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Saponin and Sapogenol. XX.[1] Selective Cleavage of the Glucuronide Linkage in Saponin by Acetic Anhydride and Pyridine Treatment

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Following the previously described two selective cleavage methods (*i.e.* photolysis and lead tetraacetate degradation) for the glucuronide linkage in the oligoglycosides, it has been found that acetic anhydride and pyridine treatment is also an effective method to selectively cleave the glucuronide-saponin at the glucuronide moiety. The degradation method has been shown to be different from ordinary acetolysis and by the present method, the glucuronide-saponin, which possesses a free carboxyl function and two carbohydrate residues at C-2′ and C-4′ in its glucuronide moiety, is decomposed to furnish the acetylated derivatives of the genuine sapogenol and those of the carbohydrate residues. The reaction pathway initiated by mixed anhydride formation and decarboxylative elimination has been presumed.

Keywords——selective cleavage of glucuronide linkage; acetic anhydride and pyridine treatment; oligoglycoside; glucuronide-saponin; PMR; sakuraso-saponin; desacyl-jegosaponin; soyasaponin I; chikusetsusaponin

During the course of studies searching for new cleavage methods by which certain glycoside linkages in oligoglycosides are selectively cleaved, we have found two degradation methods which effect selective cleavage of the glucuronide linkage in saponin. The one is photolysis[3] and the other is lead tetraacetate oxidation followed by alkali treatment.[1,4] By both methods, a glucuronide-saponin[1] (=the abbreviation for a saponin possessing a glucuronide moiety in the oligosaccharide portion directly attached to the sapogenol) is split at the glucuronide moiety to liberate the sapogenol and the carbohydrate residues.

Recently, we have found that acetic anhydride and pyridine treatment is also an effective method to selectively cleave the glucuronide-saponin at the glucuronide moiety, and the present paper provides details of the results.[5] By the present method, a glucuronide-saponin, which possesses a free carboxyl function and two carbohydrate residues at C–2′ and C–4′ in its glucuronide moiety, is decomposed to furnish acetylated derivatives of the genuine sapogenol and the carbohydrate residues.

Degradation of Sakuraso-saponin (1)

In the course of the structure study of sakuraso-saponin (**1**),[6] we noticed that treatment of **1** in acetic anhydride and pyridine under reflux resulted in the liberation of 3,16-di-O-

1) Part XIX: I. Kitagawa, M. Yoshikawa, K.S. Im, and Y. Ikenishi, *Chem. Pharm. Bull.* (Tokyo), **25**, 657 (1977).
2) Location: *133–1, Yamada-kami, Suita, Osaka, 565, Japan*; *a*) Present address: *Shionogi Research Laboratory, Shionogi and Co., Ltd., Fukushima-ku, Osaka, 553, Japan*; *b*) Present address: *College of Pharmacy, Busan National University, Busan, 601–02, Korea.*
3) *a*) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. and 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).
4) I. Kitagawa, M. Yoshikawa, Y. Ikenishi, K.S. Im, and I. Yosioka, *Tetrahedron Letters*, **1976**, 549.
5) Presented at the 19th Symposium on the Chemistry of Natural Products held at Hiroshima, Oct. 25—27 th, 1975, Symposium paper, p. 231.
6) I. Kitagawa, Y. Ikenishi, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 2470 (1976).

acetyl-protoprimulagenin A (**2**) in a 60% yield and that the desired peracetate of **1** could not be obtained.[7] In addition to **2**, 1,2,3,4,6-penta-O-acetyl-glucopyranose (**3**, in a 78% yield) and trisaccharide nonaacetate (**4**, in a 71% yield) were isolated as respective anomeric mixtures from the total reaction product.

The structures of **2** and **3** have been elucidated from their spectral data and the respective deacetylation leading to protoprimulagenin A (**5**)[8] and glucose. The infrared (IR) spectrum of **4** shows acetoxyl absorption bands (1760, 1216 cm^{-1}) but no hydroxyl absorption band. In the proton magnetic resonance (PMR) spectrum of **4**, are observed one six-proton doublet (δ 1.23, J=6 Hz) due to two rhamnose-methyls and two duoblets (δ 5.62, J=7 Hz and δ 6.29, J=4 Hz) of totally one-proton intensity which are respectively assignable to the anomeric proton of galactoside at the reducing terminal in the anomeric mixture.

Treatment of **4** with sodium methoxide in methanol furnished a trisaccharide (**6**, an anomeric mixture) which, on acid hydrolysis, was decomposed to give galactose and rhamnose. The nona-O-methyl derivative (**7**) obtained as a major product by methylation of **6** with silver oxide-methyl iodide contains no free hydroxyl as shown by the IR spectrum. In the PMR spectrum of **7**, are observed the signals due to two rhamnose-methyls (δ 1.24, 6H, d, J=6 Hz), an anomeric proton of galactoside (δ 4.71, 1H, d, J=4 Hz), and two anomeric protons of the rhamnoside moieties (δ 4.84, 1H, d, J=1 Hz and δ 5.01, 1H, d, J=2 Hz). On methanolysis, **7** furnished methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 3,4-di-O-methyl-rhamnopyranoside, and methyl 3,4,6-tri-O-methyl-galactopyranoside. Based on these findings and comparison with the total structure of sakuraso-saponin (**1**),[6] the structure of the trisaccharide nonaacetate has been assigned **4**.

It has been clarified that, by acetic anhydride and pyridine treatment, the glucuronide-saponin such as sakuraso-saponin (**1**) is readily decomposed to afford its genuine sapogenol and carbohydrate residues attached to the glucuronide moiety as their acetylated derivatives such as **2**, **3**, and **4**

As for cleavage of the glycoside linkage using acetic anhydride as the reagent, acetolysis[9] with acetic anhydride accompanied by an acid catalyst has often been undertaken. In order to compare the present method with ordinary acetolysis, sakuraso-saponin (**1**) was subjected to boron trifluoride (BF_3) etherate–acetic anhydride–acetic acid treatment.[10] In this case, **1** furnished 3,16,28-tri-O-acetyl-primulagenin A (**8**) along with **3** and **4**, and the significant difference between the two procedures was that the acetylated genuine sapogenol (**2**) was liberated by the acetic anhydride–pyridine method while the acetylated artifact sapogenol (**8**) was obtained by the acetic anhydride-BF_3 etherate method. Therefore, it is worth mentioning that by the present acetic anhydride–pyridine method, an acid-labile moiety in the sapogenol portion such as the 13β, 28-oxide ring in protoprimulagenin A (**5**) can be kept intact during the procedure and the acetylated genuine sapogenol **2** is liberated.

The above described analysis of the reaction products has led us to presume that the acetic anhydride–pyridine degradation initially occurs at the glucuronide moiety and liberates the substituents at C–1′, C–2′ and C–4′. However, identification of the reaction product originating from the glucuronide portion has not yet been performed.

Structure Requirement for the Degradation

In order to elucidate the reaction pathway of the present degradation method, the role

7) Acetylation at room temperature affords a mixture (*ca.* 1: 1) of the peracetate (=tetradecaacetate) and tridecaacetate (with free 16α-OH), and acetylation under forced conditions results in degradation.
8) I. Kitagawa, A. Matsuda, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2226 (1972).
9) *a*) R.D. Guthrie and J.F. McCarthy, *Advan. Carbohyd. Chem.*, **22**, 11 (1967); *b*) B. Lindberg, J. Lönngren, and S. Svensson, *Advan. Carbohyd. Chem. and Biochem.*, **31**, 185 (1975).
10) U. Ohsawa, H. Kimura, and K. Igarashi, The 25th Annual Meeting of the Kinki Branch of Pharmaceutical Society of Japan, Nov. 15, 1975, Kobe, Abstract Paper, p. 34.

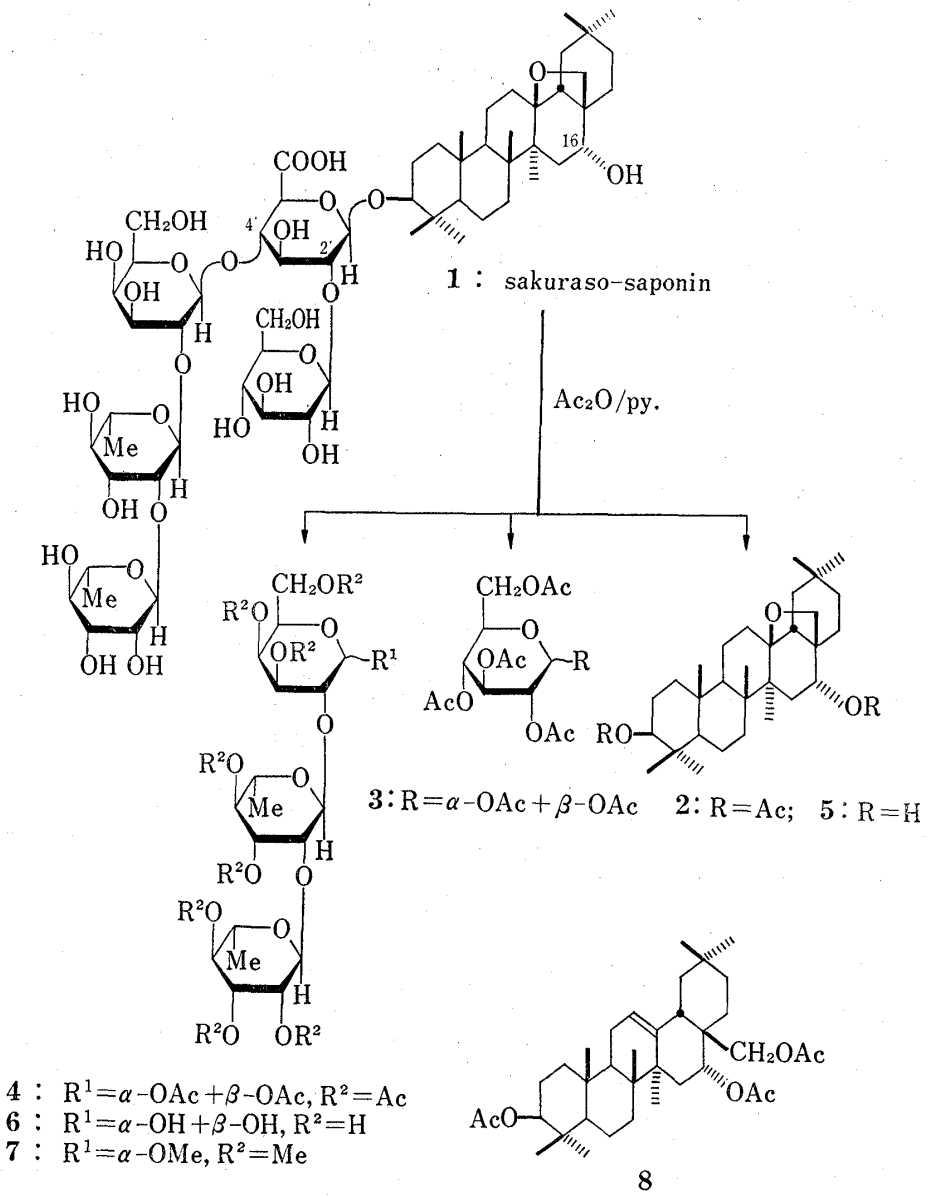


Chart 1

of both reagents (acetic anhydride and pyridine) has been examined. Thus, sakuraso-saponin (**1**) was treated as above with the acetic anhydride-pyridine mixtures of various molar ratios and it has been found that mixtures with ratios between 10: 1 and 1: 10 could realize the degradation, and that lesser amounts of either reagent give rise to unsatisfactory results (see Experimental). In addition, treatment with pyridine only was shown not to affect the parent saponin, whereas treatment with acetic anhydride only afforded a mixture of the acetylated saponins.

Next, the role of free carboxyl group in the glucuronide portion has been investigated. When a methyl ester (**9**), which was obtained previously by methanolic hydrogen chloride treatment of sakuraso-saponin (**1**),[6] was heated with acetic anhydride and pyridine under reflux for 24 hours, no degradation product was obtained but instead the peracetate (**10**) was formed. On the other hand, when a carboxylic acid (**11**) prepared from **9** by alkaline hydrolysis was subjected to the acetic anhydride-pyridine degradation, it was decomposed to afford 3,16,28-tri-O-acetyl-primulagenin A (**8**) (53%), **3**(65%), and **4** (51%), the necessity of the presence of a free carboxyl group being thus demonstrated.

It has become clear that the present degradation method differs from ordinary acetolysis

and is effective for cleavage at the glucuronide moiety having a free carboxyl function and also that both acetic anhydride and pyridine are indispensable to effect the reaction. In regard to the initial stage of the degradation, since the methyl ester **9** was unaffected, the decarboxylation reaction[11] seems to be more likely than the β-elimination reaction[9b,12] which is known to occur often in base catalyzed degradation of glucuronide containing polysaccharide.

The structure requirement in the glucuronide moiety for ready degradation has been investigated. At first, desacyl-jegosaponin (**12**),[13] which carries two carbohydrate residues at C-2′ and C-4′ of its glucuronide moiety as in **1** and **11**, was subjected to the degradation. In this case, two acetates, *i.e.* 3,21,22,28-tetra-O-acetyl-barringtogenol C (**13**)[14] (in a 49% yield) and 3,16, 21,22,28-penta-O-acetyl-barringtogenol C (**14**)[14] (12%), were obtained from the sapogenol portion, while 1,2,3,4,6-penta-O-acetyl-glucopyranose (**3**, 60%) and two anomers of the heptaacetates of rhamno-galactose (**15**, 35% and **16**, 28%) were isolated from the oligosaccharide portion.

In the IR spectrum of **15**, no hydroxyl absorption band is observed but bands due to acetoxyl are observed at 1760 and 1217 cm^{-1}. The PMR spectrum of **15** shows the signals assignable to a rhamnose-methyl (δ 1.16, 3H, d, J=6 Hz), seven acetoxyls, and two anomeric

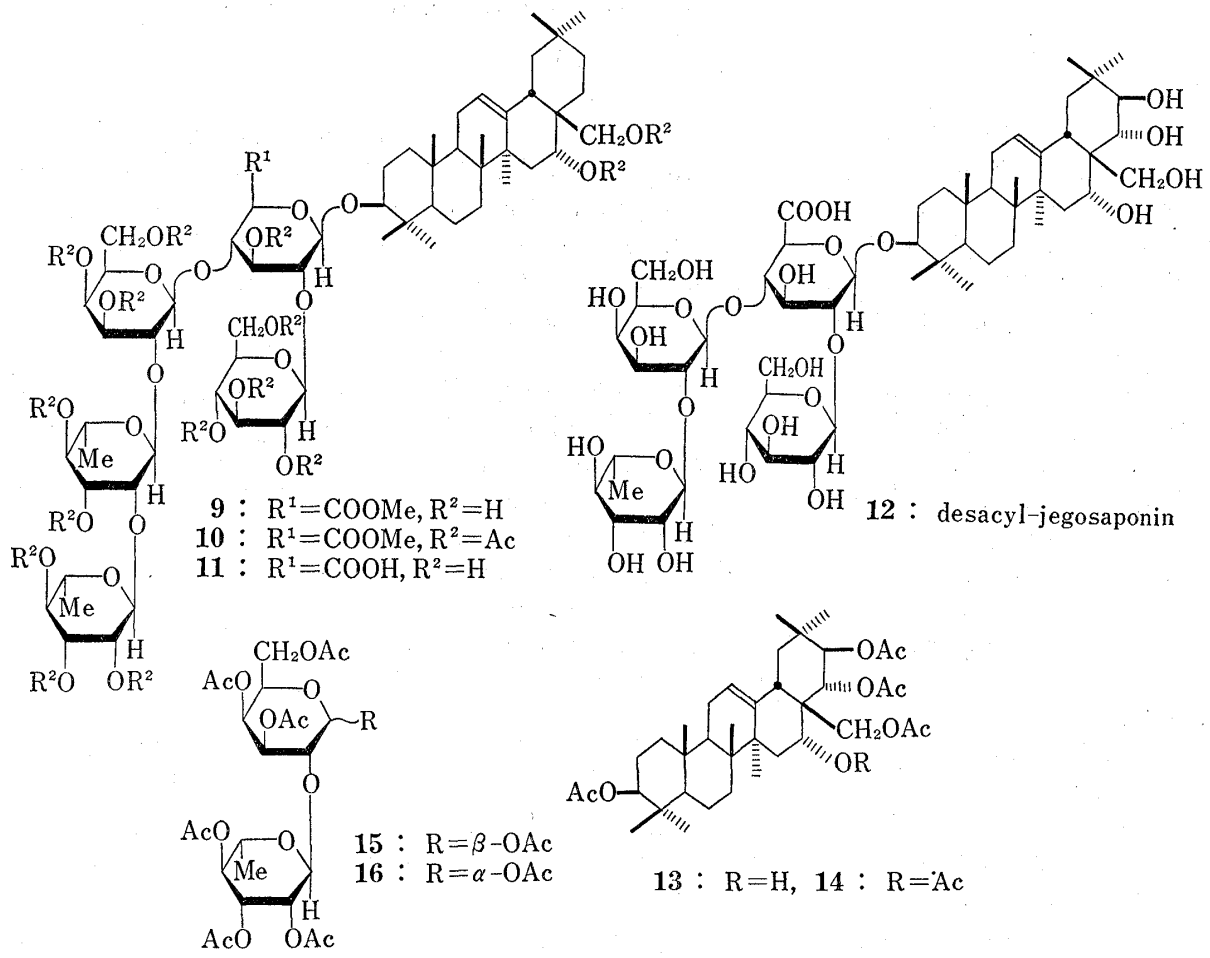
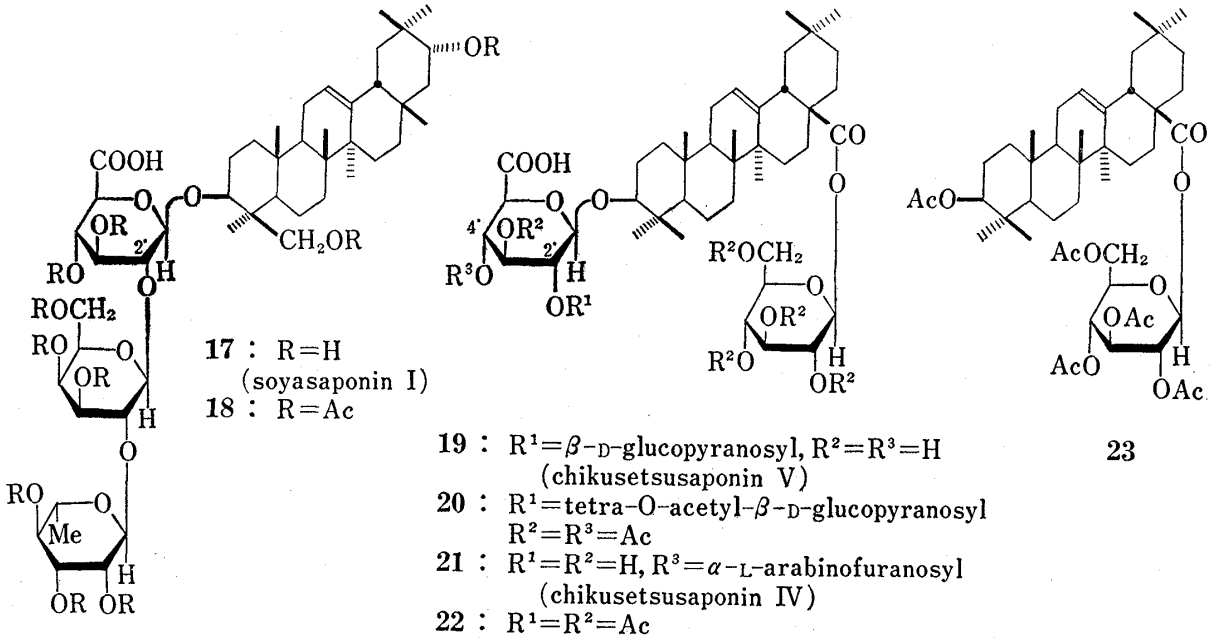


Chart 2

11) *a*) J. Zemlicka, R. Gasser, J.V. Freisler, and J.P. Horwitz. *J. Am. Chem. Soc.*, **94**, 3213 (1972); *b*) K.D. Philips, J. Zemlicka, and J.P. Horwitz, *Carbohyd. Res.*, **30**, 281 (1973).
12) J. Kiss, *Advan. Carbohyd. Chem. and Biochem.*, **29**, 229 (1974).
13) I. Kitagawa, Y. Imakura, T. Hayashi, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1520 (1975).
14) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).



17 : R=H
(soyasaponin I)
18 : R=Ac

19 : R^1=β-D-glucopyranosyl, R^2=R^3=H
(chikusetsusaponin V)
20 : R^1=tetra-O-acetyl-β-D-glucopyranosyl
R^2=R^3=Ac
21 : R^1=R^2=H, R^3=α-L-arabinofuranosyl
(chikusetsusaponin IV)
22 : R^1=R^2=Ac
R^3=tri-O-acetyl-α-L-arabinofuranosyl

23

Chart 3

protons at δ 5.58 (1H, d, J=8 Hz, on galactoside) and at δ 5.34 (1H, d, J= 3 Hz, on rhamnoside). The spectral data of **16** are like those of **15** described above, and two anomeric proton signals in this case are observed at δ 6.24 (1H, d, J=4 Hz, galactoside) and at δ 5.40 (1H, d, J=3 Hz, rhamnoside). Deacetylation followed by acid hydrolysis of **15** and **16** furnished galactose and rhamnose. Based on these findings and the structure of parent desacyl-jegosaponin (**12**), the structures **15** and **16** have been assigned to the hepta-acetates.

Secondly, soyasaponin I (**17**)[15] and chikusetsusaponin V (**19**),[16] both of which possess a carbohydrate residue (a rhamno-galactosyl residue in **17** and a glucosyl residue in **19**) only at C-2′ of the glucuronide moiety, were treated with acetic anhydride and pyridine under reflux. Even after prolonged heating, no degradation product was formed but the respective peracetates (**18** and **20**) were obtained in good yields.

Thirdly, chikusetsusaponin IV (**21**)[17] possessing an arabinofuranosyl residue at C-4′ of the glucuronide moiety was treated as above. In this case, the major reaction product was the peracetate (**22**) and only a trace amount of the degradation product was isolated and identified as the peracetate of β-D-glucopyranosyl oleanolate (= compound O peracetate (**23**)[18]).

Finally, a mixture of trideca- and teradecaacetates of sakuraso-saponin (**1**)[7] was treated as above and the reaction products were found to be identical with those obtained directly from sakuraso-saponin (**1**) described above.

In conclusion, it has been shown that the degradation is smoothly effected only for the glucuronide-saponin which possesses a free carboxyl function and two carbohydrate residues (either in acetylated form or as free) both at C-2′ and C-4′

Based on the above described evidence, the degradation reaction could presumably be explained by the pathway shown in Chart 4: 1) an initial attack at the carboxyl function

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16) N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).
17) N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969).
18) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303.

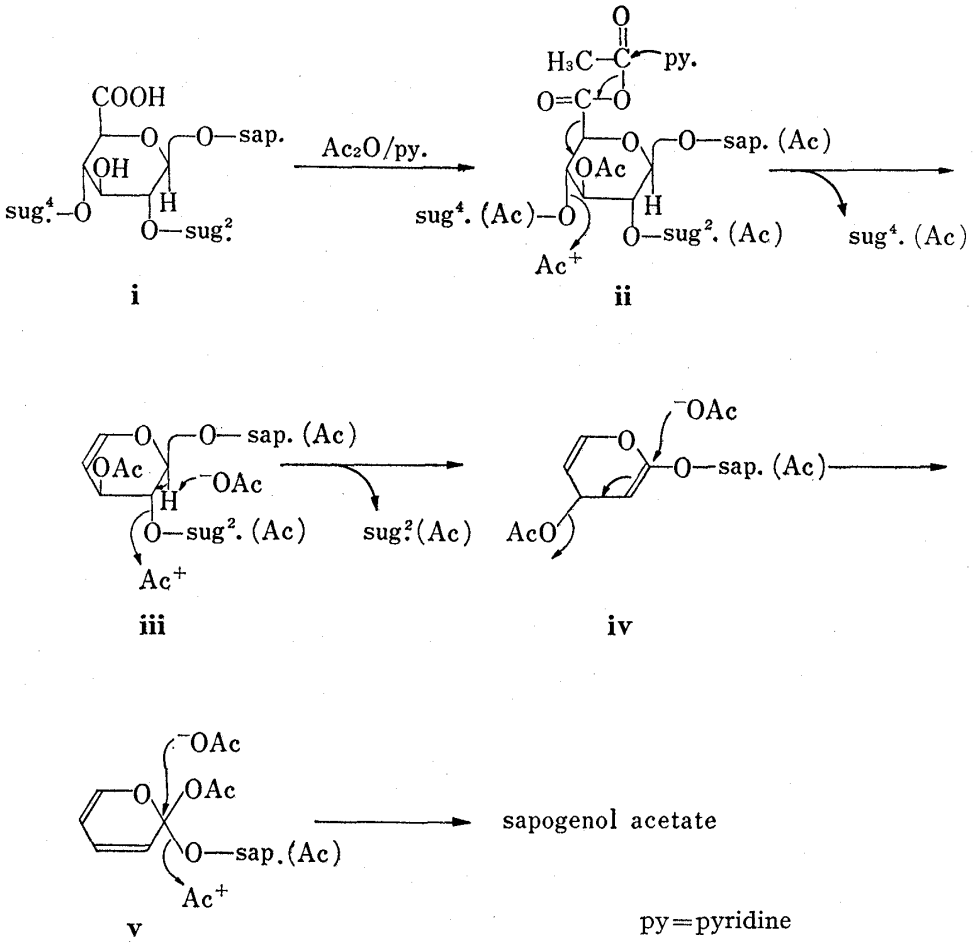

Chart 4

abbreviations: sap.=sapogenol, sap. (Ac)=acetylated sapogenol, sug.=carbohydrate residue, sug. (Ac)=acetylated carbohydrate residue

(*e.g. via* mixed anhydride formation (**ii**)) followed by the decarboxylative elimination (*e.g.* occurring between the anhydride group at C–5′ and the acetylated carbohydrate residue at C–4′ (**ii**)), 2) the elimination of the acetylated carbohydrate residue at C–2′ (**iii**), and 3) the liberation of the acetylated sapogenol (**v**). However, the details of the reaction mechanism should be the subject of the further investigation.

Experimental[19]

Treatment of Sakuraso-saponin (1) with Acetic Anhydride and Pyridine under Reflux——A solution of sakuraso-saponin (**1**, 460 mg) in Ac_2O (4.0 ml) and pyridine (2.9 ml) was heated under reflux for one hour and poured into ice-water. The precipitate collected by filtration, was purified by preparative thin-layer chromatography (TLC) (benzene–acetone=4: 1) to give 3,16-di-O-acetyl-protoprimulagenin A (**2**, 120 mg, 60%), 1,2,3,4,6-penta-O-acetyl-glucopyranose (**3**, 111 mg, 78%), and trisaccharide nonaacetate (**4**, 221 mg, 71%). **2**, amorphous, $[\alpha]_D^{13}$ −23.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{54}O_5$: C, 75.23; H, 10.03. Found: C, 75.05; H, 9.88. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1737, 1240 (OAc). PMR ($CDCl_3$) δ: 0.83, 0.90 (6H each), 0.99, 1.09, 1.16 (3H each) (all s, *tert.* $CH_3\times7$), 2.03, 2.05 (3H each, both s, OAc×2), 3.22, 3.62 (1H each, AB q, J= 7 Hz, 28-H_2), 4.51 (1H, t-like, 3α-H), 5.05 (1H, d, J=6.5 Hz, 16β-H). **3**, amorphous, IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1765, 1211 (OAc). PMR ($CDCl_3$) δ: 1.99—2.16 (15H, OAc×5), 5.62 (2/3H, d, J=7 Hz), 6.21 (1/3H, d, J= 4 Hz). **4**, amorphous, IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1760, 1216 (OAc). PMR ($CDCl_3$) δ: 1.23 (6H, d, J=6 Hz,

19) The instruments used in the experimental section and experimental conditions for chromatography were same as in our previous paper[1] unless specified otherwise. Some of the optical rotations were measured with a JASCO DIP-181 Digital Polarimeter (1=1 dm).

sec. $C\underline{H}_3\times 2$ in two rhamnosides), 1.98, 2.01, 2.02 (3H each), 2.04 (9H), 2.10, 2.13, 2.18 (3H each) (all s, OAc×9), 5.62 (1/3H, d, $J=7$ Hz, anomeric H of β-galactoside), 6.29 (2/3H, d, $J=4$ Hz, anomeric H of α-galactoside).

Deacetylation of 2 and 3——i) A solution of **2** (50 mg) in anhydrous MeOH (2 ml) was treated with 1% NaOMe–MeOH (0.1 ml) and the total mixture was kept stirring at room temperature for 2 hr, neutralized with Dowex 50w×8 (H^+, 3.5 g), and concentrated under reduced pressure. The residue thus obtained was purified by preparative TLC ($CHCl_3$–MeOH=25:1) to give a product (36 mg), which was recrystallized from *n*-hexane–acetone and identified with protoprimulagenin A (**5**)[8] by mixed mp, IR (KBr), and TLC. 2) A solution of **3** (10 mg) in 0.05% NaOMe–MeOH (1 ml) was kept stirring at room temperature for one hour, neutralized with Dowex 50w×8 (H^+, 1 g), and evaporated under reduced pressure to give a residue which was identical to glucose by paper partition chromatography (PPC) (iso-PrOH–*n*-BuOH–H_2O=7:1:2, developing twice).

Deacetylation followed by Acid Hydrolysis of 4——To a solution of **4** (410 mg) in anhydrous MeOH (10 ml) was added 1% NaOMe–MeOH (3 ml) and the total solution was kept stirring at room temperature for 1.5 hr, neutralized as above, and evaporated under reduced pressure to give **6** (240 mg), IR ν_{max}^{Nujol} cm^{-1}: 3350 (br, OH), no OAc. **6** (10 mg) was dissolved in aq. 20% H_2SO_4–MeOH (1:1) (1 ml) and the solution was heated under reflux for 4.5 hr, neutralized with aq. sat. $Ba(OH)_2$, and centrifuged to remove the precipitate. The supernatant was taken and evaporated under reduced pressure to give a syrup which was subjected to PPC (as above) to identify with galactose and rhamnose.

Methylation of 6 giving 7 and Methanolysis of 7——To a solution of **6** (113 mg) in dimethylformamide (4 ml) was added silver oxide (500 mg) and methyl iodide (10 ml), and the total mixture was kept stirring in the dark at 37° overnight and filtered. The filtrate was diluted with ether, washed with water, and dried over $MgSO_4$. Evaporation of the solvent gave a product (93 mg), wihich was purified by preparative TLC ($CHCl_3$–acetone=5:1) to furnish the nona-O-methyl derivative (**7**), colorless viscous oil.[20] IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1115 (C–O–C). PMR ($CDCl_3$) δ: 1.24 (6H, d, $J=6$ Hz, *sec.* $C\underline{H}_3\times 2$ in two rhamnosides), 3.34, 3.36, 3.44, 3.47, 3.48, 3.49 (3H each), 3.52 (9H) (all s, $OC\underline{H}_3\times 9$), 4.71 (1H, d, $J=4$ Hz, anomeric H of α-galactoside), 4.84 (1H, d, $J=1$ Hz), 5.01 (1H, d, $J=2$ Hz) (anomeric H×2 of two rhamnosides).

A solution of **7** (15 mg) in anhydrous 10% HCl–MeOH (4 ml) was heated under reflux for one hour, neutralized with Ag_2CO_3, and filtered to remove the precipitate. The filtrate was concentrated under reduced pressure to give a syrup which was subjected to TLC and gas–liquid chromatography (GLC) and identified as methyl 2,3,4-tri-O-methyl-rhamnopyranoside (a), methyl 3,4-di-O-methyl-rhamnopyranoside (b), and methyl 3,4,6-tri-O-methyl-galactopyranoside (c). TLC: benzene–acetone=1:1, benzene–MeOH=5:1. GLC: column: 15% polyneopentylglycol succinate on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp. 185°; carrier gas: N_2 flow rate 30 ml/min; t_R: a=2′12″ (major), 2′53″ (minor); b=3′51″ (major), 5′52″ (minor); c=11′52″ (major), 17′11″ (minor).

Acetolysis of 1 with BF_3-etherate——A solution of **1** (150 mg) in Ac_2O (0.8 ml)–AcOH (1.5 ml) was treated with BF_3-etherate (0.2 ml) and the total mixture was heated with stirring at 100° for one hour and poured into ice-water. The product (178 mg) collected by filtration was purified by preparative TLC separation (*n*-hexane–ether=4:1) to furnish 3,16,28-tri-O-acetyl-primulagenin A (**8**, 44 mg, 63%), **3** (33 mg, 71%), and **4** (65 mg, 65%). **8**, mp 158—160° (colorless needles from MeOH), $[\alpha]_D^{24}$ −0.15° ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65. Found: C, 73.71; H, 9.61. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1744, 1245 (OAc). PMR ($CDCl_3$) δ: 0.82 (3H), 0.91 (12H), 0.95, 1.25 (3H each) (all s, *tert.* $C\underline{H}_3\times 7$), 2.02 (6H), 2.06 (3H) (both s, OAc×3), 3.67, 3.89 (1H each, AB q, $J=11$ Hz, 28-$\underline{H}_2$), 4.48 (1H, t-like, 3α-H), 5.11 (1H, m, $W_{h/2}=9$ Hz, 16β-H), 5.30 (1H, t-like, 12-H).

Treatment of 9 with Acetic Anhydride and Pyridine under Reflux——A solution of **9** (104 mg) in Ac_2O (2.4 ml)–pyridine (1.0 ml) was heated under reflux for 24 hr. Detailed examination by TLC of the total reaction mixture showed the absence of any degradation product. The reaction mixture was poured into ice-water and the product was collected by filtration and purified by preparative TLC (benzene-acetone=5:2) to furnish **10** (91 mg), amorphous, $[\alpha]_D^{22}$ −31.9° ($c=0.5$, $CHCl_3$). *Anal.* Calcd. for $C_{91}H_{130}O_{42}$: C, 57.64; H, 6.91. Found: C, 57.55; H, 6.97. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1762, 1226 (OAc). PMR ($CDCl_3$) δ: 0.82 (3H), 0.92 (12H), 1.06, 1.26 (3H each) (all s, *tert.* $C\underline{H}_3\times 7$), 1.22 (6H, d, $J=7$ Hz, *sec.* $C\underline{H}_3\times 2$ in two rhamnosides), 1.98, 1.99, 2.05, 2.07, 2.10, 2.13, 2.16 (totally 45H, all s, OAc×15), 3.74 (3H, s, $OC\underline{H}_3$). Deacetylation of **10** (91 mg) with 1% NaOMe–MeOH (2 ml) as for **3** followed by neutralization with Dowex 50w×8 (H^+, 5 g) furnished **9** (65 mg).

Alkaline Hydrolysis of 9 giving 11——A solution of **9** (185 mg) in MeOH (5 ml) was treated with aq. 10% K_2CO_3 (5 ml) and the total mixture was heated under reflux for 1.5 hr, neutralized with Dowex 50w×8 (H^+, 5 g), and evaporated under reduced pressure to give a product (160 mg), which was crystallized from MeOH. **11**, mp 224—225° (colorless needles), $[\alpha]_D^{24}$ −0.30° ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{60}H_{98}O_{27}\cdot 3H_2O$: C, 55.21; H, 7.98. Found: C, 55.54; H, 8.16. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1735 (COOH).

Treatment of 11 with Acetic Anhydride and Pyridine under Reflux——A solution of **11** (160 mg) in Ac_2O

20) After purity was secured by TLC, the oil was directly subjected to methanolysis.

(3.0 ml)–pyridine (1.2 ml) was heated under reflux for one hour. Treatment of the reaction mixture followed by purification of the products as for **1** gave **8** (40 mg, 53%, identified by IR (CCl_4), TLC (*n*-hexane–ether=4:1; benzene-acetone=30:1)), **3** (33 mg, 65%), and **4** (56 mg, 51%) (both identified by TLC (*n*-hexane–ether=1:4; benzene–acetone=4:1; $CHCl_3$–acetone=5:1)).

Treatment of Sakuraso-saponin (1) with Acetic Anhydride and Pyridine of Various Molar Ratios——A solution of **1** (10 mg each) in 0.6 ml of the Ac_2O–pyridine mixture (1000:1, 100:1, 10:1, 1:1, 1:10, 1:100, or 1:1000) was heated under reflux while monitoring the reaction by TLC (benzene–acetone=4:1). The Ac_2O–pyridine mixtures of the ratios (10:1, 1:1, and 1:10) afforded the degradation products (**2**, **3**, and **4**) after heating for one hour, but the mixtures of the ratios (1000:1, 100:1, 1:100, and 1:1000) gave only trace amounts of **2**, **3**, and **4** even after heating for 4 hr.

Treatment of 1 with Pyridine under Reflux——A solution of **1** (20 mg) in pyridine (0.6 ml) was heated under reflux for 4 hr and evaporated under reduced pressure to remove pyridine. The residue thus obtained was identical to parent saponin **1** by TLC ($CHCl_3$–MeOH–H_2O=65:35:10, lower layer), and no degradation product was detected.

Treatment of 1 with Acetic Anhydride under Reflux——A solution of **1** (15 mg) in Ac_2O (0.6 ml) was heated under reflux for 5 hr, poured into ice-water, and extracted with ether. The ether extract was washed with aq. sat. $NaHCO_3$ and water successively, and dried over $MgSO_4$. The product (10 mg) obtained by evaporation of the solvent was identified by TLC ($CHCl_3$–MeOH=30:1; benzene–acetone=1:1) with the mixture of sakuraso-saponin tetradecaacetate and tridecaacetate which was prepared by acetylation of **1** with Ac_2O–pyridine (1:1) at room temperature. No degradation product was detected in the total reaction mixture by TLC.

Acetylation followed by Degradation with Acetic Anhydride and Pyridine of 1——A solution of **1** (1.2 g) in Ac_2O–pyridine (1:1, 40 ml) was kept stirring at room temperature overnight and poured into ice-water. The product (1.5 g) obtained by filtration was a mixture (*ca.* 1:1) of tetradecaacetate and tridecaacetate[7] which was shown as two spots on TLC ($CHCl_3$–MeOH=30:1). The acetate mixture (1.5 g) was dissolved in Ac_2O (80 ml)-pyridine (8 ml) and the total solution was heated under reflux for 1.5 hr, poured into ice-water, and extracted with ether. After the usual work-up, the reaction product (1.6 g) was purified by column chromatography using silica gel (Merck, 70—230 mesh, 120 g) and eluting with benzene–acetone mixture to furnish **2** (123 mg), **3** (230 mg), and **4** (500 mg) which were respectively identified with the samples obtained above directly from **1**.

Acetic Anhydride-Pyridine Degradation of Desacyl-jegosaponin (12)——A solution of **12** (250 mg)[13] in Ac_2O (2.5 ml) and pyridine (2.0 ml) was heated under reflux for one hour and treated as for **1**. The product (295 mg) thus obtained was purified by preparative TLC (benzene–acetone=10:1) to afford 3,21,22,28-tetra-O-acetyl-barringtogenol C (**13**, 70 mg, 49%), 3,16,21,22,28-penta-O-acetyl-barringrogenol C (**14**, 19 mg, 12%), **3** (52 mg, 60%), and two disaccharide heptaacetates (**15**, 42 mg and **16**, 53 mg, totally 64%). **13** and **14** were identified with the authentic samples[14] by mixed mp, IR (CCl_4), and TLC, and **3** was identified by TLC (benzene–acetone=8:1; *n*-hexane–ether=1:5). **15** (β-anomer), mp 183° (colorless needles from acetone), $[\alpha]_D^{13}$ +17.6° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.63; H, 5.93. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1760, 1217 (OAc). PMR ($CDCl_3$) δ: 1.16 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$ in rhamnoside), 1.95 (3H), 2.01 (6H), 2.06 (3H), 2.11 6H), 2.13 (3H) (all s, OAc×7), 5.34 (1H, d, J=2 Hz, anomeric H of rhamnoside), 5.58 (H, d, J=8 Hz, anomeric H of galactoside). **16** (α-anomer), mp 195—196.5° (colorless needles from acetone), $[\alpha]_D^{22}$ +27.5° (c=1.6, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.28; H, 5.86. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1758, 1219 (OAc). PMR ($CDCl_3$) δ: 1.20 (3H, d, J=6 Hz, *sec.* C$\underline{H}_3$ in rhamnoside), 1.98 (3H), 2.01 (9H), 2.11 (6H), 2.19 (3H) (all s, OAc×7), 5.40 (1H, d, J=3 Hz, anomeric H of rhamnoside), 6.24 (1H, d, J=4 Hz, anomeric H of galactoside).

15 or **16** (10 mg each) was dissolved in 0.1% NaOMe–MeOH (1.0 ml) and the solution was kept stirring at room temperature for one hour, neutralized with Dowex 50w×8 (H^+, 1 g) by stirring for one hour, and evaporated under reduced pressure. The residue thus obtained was dissolved in aq. 10% HCl (2 ml) and the solution was heated under reflux for 1.5 hr, neutralized with Amberlite IRA −400 (OH^-, 1 g) by stirring for one hour, and concentrated under reduced pressure to give a syrupy product, from which galactose and rhamnose were identified by PPC (iso-PrOH–*n*-BuOH–H_2O=7:1:2, developing twice).

Treatment of Soyasaponin I (17) with Acetic Anhydride and Pyridine under Reflux——A solution of **17** (30 mg)[15] in Ac_2O (0.5 ml) and pyridine (0.4 ml) was heated under reflux for 25 hr and poured into ice-water. The precipitated product was collected by filtration and purified by preparative TLC ($CHCl_3$–acetone=4:1) to furnish the peracetate (**18**, 32 mg), amorphous, $[\alpha]_D^{22}$ +17.9° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{68}H_{98}O_{28}$: C, 59.90; H, 7.25. Found: C, 59.96; H, 7.19. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1756, 1235 (OAc). Treatment of **18** (32 mg) with 1% NaOMe–MeOH (2 ml) with stirring at room temperature for one hour recovered **17** (22 mg).

Treatment of Chikusetsusaponin V (19) with Acetic Anhydride and Pyridine under Reflux——A solution of **19** (50 mg) in Ac_2O (0.9 ml) and pyridine (0.7 ml) was heated under reflux for 25 hr and treated as for **17**. The product was purified by preparative TLC (benzene–acetone=6:1) to furnish the peracetate (**20**, 45 mg), amorphous, $[\alpha]_D^{22}$ −18.3° (c=1.5, $CHCl_3$). *Anal.* Calcd. for $C_{68}H_{96}O_{29}$: C, 59.29; H, 7.03. Found: C, 58.85; H, 6.89. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1768, 1225 (OAc). PMR ($CDCl_3$) δ: 0.74 (6H), 0.84 (9H), 1.11, 1.24 (3H each) (all s, *tert.* C$\underline{H}_3$×7), 2.01 (15H), 2.06 (6H), 2.09 (6H) (all s, OAc×9), 5.58 (1H, d, J=8 Hz, anomeric

H of ester glucoside). **20** was identified by IR (CCl_4) and TLC (benzene–acetone=6: 1; *n*-hexane–ether=1: 3) with the peracetate which was prepared by ordinary acetylation of **19** with Ac_2O and pyridine (1: 1, 24 hr) at room temperature.

Treatment of Chikusetsusaponin IV (21) with Acetic Anhydride and Pyridine under Reflux——A solution of **21** (142 mg) in Ac_2O (1.5 ml) and pyridine (1.2 ml) was heated under reflux for 25 hr and poured into ice-water. The precipitated product was collected by filtration and purified by preparative TLC (benzene–acetone=6: 1) to furnish the peracetate (**22**, 140 mg) and **23** (1 mg). **22**, amorphous, $[\alpha]_D^{22}$ −11.6° (c=1.4, $CHCl_3$). *Anal.* Calcd. for $C_{65}H_{92}O_{27}$: C, 59.80; H, 7.10. Found: C, 59.91; H, 7.10. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1763, 1229 (OAc). PMR ($CDCl_3$) δ: 0.72, 0.76 (3H each), 0.91 (9H), 1.11, 1.24 (3H each) (all s, *tert.* $CH_3\times 7$), 2.02, 2.07 (6H each), 2.10 (9H), 2.13 (3H) (all s, OAc×8), 5.57 (1H, d, J=7 Hz, anomeric H of ester glucoside). **22** was identified by IR (CCl_4) and TLC (*n*-hexane–ether=1: 2, benzene–acetone=6: 1) with the peracetate which was prepared by ordinary acetylation of **21** with Ac_2O and pyridine at room temperature. **23** was identified with compound O peracetate[18] by TLC ($CHCl_3$–AcOEt=6: 1; *n*-hexane–ether=1: 1; benzene–acetone=10: 1).

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