) 1722(ester)	+128.6°
α,β-Unsaturated ketone	$C_{31}H_{44}O_6$	275~276°	253(4.26)		
Dehydroproduct	$C_{31}H_{44}O_5$	198~202°	232(3.94) 242(4.03) 251(4.06) 260(3.90)		

* in EtOH-KOH　　　** in CHCl$_3$
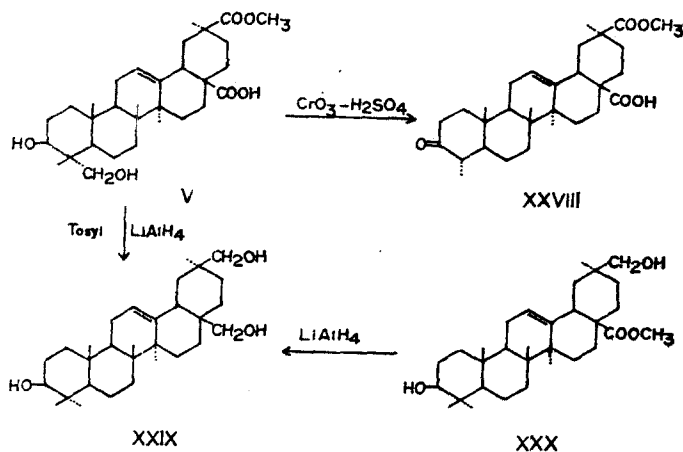
278~280°, LiAlH$_4$ reduction product of queretaroic acid methylester[50] (**XXX**), $C_{31}H_{50}O_4$, mp 223~224°, or spergulagenic acid dimethylester[48] (**XXVII**).

Table III shows the physical data of derivatives of esculentic acid.

The NMR spectrum of dimethyl esculentate diacetate is in complete agreement with the structure and shows five tertiary methyl signals at δ 0.72(3 H), 0.83(3 H), 0.98(3 H), 1.12(3 H), and 1.14(3 H) due to C-26, 24, 25, 27, and 29 methyls, respectively; two acetyl signals at 2.02(3H) and 2.06(3H); two methyl ester signals at 3.57(3H) and 3.69(3H); a multiplet centered at 5.37(1H) due to H-12; a triplet like multiplet centered at 4.8(1H) due to H-3; an AB quartet at 3.72 and 3.88(1H each, J=12Hz) due to two methylene pro-

Table III—Physical data on esculentic acid derivatives:

Name	Formula	Mp	UV nm(log ε) (in EtOH)	IR (cm⁻¹ KBr)	[α]ᴅ (in EtOH)
Esculentic acid	$C_{30}H_{46}O_5$	>360°	204(3.70)	3450(OH) 1701(acid)	+85.6°
Diacetate		103~106°			
Bromolactone	$C_{30}H_{45}O_5Br$	231~233°		1767(γ-lactone) 1747(ester) 1718(acid)	
Dimethyl ester	$C_{32}H_{50}O_6$	151~153°		1710(ester) 1730(ester)	+89.2°
28-Monomethylester	$C_{31}H_{48}O_6$	306~307°		1740(ester) 1710(acid)	+85.1°
Phytolaccagenic acid	$C_{31}H_{48}O_5$	309~311°	204(3.79)	3400(OH) 1730(ester) 1700(acid) 825(CH=C)	+86.5°
Diacetate	$C_{35}H_{52}O_8$	138~142°		1738(ester) 1240(acetate)	
Bromolactone	$C_{41}H_{57}O_6Br$	238°		1770(γ-lactone) 1725(ester)	
Dimethyl acetonide	$C_{35}H_{54}O_6$	265~266°		1365(gem-CH₃) 1383	
Dimethyl diacetate	$C_{36}H_{54}O_8$	100~103°		1730(ester) 1235(acetate)	
α,β-Unsaturated ketone	$C_{36}H_{52}O_9$	197~199°	249(3.73)		
Dehydroproduct	$C_{36}H_{52}O_8$	77~79°	243(4.02) 251(4.09) 260(3.88)		

tons on 23-acetoxymethyl group.

Johnson and Shimizu[47] of University of Rhode Island recently reported the isolation of a new compound from the berries of *P. americana* and named it as phytolaccinic acid, which is identical with our phytolaccagenic acid.

Spergulagenic Acid and 30-Monomethylester(Serjanic Acid)—Spergulagenic acid, $C_{30}H_{46}O_5$, mp 320~322°, has been first isolated* from *Mollugo spergula* (Ficoidaceae) by Chakrabarti, Makherjee, Chatterjee, and Barua[48] of Bose Institute in India, in 1965, and its structure was elucidated[51] as **II**, in 1968. The isolation of its 30-methylester, *i.e.* serjanic acid(**III**), $C_{31}H_{48}O_5$, mp 285~287°, from *Serjania* spp. was reported by Savoir, Tursch, and Kaisin[30] of Universite Livre de Bruxelles, in 1967. It was found that the aglycone of saponin, mollugo glycoside A(**XXXI**), $C_{41}H_{64}O_{13}$, mp 220~225°, $[\alpha]_D=+30°$(c, 0.61 in pyridine) from *M. spergula* is serjanic acid[49]. The presence of serjanic acid in *P. rivinoides* and *P. octandra* was recently reported, however, we could not detect it in all of *Phytolacca* spp. grown in Korea.

* Spergulagenic acid(**II**) has been isolated as dimethylester and not obtained in free state so far. Djerassi's research group[33] had already prepared dimethylester(**XXVII**) from queretaroic acid by CrO₃ oxidation and NaBH₄ reduction in 1957, before isolation from nature.

XXXI
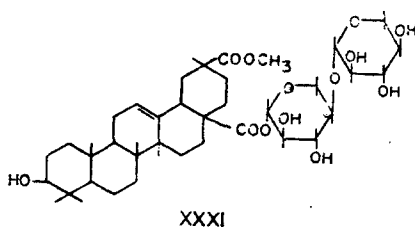
The structure of spergulagenic acid is identical to those of jaligonic acid and esculentic acid but in number of OH group on ring A. Therefore its physico-chemical properties are similar to those of jaligonic acid and esculentic acid.

Irradiation of spergulagenic acid(II) in acidified ethanolic solution (pH 2) affords an epoxy-γ-lactone[52](XXXII), $C_{30}H_{44}O_6$, mp 320~323°, $[\alpha]_D=+116°$, IR cm^{-1}; 3440(OH),

II   XXXII   XXXIII

XXVII   XXXV   XXXIV

XXXVI   XXXVII   XXXVIII
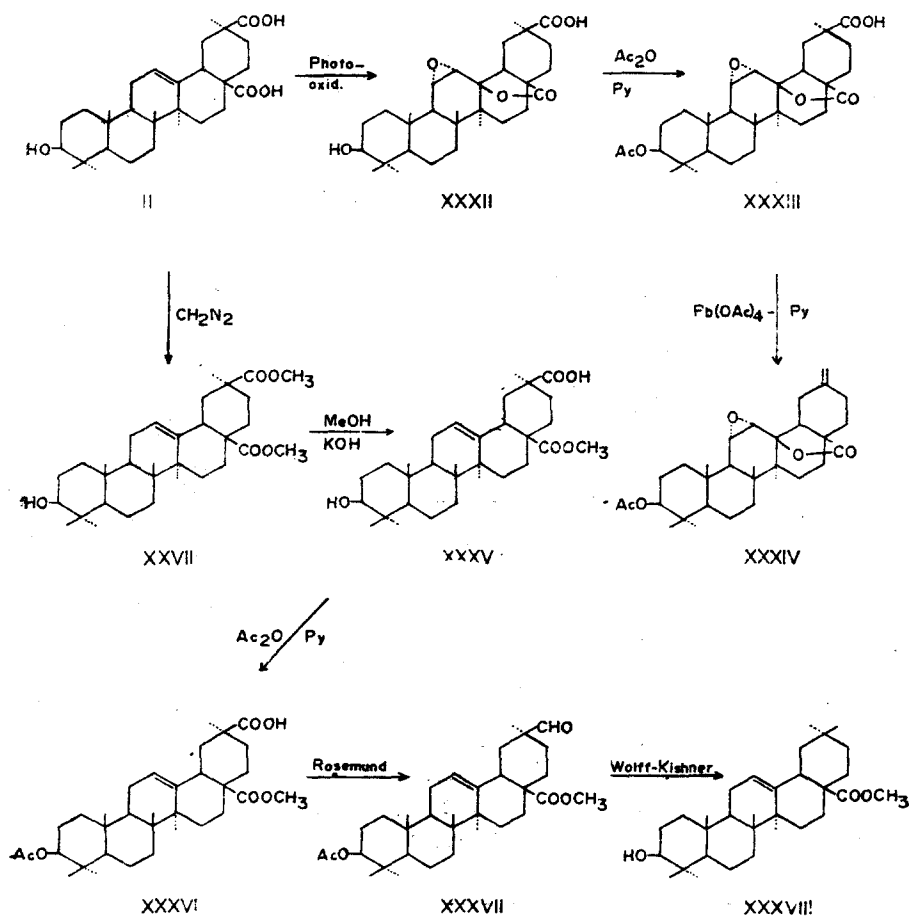
1769(γ-lactone), 1708(acid), and 872(epoxide), in 13% yield. Its acetate(**XXXIII**), $C_{32}H_{46}O_7$, mp 301~303°, $[\alpha]_D=+86°$, IR cm^{-1}; 1734 and 1247 (acetate), is oxidized by Pb(OAc)$_4$-pyridine to give eupteleogenin acetate[53] (**XXXIV**), $C_{31}H_{44}O_5$, mp 321~322°, $[\alpha]_D=+87°$, IR cm^{-1}; 1648 and 892(C=CH$_2$), 1763(γ-lactone), 873(epoxide), in 28% yield[52].

　　Rosenmund reduction of acid chloride of 28-monomethylester acetate(**XXXVI**), $C_{33}H_{50}O_6$, mp 198~204°, prepared from dimethyl ester(**XXVII**) gives the aldehyde(**XXXVII**) which is reduced by the Wolff-Kishner method and re-esterification to oleanolic acid methylester (**XXXVIII**), $C_{31}H_{50}O_3$, mp 198~200°, $[\alpha]_D=+72°$[54].

　　Table IV shows physical data of derivatives of spergulagenic acid reported.

　　The NMR spectrum of dimethylacetate shows, as expected, six tertiary methyl signals at 0.72(3 H), 0.85(6H), 0.95(3 H), and 1.15(6 H) due to C-26, 23 and 24, 25, and 27 and 29 methyls, respectively; an acetyl signal at 2.05(3 H); two methylester signals at 3.62(3 H) and 3.71(3 H); a triplet at 4.50 due to H-3; a multiplet centered at 5.38(1 H) due to H-12.

　　Saponification of Methylesters—It is well known that 28-carbomethoxyl group can be saponified only with great difficulty[44,55] but the presence of an appropriately situated oxygen function, namely β- or γ- to carbomethoxyl group, facilitates the hydrolysis(Table V). The equatorial carbomethoxyl groups are hydrolyzed more easily than the axial ones. For example, methyl 18α-glycyrrhetate with boiling 3% alcoholic KOH gives a 91.5% yield of 18α-glycyrrhetic acid in 2hr, while methyl glycyrrhetate gives only a 5% yield of glycyrrhetic

Table IV—Physical data on spergulagenic acid derivatives.

Name	Formula	Mp	UV nm(log ε) (in EtOH)	IR cm^{-1}	$[\alpha]_D$ (in EtOH)
Spergulagenic acid	$C_{30}H_{46}O_5$	347~350°		1685(acid)	+116°
Bromolatone	$C_{30}H_{65}O_5Br$	298~300°			+118°
Serjanic acid	$C_{31}H_{48}O_5$	285~287°		1730(ester) 1710(acid) 825(CH=C)	+100°
Acetate	$C_{33}H_{50}O_6$	230~235°		1728(ester) 1245(acetate)	+96°
Bromolactone	$C_{31}H_{47}O_5Br$	254°		1770(γ-lactone)	
28-Monomethylester	$C_{31}H_{50}O_5$	292~294°		1733(ester) 1712(acid)	+97°
Acetate	$C_{33}H_{50}O_6$	198~204°		1735(ester) 1715(acid) 1242(acetate)	+94°
Dimethylester	$C_{32}H_{50}O_5$	235~237°	208(3.7)	1715(ester)	+101.3°
Acetate	$C_{34}H_{52}O_6$	292~293°		1235(acetate)	+93.1°
3-Ketocompound	$C_{23}H_{48}O_5$	204~206°			+113°
α,β-Unsaturated ketone	$C_{24}H_{50}O_7$	293~295°	249(4.1)		+118°
Dehydroproduct	$C_{24}H_{50}O_5$	236~239°	242(4.27) 250(4.32) 260(4.13)		

acid[59].

The relative ease of saponification of the equatorial 23-carbomethoxyl group[33,60,61] and the resistance of the axial 24-carbomethoxyl group to hydrolysis[62,63] are also well known. Thus, several authors have used the saponification rate to determine the stereochemistry of the carboxyl groups at C-4[61] or C-20[54,55,58].

A difference in the reactivity of the two carboxyl groups was, as expected, noted[16,45,54] when 28, 30-dimethylesters were heated in refluxing methanolic KOH under the conditions where 28-carbomethoxyl group lacking activating functions is not affected, and the products are proved to be 28-monomethyl esters. However, hydrolysis of the 30-carbomethoxyl group occurs with surprising ease and the saponification rate is greater than those which have been observed in other triterpenoids[55,58].

This result implies that the presence of the carboxyl group at C-17 has a marked accelerating effect on the saponification of the 30-carbomethoxyl group. Thus it would clearly be unjustifiable to make stereochemical deductions from the saponification rate for the carboxyl group at C-20 of the triterpene-28-carboxylic acids.

Mass Spectral Fragmentation Pattern of Jaligonic Acid and Related Compounds— Table VI shows mass spectral fragmentation of jaligonic acid and related compounds. Typical

Table V—Saponification rates of triterpene methylester with methanolic KOH(%).

| Ester	Substituent	1% 3hr	5% 8hr	7% 8hr	10% 8hr	20% 3hr	Reference
Oleanolate	28-COOH 28-COOH		0	0	0	5*	(44, 55)
Cochalate	16β-OH 28-COOH		0	3	20		(55)
Echinocystate	16α-OH 28-COOH					60*	(44)
Entagenate	15α,16α-OH 28-COOH					37*	(56)
Icterogenin	22β-Angelyl					60*	(44)
Machaerate	23-COOH 21-C=O		100				(57)
Deoxyglycyrrhetate	30-COOH		5	13~17	40~47		(55)
Myrtillogenate	29-COOH 28-CH₂OH 16-OH		11	31	85~93		(55)
Acid from cyclamigenate	30-COOH 13, 28-CH₂O				58~65		(58)
Spergulagenate^b	30-COOH 28-COOH	42*			99*		(54)
Esculentate^b	30-COOH 28-COOH				83		(45)
Jaligonate^b	30-COOH 28-COOH	11~18	33.2	54.8	78~89		(16)

*EtOH-KOH,   ᵇ28-COOCH₃ was not hydrolyzed.

Table VI—Mass spectral fragmentation of jaligonic acid and related compounds.

Compound	M+	a	b	c	d	e	b'	c'	d'	f	g	h	Ref.
Jaligonic acid	518 (0.5)	278 (99)	233* (83)	232* (98)	187* (100)	133 (40)	233* (83)	232* (98)	187* (100)	219 (50)	173 (71)	239 (25)	(16)
30-Methylester	532 (1.4)	292 (54)	233* (19)	232 (26)	187* (93)	133 (18)	247 (42)	246 (100)	187* (93)	233* (19)	173 (40)	239 (12)	(17)
28-Methylester	532 (0.5)	292 (29)	247 (20)	246 (52)	187 (100)	133 (51)	233 (48)	232 (57)	187* (100)	219 (26)	173 (69)	239 (12)	(17)
Dimethylester	546 (0.7)	306 (74)	247* (61)	246* (88)	187* (100)	133 (29)	247* (61)	246* (88)	187* (100)	233 (29)	173 (48)	239 (23)	(16)
Phytolaccagenic acid	516 (0.5)	292 (39)	233* (14)	232 (20)	187* (100)	133 (22)	247 (32)	246 (77)	187* (100)	233* (14)	173 (34)	223 (8)	(19, 22)
Methylester	530 (1.6)	306 (49)	247* (50)	246* (66)	187* (100)	133 (22)	247* (50)	246* (66)	187* (100)	233 (16)	173 (36)	223 (9)	(19, 22)
Genin A	484 (1.4)	292 (19)	233* (20)	232 (19)	187* (100)	133 (19)	247 (21)	246 (57)	187* (100)	233* (20)	173 (34)	191 (10)	(22, 42)
Methylester	498 (5.1)	306 (39)	247* (37)	246* (45)	187* (100)	133 (30)	247* (37)	246* (45)	187* (100)	233 (41)	173 (56)	191 (15)	(22, 42)
Spergulagenic acid	486 (6)	278 (100)	—	232* (100)	187* (100)	—	—	232* (100)	187* (100)	—	—	207 (100)	(25)
Dimethyl acetate	556 (3.9)	306 (82)	247* (41)	246* (70)	187* (100)	133 (14)	247* (41)	246* (70)	187* (100)	233 (20)	173 (29)	249 (6)	(51)
3-Oxo-dimethyl spergulagenate	512 (44)	306 (71)	—	—	187* (86)	—	—	—	187* (80)	233 (50)	—	205 (21)	(25)

* indicates more than one possible fragmentations giving the same ion.

Figures in parentheses are % of base peak.
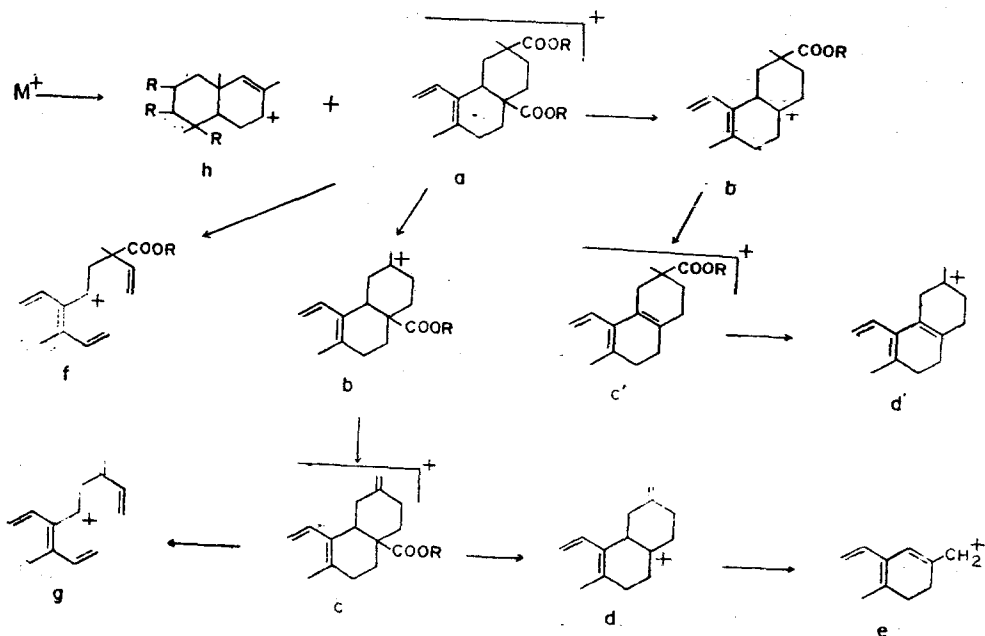
retro-Diels-Alder fragmentation patterns are shown in all mass spectra and the rest of the peaks due to further cracking are similar to the usual cascading patterns expected for olean-12-ene series. Fragment 'c' corresponding to the loss of the C-17 substituent plus one proton from ion a is very abundant in all cases compared with fregment c, corresponding to the loss of the C-20 substituent plus one proton.

In the high mass range, the intensity of peak due to the loss of C-17 substituent plus one proton from molecular ion is also much higher than that of peak due to the loss of the C-20 substituent plus one proton. For example, both of mass spectra of two monomethyl jaligonate show equally the high mass peaks at m/e 532(M^+), 514(M^+−H_2O), 496(M^+−2H_2O), 486 [M^+−(COOH+H)], 484[M^+−(H_2O+HCHO)], and 472[M^+−(COOMe+H)]. However, the peak at m/e 472 is abundant in the case of 28-monomethylester, while the peak at m/e 486 is abuntant in the case of 30-monomethylester[17] as shown in Fig. 3.

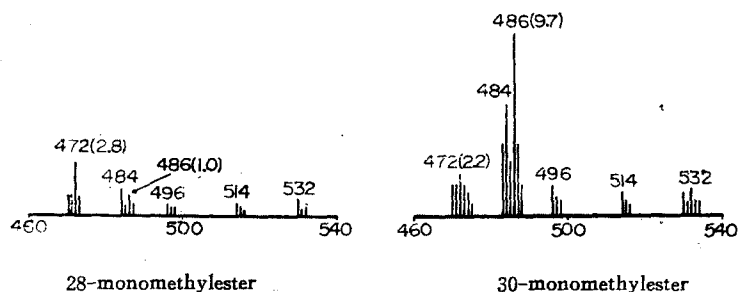28-monomethylester   30-monomethylester

Fig. 3—Intensity of high mass peaks above m/e 460 in monomethylesters of jaligonic acid. The parenthesized figures represent % of base peak.

These peaks would be therefore used as a tool for distinection between two monomethyl-esters.

The Effect of 30-carbomethoxyl Group on NMR Chemical Shift of Angular Methyl Groups—It is known that for steroid[64] and triterpenoid[65~68] skeletons, modifications in substitution pattern are accompanied by systematic changes in the NMR chemical shifts of the angular methyl groups. Such changes are, to a first approximation, additive. A complete assignment of methyl resonances in a series of compound is, therefore, of use in the eluci-dation of related structures. Effect of substitution on the chemical shifts of methyl groups in the Δ^{12}-oleanene series has been extensively studied by Tursch[65, 66], Cheung[67], and Ito[68]. However, no work has appeared in the literature regarding the effect of 30-carbomethoxyl group (because few substances with 30-carboxylic acid has been known at that time).

The observed methyl frequencies are listed in Table VII. From these data one can deduce the deshielding effect of 30-carbomethoxyl group. For all the compounds examined here, the error is less than 2 Hz. Applicability of this data is shown in Table VIII for dimethyl jaligo-nate triacetate as an example. The observed values of the chemical shifts for the C-methyl groups are in excellent agreement with the values calculated for 30-carbomethoxyl structure,

Table VII—Deduction of the additive deshilding effect of 30–COOCH₃.

Compound	Substituent					Resonance frequencies* of methyl						Ref.
	2β	3β	23	28	30	23	24	25	26	27	29	
Dimethyl spergulagenate	H	OH	H	COOMe	COOMe	60	47.5	55	44	69	69	(30)
Methyl oleanolate	H	OH	H	COOMe	Me	60	47.5	55~57	44.5	69	55~57	(66)
Dimethyl spergulagenate	H	OAc	H	COOMe	COOMe	51	51	57	43	69	69	(24)
Methyl oleanolate acetate	H	OAc	H	COOMe	Me	52	52	55~57.5	44.5	68.5	55~57.5	(66)
Serjanic acid acetate	H	OAc	H	COOH	COOMe	53.5	53.5	57	44	68.5	68.5	(24)
Oleanolic acid acetate	H	OAc	H	COOH	Me	52	52	55~57	45.5	69	55~57	(66)
Dimethyl jaligonate triacetate	OAc	OAc	OAc	COOMe	COOMe	—	62	73	44.5	67	68.5	(16)
Methyl bayogenin triacetate	OAc	OAc	OAc	COOMe	Me	—	62	73	45.5	67	54.5	(67)
Dimethyl esculentate diacetate	H	OAc	OAc	COOMe	COOMe	—	49.5	58.5	43	67	68	(45)
Methyl hederagenin diacetate	H	OAc	OAc	COOMe	Me	—	50.5	58	44.5	67	56	(67)
Increments in resonance frequencies						0	0	0	−1	0	+13	

* In Hz relative to TMS, for CDCl₃ solutions measured at 60 MHz.

Table VIII—The chemical shifts for C-methyl groups of dimethyljaligonate triacetate.

Substituent	Me–24	Me–25	Me–26	Me–27	Me–29	Me–30
△¹²-Oleanene[a]	50.5	56.5	59	69	53	53
2β-OAc[b]	+10.5	+15.5	+1.5	0	0	0
3β-OAc[a]	+3	+1	0	0	+0.5	+0.5
23-OAc[b]	−1.5	+2	+0.5	−1.5	−1	−1
28-COOMe[a]	−0.5	−1	−15.5	0	+2.5	+3
30-COOMe	0	0	−1	0	+13	
Calcd for 30-COOMe compd.	62	74	44.5	67.5	68	
29-COOMe[a]	0	+1	−0.5	0	...	+20
Calcd for 29-COOMe compd.	62	75	45	67.5		75.5
Found	62	73	44.5	67	68.5	

a, Taken from ref. 66. b, taken from ref. 67.

but they are not in accord with the values for the corresponding 29-carbomethoxy structure.

Bayogenin—Bayogenin(V), C₃₀H₄₈O₅, mp 328~330°, $[\alpha]_D^{27} = +98°$ (dioxane), was initially isolated from the wood of *Castanospermum australe*(Papilionaceae) by Eade, Simes, and Stevenson[69] of the University of New South Wales. It was found in *P. dodecandra*[26] but was not detected *Phytolacca* plants grown in Korea.
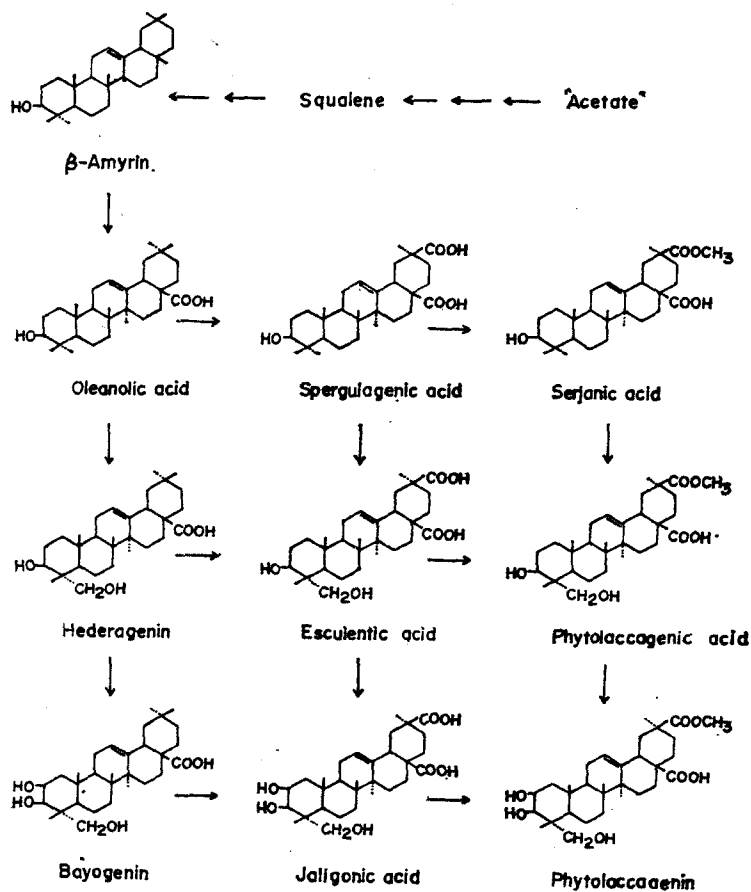
Table IX—Physical data on bayogenin derivatives.

Name	Formula	Mp	IR cm^{-1} (CHCl$_3$)	$[\alpha]_D$ (in EtOH)
Bayogenin	C$_{30}$H$_{48}$O$_5$	328~330°	1680(acid)*	+95°**
Lactone	C$_{30}$H$_{48}$O$_5$	305~308°		
Bromolactone	C$_{30}$H$_{48}$O$_5$Br	208~211°	1775(γ-lactone)	
Triacetate	C$_{36}$H$_{54}$O$_8$	257~259°	1740(ester) . 1700(acid)	+86°
Lactone triacetate	C$_{36}$H$_{54}$O$_8$	215~218°	1765(lactone) 1750(ester)	
Methylester	C$_{31}$H$_{50}$O$_5$	242~244°	1730(ester)	+84°
Methyl triacetate	C$_{37}$H$_{56}$O$_8$	196~197°	1738(ester)	+79°

* in nujol,-   ** in pyridine

Scheme 1—Plausible biogenesis of jaligonic acid.

Table IX shows physical data of bayogenin derivatives reported[70]. NMR spectrum of methyl bayogenin triacetate is very similar to that of dimethyl jaligonate triacetate since ring A is identical, showing six methyl signals at δ 0.83(3H, C-26), 0.9(6H, C-29 and 30), 1.05(3 H, C-24), 1.1 (3H, C-27), and 1.2(3H, C-25); three acetyl signals at 2.0(3H) and 2.1 (6H); a methyl ester signals at 3.65(3H); a multiplet centered at 5.4(2H) due to H-2 and H-12; a doublet centered at 4.95(1H, $J=4$) due to H-3; and the ill defined close AB system centered at 3.8(2H) assignable to the two methylene protons of the 23-acetoxymethyl group attached to C-4.

Oleanolic Acid—Oleanolic acid(I), $C_{30}H_{48}O_3$, mp 303~306°, $[\alpha]_D=+85°$ (c, 1.22 in CHCl₃), one of widespread terpenoids, was isolated from *P. dodecandra* by Powell and Whalley[26] of the University in London.

The seeds of *P. americana* contain acetyloleanolic acid, $C_{32}H_{50}O_4$, mp 290~300°, whereas traces or none of oleanolic acid were detected in this plant[20].

Plausible Biogenesis—The pentacyclic triterpene constituents isolated from *Phytolacca* species so far, differ from each other in the degree of oxidation at positions 2, 3, 23, 28, and 30. Such oxidation modifications are generally assumed to result from secondary reactions which occur after the formation of the parent β-amyrin. It would appear that in the plants, if reductive processes were not important, secondary oxidation might proceed by successive attacks, and therefore suggesting plausible genesis of the oleanane constituents as shown in scheme 1, although hederagenin has not been detected in the genus *Phytolacca*.

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