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A New Triterpene Acid from *Phytolacca esculenta* Possessing Anti-inflammatory Action

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禹源植：商陸의 抗炎症成分, Triterpene Acid 에 關한 硏究

商陸(*Phytolacca esculenta*)에서 새로운 物質 trihydroxyolean-12-ene dicarboxylic acid, $C_{30}H_{46}O_7$, m.p.318—320°, $[\alpha]_D^6 = +113.7°$ 를 分離하고 jaligonic acid라고 命名하였다. 本物質은 carrageenin으로 誘發시킨 edema形成을 强力하게 抑制하였다.

Phytolacca esculenta (Korean name, jaligong) is common plant of Korea, roots of which has been used an indigenous medicine against edema and rheumatism. However, little work has appeared in the literature regarding the chemistry of this plant. Nagai[1] first reported a toxic substance $C_{24}H_{30}O_9$ from the roots of this plant which he named "phytolaccatoxin". At a later period, however, Iwakawa[2] denied the presence of this substance in this plant.

In this communication, chemical and pharmacological investigations of this plant are briefly described.

Chemistry——The dried roots (500 g from 5.5 kg of fresh material collected in Chun-Ra-Buk-Do) were coarsely crushed and continuously extracted with Et_2O. During extraction with Et_2O a white crystalline powder was separated out. Removal of the solvent deposited some more solid. The total solid was dissolved in 10% $NaHCO_3$ solution and filtered. The filtrate was acidified with dilute HCl. The resulting precipitate was collected, washed well with H_2O and dried; yield 2.1 g. Acidic material was leached with boiling C_6H_6 until no more color was removed, and an undissolved

residue was dissolved in MeOH. When the solvent partially removed, nearly colorless product was obtained, which, after recrystallization from MeOH, gave thick prisms of the compound (0.5g), m.p. 318—320°, $[\alpha]_D^{26} = +113.7°$ (c, 0.89 in MeOH), λ_{max}^{MeOH} 206 mu(log ε 3.65), having the molecular formula $C_{30}H_{46}O_7$ [*Anal.* Calcd: C, 69.47; H, 8.94; Mol. wt., 518.70, Found: C, 68.88; H, 8.93; Mol. wt. (two carboxyl groups being assumed), 522.84 (by direct titration with 0.1 N NaOH); 512.84 (by analysis of the silver salt)].

The compound was moderately soluble in MeOH, EtOH, or dioxan, slightly soluble in Et_2O and $CHCl_3$, and practically insoluble in light petroleum or C_6H_6. It gave a pink color in Liebermann-Burchard test. The solubility, the color reaction, the specific rotation and together with the fact that it contains 30 carbon atoms support the idea that it belongs to the triterpene acid.[3] Its ir absorption spectrum showed a prominent hydroxyl peak at 3450 cm^{-1} and carboxyl peak at 1695 cm^{-1}. The compound was esterified when dissolved in MeOH and treated with ethereal diazomethane, dimethyl ester crystallized from MeOH in needles, $C_{32}H_{50}O_7$ [*Anal.* Calcd: C, 70.30; H, 9.33; CH_3O, 11.35, Found: C, 69.94; H, 9.13; CH_3O, 12.12 (Zeisel method)], m. p. 213-215°, $[\alpha]_D^{25} = +117.0°$ (c, 0.94 in MeOH), λ_{max}^{MeOH} 204 mu (log ε 3.66). Acetylation of a sample with acetic anhydride-pyridine (room temperature) or with acetic anhydride-NaAc (heating) followed by recrystallization from Et_2O led to triacetate, $C_{36}H_{52}O_{10}$ (*Anal.* Calcd: C, 67.06; H, 8.13, Found:C, 66.01; H, 8.53), m.p. 224—226°, $[a]_D^{25} = +106.0°$ (c, 0.95 in MeOH), λ_{max}^{MeOH} 204 mu (log ε 3.72).

The above methylated product was acetylated with acetic anhydride-pyridine at room temperature, to gave the dimethyl ester triacetate, but which failed to crystallize, when precipitated from MeOH solution with H_2O and dried at 110°, it was obtained as a white powder, $C_{38}H_{56}O_{10}$ (*Anal.* Calcd: C, 67.83; H, 8.39, Found: C, 66.45; H, 8.41), m.p. 115—120°. The homogeneity of this compound was checked by thin layer chromatography. This compound did not absorb in the region corresponding hydroxyl group and free carboxyl group. However, its ir spectrum showed band at 1235 cm^{-1} (acetoxy group).

The acid resisted catalytic hydrogenation over platinum oxide, but was shown to be unsaturated by means of tetranitromethane and nearly one mole of perbenzoic acid was consumed by dimethyl ester and its triacetate. Thus, these data account for all oxygen functions. The acid, molecular formula $C_{30}H_{46}O_7$, with three hydroxyl and and two carboxyl groups and a double bond appeared to be a pentacyclic triterpene.

The presence of trisubstituted double bond in compound is evident for uv absorption spectra of the acid and its derivatives.[4] Moreover, the ir spectra of the acid and its

derivatives contained bands in the 800—840cm^{-1} region. These peaks have been ascribed to a trisubstituted double bond[5] and their number and position, namely about 825, 817, and 804 cm^{-1} strongly suggested that the double bond is located in the 12:13 position of the pentacyclic triterpene.[6]

The slow rate of uptake of the per-acid was characteristic of members of the β-amyrin group possessing a hindered double bond.[7] Moreover, during oxidation with CrO_3-HAc, the dimethyl triacetate afforded an α, β unsaturated ketone (250 mu, log ε 3.92), which may, therefore, be regarded as 11-ketoolean-12-ene,[8] thus relegating the acid to group of β-amyrin series rather than the α-amyrin group.

This was confirmed by oxidation of dimethyl triacetate with selenium dioxide in glacial acetic acid leading to heteroannular diene which showed triple uv absorption maxima at 242, 251 and 260 mu (log ε 4.18, 4.23, and 4.15), typical of the 11 : 12, 13 : 18 dienes of the oleanane series.[9] On the basis of the above preliminary experiments, the new triterpene is a trihydroxyolean-12-ene dicarboxylic acid.

The triterpene acids which have been encountered so far among the Phytolaccaceae are phytolaccagenin from *P. americana*[10] and oleanolic acid and bayogenin from *P. dodecandra*[11]. These workers, however, were not able to detect the presence of the free dibasic acid in those plant. A search of the literature disclosed no description of a triterpenoid coinciding in all respects with that of the present substance. A trihydroxy dicarboxylic acid, m.p. 285°, $[\alpha]_D^{30}=+19.6°$ obtained by Sastry and Row[12] from the Et_2O extract of the wood of *Barrigtonia acutangula* and presenegenin, m. p. 310—311°, $[\alpha]_D=+91.0°$ by Dugan and de Mayo[13] from the wood of *Polygala senega* apparently has the same molecular formula as present substance, however, melting points and specific rotations reported for them differ from those of the compound isolated from *Phytolacca esculenta*. These facts suggest that it is a new triterpene acid, the author now proposes to name it as jaligonic acid. Detailed work leading to the structure elucidation of jaligonic acid will be reported elsewhere.

Pharmacology——The animals used in the experiments were adult albino rats weighing between 80 and 100 g. They were divided into groups of six animals each. The anti-inflammatory effect was investigated by means of the rat-foot edema test employing 1.0% carrageenin in 0.9% saline as phlogistics according to the method described by Winter, *et al.*[14] The drug was administered orally one hour before the carrageenin injection. The volume of the foot was measured by Harris and Spencer's method[15] before and three hours after the injection of carrageenin. Results are shown in Table I, which also includes results obtained with the hydrocortisone acetate as a reference drug. These results indicate that jaligonic acid exhibited remarkable reduction of edema formation comparable with hydrocotisone.

Table I—Anti-inflammatory activity of jaligonic acid against carrageenin-induced edema in albino rats.

Compounds	Dose mg/kg *p. o.*	Mean volume of edema *ml*±S.E.	inhibition %	P
Saline(control)	0.5m*l*	0.97±0.01	—	—
Jaligonic acid	10	0.70±0.04	27.84	<0.05
Hydrocortisone acetate	10	0.50±0.02	48.45	<0.001
Saline(control)	0.5m*l*	0.75±0.06	—	—
Jaligonic acid	40	0.43±0.09	42.67	<0.02
Hydrocortisone acetate	40	0.36±0.02	52.67	<0.001

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