

Isolation of A New Saponin from *Albizzia julibrissin*

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Abstract □ From the hydrolysate of the strong uterotonic active saponin of *Albizzia julibrissin* a new saponin, machaerinic acid methylester together with acacic acid lactone was isolated and characterized.

Keywords □ *Albizzia julibrissin*, Leguminosae, Acacic acid lactone, Machaerinic acid methylester.

Previous workers reported the isolation of quercetin-3-O-galactoside, quercetin-3-O-rhamnoside¹⁾, 3', 4', 7-trihydroxy flavone and α -spina-sterol glucoside²⁾ from *Albizzia julibrissin* (Leguminosae). In the course of our work on this plant, from the strong uterotonic active saponin fraction two saponins were isolated. We describe here the structure elucidation of the saponins.

Acid hydrolysis of the strong uterotonic active saponin fraction afforded a complex mixture of triterpenic saponins.

Separation of the individual saponins by column chromatography furnished two pure saponins named A(I) and B(XII) tentatively. Saponin A(I), C₃₀H₄₆O₄, mp 250-1°, $[\alpha]_D^{20} + 10^\circ$, showed positive Liebermann-Burchard and tetranitromethane tests and strong absorption bands at 3400(OH), 1755(γ -lactone) and 806cm⁻¹(trisubstituted double bond) in its IR spectrum. The lactone ring in the compound was opened to a free acid(II), mp 292-3°, on treatment with alkali solution and closed easily to the original compound under acidic conditions. Acetylation of I with Ac₂O/pyridine yielded a

diacetate(III), mp 235-6°, which on oxidation with SeO₂ gave a diene dione(IV), mp 310-2°, UV 231 and 287nm³⁾.

On the basis of the above preliminary experiments, the compound was supposed to be a dihydroxy olean-12-en lactone. Oxidation of I with CrO₃-pyridine complex gave a diketo lactone(V), mp 340-2°, which showed a strong negative multiple Cotton effect. The diketo lactone on treatment with alkali yielded a diketo norcompound(VI), mp 250-4°, which on acetylation with Ac₂O/pyridine at room temperature gave a diketo norcompound monoacetate(VII), mp 190-3°. These results indicated the carboxyl group was located at β -position to a keto group⁴⁾, suggesting the partial structure VIII or IX. However, the latter was precluded by the fact that a LiAlH₄ reduction product, tetrol(X), mp 330-4°, was inert to HIO₄ oxidation.

Its MS showed a molecular ion peak at m/e 470 and typical retro Diels-Alder fragmentation peaks at m/e 263(D/E ring) and 207(A/B ring). These peaks provide partial information on the presence of lactone ring and one hydroxyl group on rings D/E and one hydroxyl group on rings A/B. The highly favored loss of 45 mass units from the m/e 244 formed from RDA fragment with D/E ring by the loss of H₂O suggested that the carboxyl group forming lactone ring was located at C-17 position⁵⁾. The hydroxyl group forming the lactone ring was attached at C-21 rather than C-15 and 19 due to easy acetylation of VI under mild condition.

The latter two positions are highly resistant to the acetylation^{6,7}. The hydroxyl group forming the lactone ring must be axially oriented in order to be involved in lactone formation.

This was supported by the NMR spectra of the sapogenin A(I) and its acetate(III). The NMR spectrum of the sapogenin A showed seven angular methyl groups at 0.80, 0.90, 0.93, 1.00(3×CH₃) and 1.23ppm. Signal at 5.39ppm as a triplet-like characteristic of the proton at the β-amyrin group corresponding to Δ¹²-olefinic proton⁸. The lactonic proton signal appeared at 4.23ppm as a doublet (J=6Hz) which did not shift on acetylation.⁵ A methine proton signal at 4.03ppm downfield from 3-H signal(3.23ppm) as a doublet of doublets(J=5 & 12Hz) assigned 16β-H⁹. Both signals shifted to 5.02 and 4.47 ppm respectively on acetylation.

The mentioned spectral data coupled with physicochemical data were completely identical to reported data of acacic acid lactone⁹.

Sapogenin B(XI), C₃₁H₅₀O₄, mp 222-4°, [α]_D²⁰+80°, gave positive results in Liebermann-Burchard and tetranitromethane tests. The IR spectrum showed absorptions at 3240(OH), 1724 (ester) and 805cm⁻¹(trisubstituted double bond) which assumed that sapogenin B(XI) belongs to a triterpene group having methylester group in its structure. The sapogenin B on acetylation with Ac₂O/pyridine at room temperature gave a diacetate(XII), mp 225-30° and easily saponified with alkali to yield free acid(XIII), mp 312-8°, which on acetylation at room temperature afforded a demethylated diacetate(XIV), mp 246-9°.

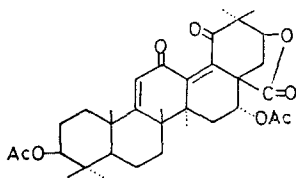
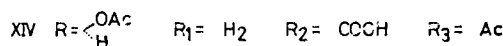
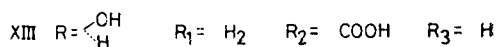
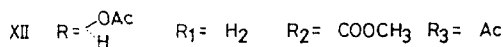
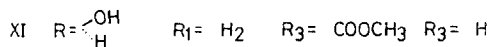
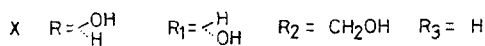
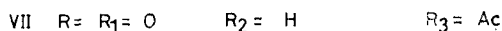
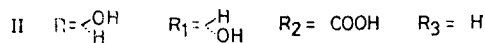
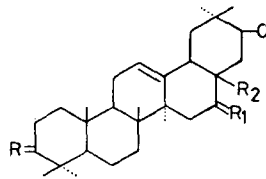
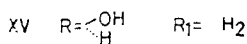
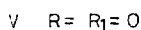
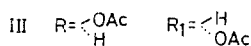
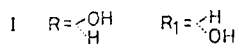
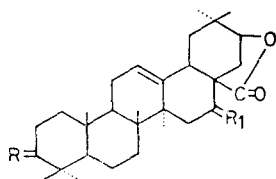
The MS spectrum of sapogenin B(XI) showed molecular ion peak at m/e 486 with sequential losses of two H₂O and one methylester groups at m/e 468, 450 and 426. The molecular ion underwent a typical RDA fragmentation to

furnish a characteristic ion peaks at m/e 278 (D/E ring) and m/e 207(A/B ring). This result indicated that sapogenin B has an α-amyrin or a β-amyrin skeleton with one hydroxyl group and one carbomethoxyl group at rings D/E and one hydroxyl group at rings A/B¹⁰. The base peak at m/e 201 must be formed from RDA fragment with D/E ring by the loss of carbomethoxyl group suggesting the presence of carbomethoxyl group at C-17^{10,11}.

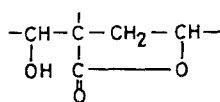
The unusually facile saponification of the methylester at C-17 and easy acetylation of hydroxyl group assumed that the hydroxyl group at rings D/E was located at C-21 with equatorial configuration^{12,13}. This assumption was supported by the γ-lactone(XV), mp 239-42°, formation of the free acid(XIII) on refluxing with acid.

From the above results, structure of sapogenin B was elucidated as 3β, 21β-dihydroxy olean-12-en-28-oic acid methylester(machaerinic acid methylester)¹³.

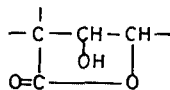
This structure was supported by the NMR spectrum of sapogenin B acetate(XII). The NMR spectrum showed seven angular methyl groups at 0.72, 0.87(3×CH₃), 0.93, 0.98 and 1.13ppm, two acetyl groups at 2.02 and 2.04 ppm and one carbomethoxyl group at 3.62ppm. The chemical shifts for the angular methyl groups were in excellent agreement with the values(0.72, 0.87, 0.88, 0.88, 0.94, 0.99 and 1.14ppm for C-26, 29, 23, 24, 25, 30 and 27, respectively) calculated¹⁴ and appearance of the highest angular methyl signal in upfield region from 0.77ppm supported the presence of carbomethoxyl group at C-28⁹. The signals at 4.48ppm as a triplet(J=7Hz) and at 5.33ppm as a triplet-like characteristic of the protons at the β-amyrin group corresponding to C-3 and Δ¹²-olefinic proton respectively⁸. The signal at 4.78ppm as a doublet of doublets(J=6 & 11Hz) was ass-



IV



VIII



IX

igned axially oriented C-21 methine proton⁵⁾.

Machaerinic acid was first isolated from *Machaerocereus gummosus* in 1955¹³⁾. However, the occurrence of machaerinic acid methylester in nature has not yet been reported.

EXPERIMENTAL METHODS

The mps were taken on a Mitamura-Riken apparatus and are uncorrected. The UV spectrum was run on a Shimadzu model MPS-50L recording spectrophotometer and the IR spectra

were determined in KBr tablets on a Beckman model IR-20A spectrophotometer. The NMR spectra were recorded on a Varian model EM-360 spectrometer with TMS as internal standard. Mass spectra were obtained with Hewlett Packard model 5985B GC/MS spectrometer. Optical rotations were obtained on a Perkin Elmer model 243 polarimeter and CD spectrum was run on a JASCO model J-20 spectrophotometer.

Isolation of the Sapogenins

The powdered stem bark of *A. julibrissin*

(8kg) was refluxed with MeOH. The MeOH extract(1.1kg) was partitioned with hexane (132g), CHCl₃(173g), ethylacetate(25g) and EtOH(32g) successively. The biologically active saponin fraction, EtOH soluble portion, was hydrolyzed with 5% H₂SO₄ in 50% dioxane solution for 5hr. The solution was concentrated to a half under reduced pressure and added to ice-water solution. The precipitate was filtered, washed with water and dried to give a brown solid, which was chromatographed over SiO₂ column eluting with benzene-ether(4:1) to give saponin A and B. The saponin A(I) was crystallized from MeOH as needles.

mp 256-1°, $[\alpha]_D^{20} + 10^\circ$ (C=0.04, MeOH)

LB and TNM tests: positive.

IR ν_{\max}^{KBr} cm⁻¹ 3400(OH), 1755(γ -lactone), 806(trisubstituted double bond).

NMR(CDCl₃, TMS) δ 0.80(3H, s, CH₃), 0.90(3H, s, CH₃), 0.93(3H, s, CH₃), 1.00(9H, s, 3 \times CH₃), 1.23(3H, s, CH₃), 3.23(1H, t, J=8Hz, 3-H), 4.03(1H, dd, J=5 & 12Hz, 16-H), 4.23(1H, d, J=6Hz, 21-H), 5.39(1H, t-like, 12-H) MS m/e(%) 470(M⁺, 1.2), 452(M⁺-H₂O, 2.3), 263(D/E ring+H, 29), 262(D/E ring, 8.4), 245(D/E ring+H-H₂O, 20.7), 244(D/E ring-H₂O, 64.5), 208(A/B ring+H, 24.3), 207(A/B ring, 37.5), 199(244-COOH, 18.0), 190(208-H₂O, 100), 189(A/B ring-H₂O, 27.5), 175(190-CH₃, 48.4).

Saponin B(XI) was crystallized from MeOH as needles.

mp 222-4°, $[\alpha]_D^{20} + 80^\circ$ (C=0.1, MeOH)

LB and TNM tests: positive.

IR ν_{\max}^{KBr} cm⁻¹ 3240(OH), 1724(ester), 805 (trisubstituted double bond).

MS m/e(%) 486(M⁺, 1.9), 468(M⁺-H₂O, 8.3), 450(M⁺-2H₂O, 6.0), 426(M⁺-CH₃COOH, 4.7), 408[M⁺-(CH₃COOH+H₂O), 5.1], 391(450-CH₃COO, 3.9), 278(D/E ring, 3.2), 260

(278-H₂O, 38.5), 247(43.4), 208(A/B ring+H, 5.5), 207(A/B ring, 17.7), 201[278-(H₂O+CH₃COO), 100], 190(208-H₂O, 33.9), 189(207-H₂O, 27.9), 175(190-CH₃, 26.7).

Alkaline Hydrolysis of I

A sample(100mg) of I was refluxed with 10% alcoholic potassium hydroxide for 4hr and the volume was reduced to a half in vacuo. The reaction mixture was poured onto crushed ice, acidified with d-HCl and filtered. The filtrate was crystallized from MeOH in cold room to yield II as colorless needles. mp 292-3°

IR ν_{\max}^{KBr} cm⁻¹ 3480, 3380(CH), 1700(acid).

It was easily lactonized in acidic solution to yield original saponin(I).

Acetylation of I

To the saponin A(50mg) Ac₂O and pyridine(1ml each) was added and allowed to stand at room temperature overnight. The reaction mixture was poured onto crushed ice and filtered. The filtrate was crystallized from MeOH-H₂O to give III as colorless needles.

mp 235-6°, $[\alpha]_D^{20} - 23.8^\circ$ (C=0.042, MeOH).

IR ν_{\max}^{KBr} cm⁻¹ 1770(γ -lactone), 1725, 1240, 1220 (acetate).

NMR(CDCl₃, TMS) δ 0.82(6H, s, 2 \times CH₃), 0.89(3H, s, CH₃), 0.96(3H, s, CH₃), 0.98(3H, s, CH₃), 1.18(3H, s, CH₃), 1.20(3H, s, CH₃), 1.94(3H, s, acetyl), 2.00(3H, s, acetyl), 4.17(1H, d, J=6Hz, 21-H), 4.47(1H, t, J=8Hz, 3-H), 5.02(1H, dd, J=5 & 12Hz, 16-H), 5.40(1H, m, 12-H).

SeO₂ Oxidation of III

A solution of III(10mg) and freshly prepared SeO₂(10mg) in HOAc(1ml) was refluxed for 3hr. The solution was filtered, diluted with water and extracted with ether. The residue was crystallized from MeOH to yield IV as pale yellowish needles.

mp 310-2°, UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm(log ϵ) 231

(4.25), 287(4.25).

CrO₃ Oxidation of I

A sample(100mg) of I in pyridine(5ml) was added to CrO₃-pyridine complex(CrO₃ 100mg+pyridine 2ml) and allowed to stand at room temperature overnight. The reaction mixture was poured onto crushed ice and filtered. The filtrate was crystallized from MeOH to give V as needles.

mp 340-2°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1788(γ -lactone), 1720, 1703 (C=O).

CD $A_{\epsilon 291}$ -2.28(MeOH, C=0.033), $A_{\epsilon 298}$ -2.49, $A_{\epsilon 307}$ -2.07.

Decarboxylation of V

Compound V(50mg) was refluxed with 2g of KOH and 20ml of Me OH for 3hr. The reaction mixture was diluted with water, extracted with ether and crystallized from MeOH to yield VI as amorphous powder.

mp 250-4°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3360(OH), 1710, 1698(C=O).

MS m/e(%) 440(M⁺, 4.3), 422(M⁺-H₂O, 24.8), 407(422-CH₃, 3.6), 379(407-CO, 18.9), 354[M⁺-(C₆H₈O+2H), 12.3], 234(D/E ring, 34.4), 216(234-H₂O, 32.8), 205(A/B ring, 23.4), 201(216-CH₃, 50.0), 173(201-CO,100).

Acetylation of VI

The acetate of VI was prepared as described above and crystallized from MeOH.

mp 190-3°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1730, 1220(acetate), 1710, 1700(C=O).

LiAlH₄ Reduction of I

A sample of I(100mg) was dissolved in dry tetrahydrofuran(100ml) and LiAlH₄(200mg) was added slowly with stirring and then refluxed for 3 hr. The reaction mixture was filtered, concentrated and subjected to SiO₂ column chro-

matography eluting with benzene-ether(4:1). The reaction product was crystallized from MeOH-CHCl₃ to yield X as plate.

mp 330-4°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3380, 3300, 1095, 1070, 1030 (OH), 1625, 820(trisubstituted double bond).

The tetrol(X) did not oxidized with HIO₄.

Acetylation of XI

Treatment of XI(30mg) as described in acetylation of I and crystallized from MeOH afforded XII as needles.

mp 225-30°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 1730, 1235(acetate).

NMR(CDCl₃, TMS) δ 0.72(3H, s, CH₃), 0.87(9H, s, 3×CH₃), 0.93(3H, s, CH₃), 0.98(3H, s, CH₃), 1.13(3H, s, CH₃), 2.02(3H, s, acetyl), 2.04(3H, s, acetyl), 2.93(1H, dd, J=6 & 14 Hz, 18-H), 3.62(3H, s, CH₃CO), 4.48(1H, t, J=7Hz, 3-H), 4.78 (1H, dd, J=6 & 11Hz, 21-H), 5.33(1H, t-like, 12-H).

MS m/e(%) 570(M⁺, 0.12), 510(M⁺-CH₃COOH, 5.0), 450(M⁺-2CH₃COOH, 7.1), 260(D/E ring-CH₃COOH, 22.7), 201(260-CH₃COO, 50.2), 200(260-CH₃COOH, 17.9), 190[(A/B ring+H)-CH₃COOH, 16.1], 189(A/B ring-CH₃COOH, 14.7).

Alkaline Hydrolysis of XI

The sapogenin B(15mg) was refluxed with 10 % ethanolic KOH for 8hr and followed by the usual work-up. It was crystallized from MeOH to give a free acid(XIII) as needles.

mp 312-8°

IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ 3430(OH), 1700(acid), 820, 810(trisubstituted double bond).

Acetylation of XIII

The free acid(XIII, 10mg) was acetylated as described in sapogenin B and followed by the usual work-up. It was crystallized from MeOH-

H₂O to afford a diacetate(XIV) as needles.

mp 246-9°

IR ν_{\max}^{KBr} cm⁻¹ 1736, 1240(acetate), 1700

(acid).

Lactonization of XIII

The free acid(XIII, 10mg) was treated under reflux with 5% H₂SO₄ in dioxane for 3hr. The solution was concentrated to a half in vacuo, poured onto crushed ice and filtered. The filtrate was crystallized from MeOH to give lactone (XV) as needles.

mp 239-42°

IR ν_{\max}^{KBr} cm⁻¹ 3450(OH), 1755(γ -lactone).

NMR(CDCl₃, TMS) δ 0.75(3H, s, CH₃), 0.81(3H, s, CH₃), 0.91(3H, s, CH₃), 1.03(9H, s, 3 \times CH₃), 1.10(3H, s, CH₃), 3.30(1H, m, 3-H), 4.20(1H, d, J=6Hz, 21-H), 5.40(1H, m, 12-H).

ACKNOWLEDGMENT

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