

**Structure of Desacyl-jegosaponin, a Common Desacyl Derivative of
Jegosaponin isolated from Pericarps of *Styrax
japonica* SIEB. et ZUCC.**

Previously, we reported that jegosaponin isolated from the pericarps of *Styrax japonica* SIEB. et ZUCC. comprises several saponins whose aglycones are the acylated (acetyl, tigloyl, or 2'-*cis*-hexenoyl) derivatives of barringtogenol C (I) on the basis of chemical evidence¹⁾ and soil bacterial hydrolysis study.²⁾ Due to difficulty in the separation of each saponin component of jegosaponin and based on a finding that alkaline hydrolysis of jegosaponin gives rise to a common desacyl derivative as a single product, we have conducted structure elucidation of the desacyl derivative now named desacyl-jegosaponin. The present communication provides chemical evidence supporting a formulation (II) for desacyl-jegosaponin.

On acid hydrolysis, desacyl-jegosaponin (II), $C_{54}H_{88}O_{25} \cdot H_2O$,³⁾ mp 248—251° (EtOH-H₂O), $[\alpha]_D^{20} -12.0^\circ$ (MeOH), infrared spectrum (IR) $\nu_{max}^{KBr} \text{ cm}^{-1}$: 3400 (OH), 1730 (COOH), yielded barringtogenol C (I) and one mole each of D-glucuronic acid, D-glucose, D-galactose, and L-rhamnose.⁴⁾ Mild acid hydrolysis of II with 1N H₂SO₄-EtOH (1:2) yielded three prosapogenols designated as DJ-1, DJ-2, and DJ-3.

DJ-1 (III), $C_{38}H_{62}O_{11} \cdot 2H_2O$, mp 199—201° (CHCl₃-MeOH), $[\alpha]_D^{20} -12.0^\circ$ (MeOH), IR $\nu_{max}^{KBr} \text{ cm}^{-1}$: 3425 (OH), 1730 (COOEt),⁵⁾ is a glucuronide of I as revealed by acid hydrolysis. On methylation by Hakomori's method,⁶⁾ it gave an octa-O-methyl derivative (IIIa), which shows no hydroxyl absorption band in its IR spectrum. The proton magnetic resonance (PMR) spectrum of IIIa (CDCl₃) shows a doublet at δ 4.34 ($J=8$ Hz) assignable to an anomeric proton which indicates the presence of β -glucuronopyranoside linkage in IIIa. DJ-2 (IV) (amorphous), $[\alpha]_D^{20} -14.0^\circ$ (MeOH), IR $\nu_{max}^{KBr} \text{ cm}^{-1}$: 3425 (OH), 1740 (COOEt), comprises I and one mole each of D-glucuronic acid and D-glucose as shown by acid hydrolysis. Permethylation of IV as above gave an undeca-O-methyl derivative (IVa), which, on LiAlH₄ reduction, yielded a product (IVb), $C_{52}H_{90}O_{15}$ (amorphous), $[\alpha]_D^{20} +4.4^\circ$ (CHCl₃), IR $\nu_{max}^{CCl_4} \text{ cm}^{-1}$: 3400 (OH), no COOMe. The PMR spectrum of IVb (CDCl₃, δ) shows the presence of ten methoxyls: 3.28 (6H, s), 3.36 (3H, s), 3.51 (6H, s), 3.54 (6H, s), 3.58 (3H, s), 3.63 (6H, s), an olefinic proton: 5.28 (1H, m, C₍₁₂₎H), and two anomeric protons: 4.38 (1H, d, $J=7$ Hz), 4.67 (1H, d, $J=7$ Hz), the latter signals being indicative of that both glucuronic acid and glucose residues in DJ-2 are connected with β -linkage. Methylated carbohydrate components of IVb obtained by methanolysis were identified with methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC), while a methylated aglycone obtained in the same procedure was acetylated with Ac₂O and pyridine to give an acetate (Ia), $C_{36}H_{60}O_6$ (amorphous), $[\alpha]_D^{20} +20.0^\circ$ (CHCl₃), IR $\nu_{max}^{CCl_4} \text{ cm}^{-1}$: no OH, 1750 (acetate), whose structure has been assigned on the basis of its physicochemical properties. Thus, the PMR spectrum (CDCl₃, δ) shows the presence of four methoxyls: 3.27—3.56 (totally 12H), one acetoxyl: 2.04 (3H, s), one olefinic proton: 5.29 (1H, m, C₍₁₂₎H), and one carbonyl proton geminal to an acetoxyl: 4.53 (1H, t-like), attributable to C₍₃₎ α -H.⁷⁾ The mass spectrum of Ia (M^+ : m/e 588, M^+ -AcOH: m/e 528) gives

1) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353.

2) I. Yosioka, S. Saijoh, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 564 (1972).

3) All compounds given with the chemical formulae gave the satisfactory analytical values.

4) The carbohydrate composition has been revised from the previous report¹⁾ by the recent investigation.

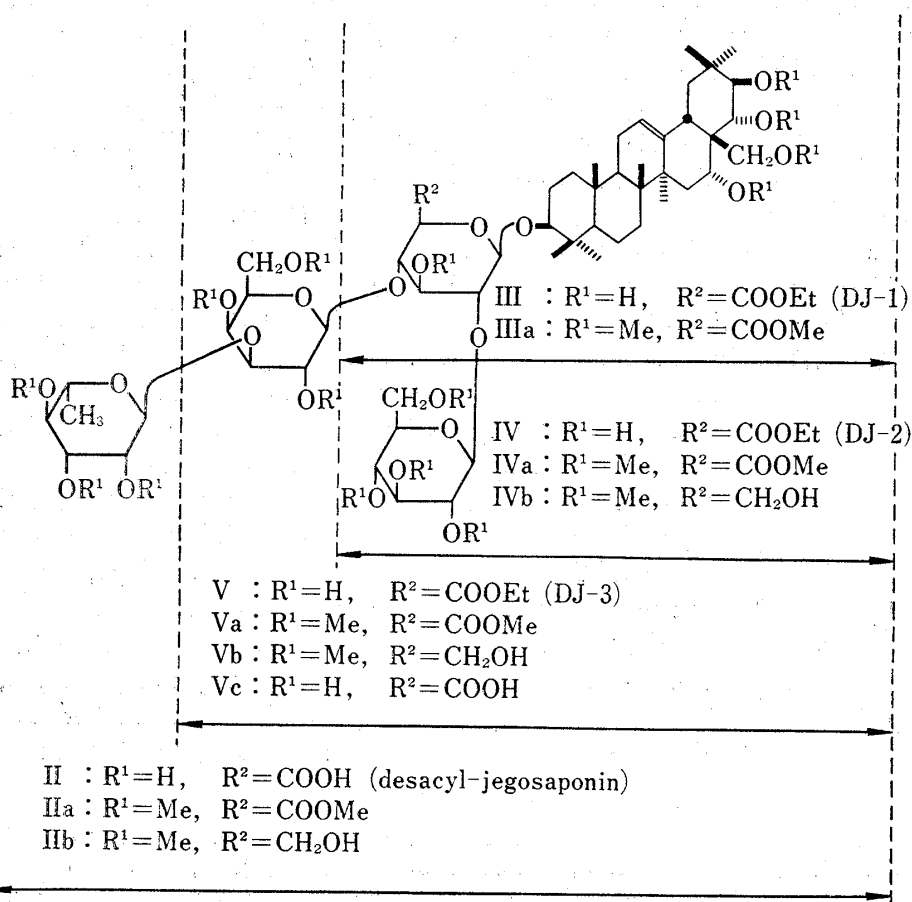
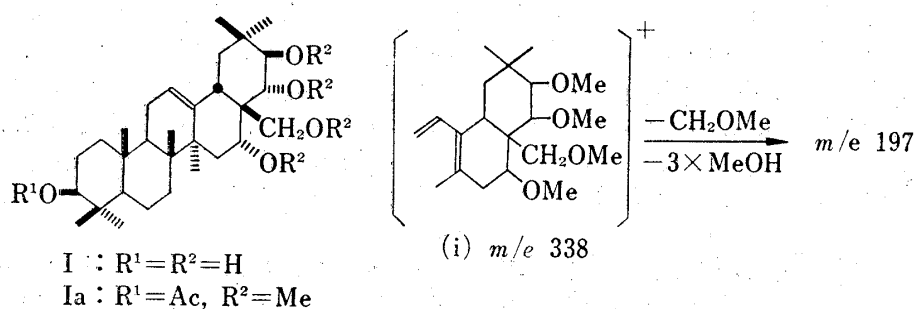
5) The ethyl function was introduced during ethanolic acid hydrolysis.

6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

7) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).

additional evidence by two ion peaks appeared at m/e 338 (i) and 197, which are derived through a reverse Diels–Alder type fragmentation of the ring C.^{7,8)} The structure of DJ-2 is therefore assigned IV.

DJ-3(V), $C_{50}H_{82}O_{21}$, mp 279–282° (EtOH–H₂O), $[\alpha]_D^{25} -4.7^\circ$ (MeOH), IR $\nu_{\max}^{KBr} \text{ cm}^{-1}$: 3460–3360 (br, OH), 1736 (COOEt), is a galactoside of DJ-2 (IV). On methylation as above, DJ-3 gave a tetradeca-O-methyl derivative (Va), $C_{62}H_{106}O_{21}$ (amorphous), $[\alpha]_D^{25} -6.3^\circ$ (CHCl₃) IR $\nu_{\max}^{CCl_4} \text{ cm}^{-1}$: no OH, 1755 (COOMe), whose PMR spectrum (CDCl₃+C₆D₆, δ) shows the presence of three anomeric protons: 4.50 (1H, d, $J=7$ Hz), 4.86 (1H, d, $J=7$ Hz), 4.97 (1H, d, $J=7$ Hz), one olefinic proton: 5.26 (1H, m, C₍₁₂₎H), and fourteen methoxyls: 3.21–3.59 (totally 42H). The coupling pattern of the signals due to the anomeric protons substantiates β -linkage of three carbohydrate components. Methanolysis of a reduction product (Vb), obtained by LiAlH₄ treatment of Va, yielded methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl



8) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 121.

2,3,4,6-tetra-O-methyl-D-galactopyranoside, and methyl 3-O-methyl-D-glucopyranoside (identified by GLC and TLC) in addition to a methylated aglycone which, after acetylation, was identified with Ia. Therefore, DJ-3 is formulated as V.

Finally, the structure of desacyl-jegosaponin (II) has been determined as described below. On permethylation as above, II gave a hexadeca-O-methyl derivative (IIa), $C_{70}H_{120}O_{25}$, mp 189–191° (EtOH-H₂O), $[\alpha]_D^{25} -26.7^\circ$ (CHCl₃), IR $\nu_{max}^{CCl_4} cm^{-1}$: no OH, 1755 (COOMe), whose PMR spectrum (CDCl₃+C₆D₆, δ) shows the presence of sixteen methoxyls: 3.09–3.66 (totally 48H) and four anomeric protons: 4.47 (1H, d, $J=7$ Hz), 4.96 (1H, d, $J=7$ Hz), 5.07 (1H, d, $J=7$ Hz), 5.29 (2H, br. s, overlapped with C₍₁₂₎H). A reduction product of desacyl-jegosaponin (IIb), which was obtained by LiAlH₄ treatment, yielded on methanolysis methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, methyl 2,4,6-tri-O-methyl-D-galactopyranoside, methyl 3-O-methyl-D-glucopyranoside (each identified by GLC and TLC), and the same methylated aglycone as in the case of DJ-3 (V) (identified as its monoacetate (Ia)). The foregoing accumulated evidence has led us to formulate desacyl-jegosaponin as II. The orientation in the linkage of each carbohydrate moiety was also corroborated by the application of Klyne's rule⁹⁾: for example, $[M]_D$ (II)– $[M]_D$ (V) = –88.5°, $[M]_D$ (II)– $[M]_D$ (Vc)¹⁰⁾ = –120.5°, $[M]_D$ (methyl α -L-rhamnopyranoside) = –109°, and $[M]_D$ (methyl β -L-rhamnopyranoside) = +169°.¹¹⁾

Elucidation of the structure of desacyl-jegosaponin (II), which possesses a glucuronide moiety directly attached to the aglycone (I) and was already shown to be cleaved by ultraviolet irradiation,^{12a)} provides an additional chemical support for the photolytic cleavage of uronide linkage in saponin which has been recently developed in our laboratory.¹²⁾

We would like to express our sincere thankness to Prof. T. Kawasaki (Kyushu University), Prof. J. Shoji (Showa University), and Prof. M. Tomoda (Kyoritsu College of Pharmacy) for their generous gifts of authentic methylated carbohydrate specimens, and to the Res. Lab. of Dainippon Pharm. Co. for elemental analyses.

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Received February 23, 1974

9) W. Klyne, *Biochem. J.*, **47**, xli (1950).

10) Prepared by alkaline treatment of V for comparison of the optical data.

11) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1972).

12) a) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. & Ind.*, **1973**, 276; b) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, **1973**, 3997; c) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1339 (1974).