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Saponin and Sapogenol. XIX.[1] Selective Cleavage of the Glucuronide Linkage in Saponin by Lead Tetraacetate Oxidation followed by Alkali Treatment

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As a continuing study in search of new selective cleavage methods for certain glycoside linkages in oligoglycosides, it has been found that lead tetraacetate oxidation followed by a brief treatment with alkali is an effective method for selective cleavage of a glucuronide linkage in glucuronide-saponin which possesses a glucuronic acid moiety directly connected to the sapogenol. When a permethylated derivative of a glucuronide-saponin, which retains its free carboxylic function, is subjected to lead tetraacetate oxidation and successive treatment with alkali, it is decomposed to the constituents from the carbohydrate portion and sapogenol in excellent yields. The glucuronic acid moiety has been found to furnish a dienic compound (**21**).

It is suggested that the present degradation method seems to be applicable to the cleavage of other kinds of uronide linkages and also to be useful for selective degradation at the glucuronide moiety in polysaccharides.

Keywords——selective cleavage of glucuronide linkage; lead tetraacetate oxidation; genuine aglycone; oligoglycoside; glucuronide-saponin; PMR; soyasaponin I; sakuraso-saponin

In recent years, in structural studies on oligoglycosides such as saponins, it has been demonstrated in many cases that acid hydrolysis often causes secondary changes of the aglycone and results in the formation of artifact aglycone. In order to avoid such undesirable side reactions, several chemical and biochemical methods have been applied, and in this connection, we have been developing the soil bacterial hydrolysis method by which microbiological hydrolysis of the glycoside linkage has been effected to liberate the genuine aglycone.[3]

In a parallel study, we have been exploiting a new chemical method by which a certain glycoside linkage in the oligoglycosides is selectively cleaved and recently, we have found that photolysis is a convenient procedure to split a glucuronide linkage in saponin.[4] By this procedure, some saponins, which possess a glucuronide moiety directly attached to the sapogenol (now abbreviated as glucuronide-saponin), are readily decomposed to extricate their genuine sapogenols in moderate yields. In a continuing study, we have found that lead tetraacetate oxidation followed by alkali treatment is also an effective method to selectively cleave a glucuronide linkage in oligoglycosides. By this method, the glucuronide-saponin is decomposed to its constituents in excellent yields. The present paper presents full details of the method. A preliminary report has already appeared.[5]

1) Part XVIII: I. Kitagawa, K.S. Im, and Y. Morii, *Chem. Pharm. Bull.* (Tokyo), **24**, 3114 (1976).
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3) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; *b*) I. Yosioka, K. Imai, Y. Morii, and I. Kitagawa, *Tetrahedron*, **30**, 2283 (1974), and the preceding papers of the series.
4) *a*) I. Kitagawa, M. Yosikawa, 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).
5) I. Kitagawa, M. Yoshikawa, Y. Ikenishi, K.S. Im, and I. Yosioka, *Tetrahedron Letters*, **1976**, 549.

Degradation of Glucuronide-Prosapogenol

Although lead tetraacetate has been known to effect an oxidative decarboxylation reaction of a carboxyl group,[6] no report has been made on its application for degradation of a glucuronide moiety. We have initially considered that when a glucuronide retaining its free carboxyl group (**1**, R= the protecting groups or the protected carbohydrate residues) is treated with lead tetraacetate, an enol (**2**) and/or acetates (**3**) would mainly be formed and the resulting products would readily be decomposed either by mild acid or mild alkali treatment to liberate the sapogenol. Thus, we have at first examined the reactions using a prosapogenol (**5**) of soyasaponin I (**4**)[7] which was isolated from soybean.

The prosapogenol (**5**) was methylated with methyl iodide (CH_3I)/dimethyl sulfoxide (DMSO)/sodium hydride (NaH)[8] to give a hexa-O-methyl derivative (**6**) which did not contain free hydroxyl as shown by its infrared (IR) spectrum. The methyl ester function in **6** was then hydrolyzed with aqueous potassium carbonate to give **7** (IR: 1735 cm^{-1} (COOH)) which was a starting material for the degradation. The structures of **6** and **7** were corroborated by their spectroscopic properties (IR and proton magnetic resonance (PMR) spectra), among which the respective anomeric proton signals observed at δ 4.32 (1H, d, J=7 Hz) for **6** and at δ 4.36 (1H, d, J=7 Hz) for **7** were essential.

Treatment of **7** with lead tetraacetate in benzene under reflux furnished two isomeric acetates **8** and **9** in 45% and 42% yields, respectively, and no enolic compound of the type **2** was obtained in this reaction. The IR and PMR spectra of **8** indicate the presence of a secondary acetoxyl (IR: 1762, 1226 cm^{-1}; PMR: δ 2.11, 3H, s, due to an acetoxyl and δ 6.21, 1H, d, J=3.5 Hz, due to 5′-H) and five methoxyls. The anomeric proton signal in the PMR spectrum of **8** is observed at δ 4.65 (1H, d, J=7 Hz), thus the structure **8** possessing an α-axial acetoxyl at C-5′ has been assigned to one acetate. The other acetate is assigned **9** having a β-equatorial acetoxyl, based on similar spectroscopic evidence. In the PMR spectrum of **9** taken in deuterochloroform, a doublet-like signal due to 5′-H is observed at δ 5.43 while a signal due to the anomeric proton is observed at δ 4.45 also as a doublet-like signal. These deformed signal

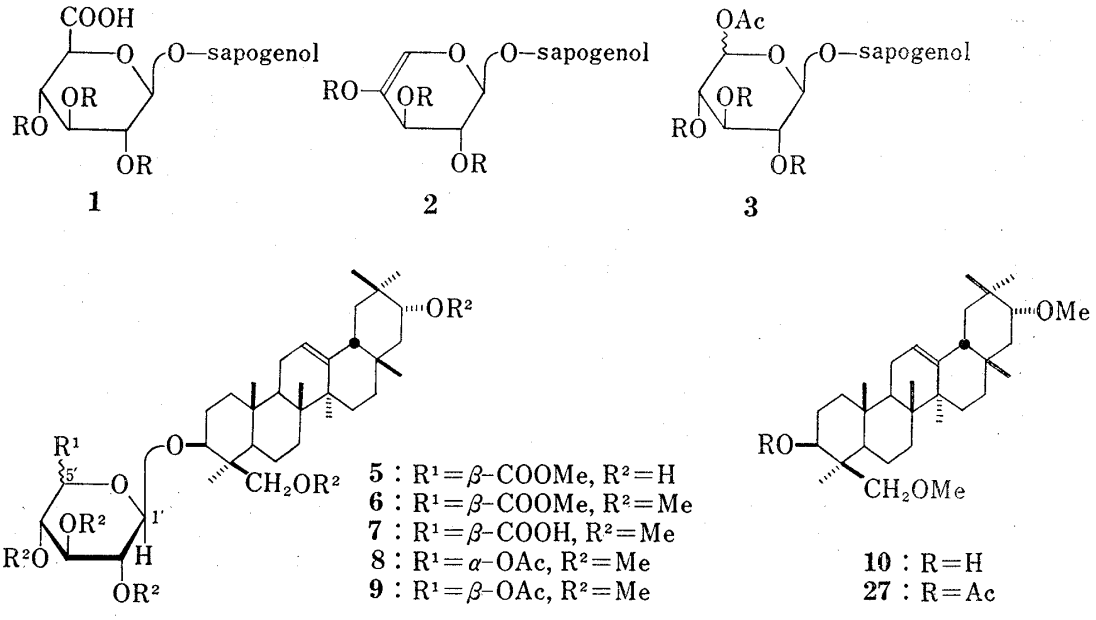


Chart 1

6) R.A. Sheldon and J.K. Kochi, *Organic Reactions*, **19**, 279 (1972).
7) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 121 (1976).
8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

patterns are attributable to the virtual long-range coupling,[3b,9] since measurement of the spectrum in other solvents, such as hexadeuteroacetone, hexadeuterobenzene, pentadeuteropyridine, and carbon tetrachloride, has revealed that the coupling constants ($J_{1',2'}$ and $J_{4',5'}$) are both 7 Hz.

The two monoacetates, **8** and **9**, were then subjected to alkali treatment with 0.1% sodium methoxide in dry methanol at room temperature to furnish 21,24-di-O-methyl-soyasapogenol B (**10**)[7] in 91% and 95% yields, respectively. Consequently, it has been substantiated that lead tetraacetate oxidation followed by alkali treatment is an effective procedure for cleavage of the glucuronide linkage in glucuronide-prosapogenol to liberate the sapogenol.

Degradation of Sakuraso-saponin (11)

Next, sakuraso-saponin (**11**) was taken as an example of glucuronide-saponins and subjected to this new degradation method. A tetradeca-O-methyl ether (**13**) preserving the free carboxyl function (IR) was prepared from sakuraso-saponin (**11**), which was isolated from the root of *Primula sieboldi* E. Morren,[10] *via* the pentadeca-O-methyl derivative (**12**). Treatment of **13** with lead tetraacetate as for **7** yielded a mixture of two monoacetates (**14**) in 94% yield. The IR and PMR spectra of **14** shows that **14** is a mixture of two epimeric acetates: i) Two singlets at δ 1.99 and δ 2.04 of totally three protons are assigned to one acetoxyl (a mixture of 5'β-OAc and 5'α-OAc) and ii) two doublets observed at δ 5.52 (J=8 Hz) and δ 6.05 (J=4 Hz), whose combined intensity counts one proton, are assigned to the respective methine protons geminal to epimeric acetoxyls. **14** was then subjected to sodium methoxide hydrolysis and acetylation with acetic anhydride and pyridine for facile separation of the products. Preparative thin-layer chromatographic (TLC) separation of the products led to the isolation of five products, one (**15**) from the sapogenol portion and four (**18**, **19**, **20**, and **21**) from the carbohydrate portion.

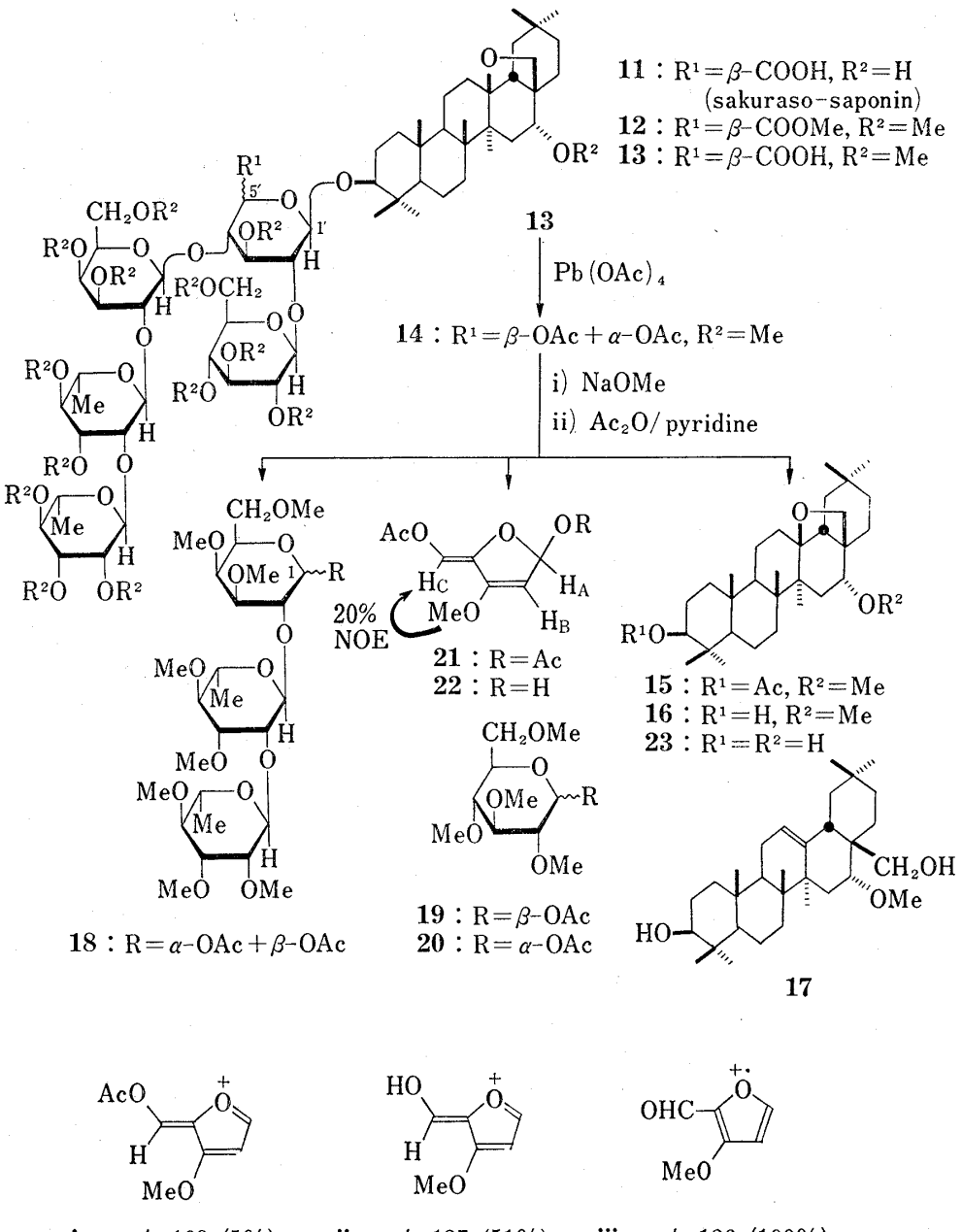
A product from the sapogenol portion was obtained in 90% yield and has been elucidated to be 3-O-acetyl-16-O-methyl-protoprimulagenin A (**15**) on the following basis. It shows the acetoxyl absorption bands at 1730 and 1238 cm^{-1} in its IR spectrum and two three-proton singlets at δ 1.94 and δ 3.21 assignable to an acetoxyl and a methoxyl and a one-proton triplet-like signal at δ 4.39 due to 3α-H in its PMR spectrum. Alkaline hydrolysis (giving **16**) followed by acid treatment converted **15** to 16-O-methyl-primulagenin A (**17**).[10]

Four products from the carbohydrate portion have been assigned as **18** (a mixture of 1α-OAc and 1β-OAc derivatives, obtained in 92% combined yield), **19** (with β-OAc), **20** (with α-OAc) (in 70% combined yield), and **21** (32%),[11] respectively.

The IR spectrum of **18** shows the presence of acetoxyl (1767, 1225 cm^{-1}), while the PMR spectrum shows the signals due to one acetoxyl (δ 2.10, 3H, s), two secondary methyls of rhamnose moieties (δ 1.21 and 1.25, 3H each, both d, J=6 Hz), and two kinds of anomeric protons of totally one proton intensity (δ 5.44 and 6.15, both d, J=7 and 4 Hz, 1-H). On methanolysis, **18** was decomposed to give methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 3,4-di-O-methyl-rhamnopyranoside, and methyl 3,4,6-tri-O-methyl-galactopyranoside. By comparison with the total structure of sakuraso-saponin (**11**),[10] the trisaccharide has been assigned as **18**.

A monosaccharide **19** shows an acetoxyl absorption band (1770, 1224 cm^{-1}) in its IR spectrum and it shows an anomeric proton doublet at δ 5.42 (J=7 Hz) in addition to signals due to one acetoxyl and four methoxyls in its PMR spectrum. On methanolysis, it was converted to methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, the structure **19** thus being corroborated. Another monosaccharide **20** has been elucidated on a simiiar basis as for **19**. The anomeric proton signal in its PMR spectrum is observed at δ 6.14 as a doublet of coupling constant J=3 Hz.

9) J.I. Musher and E.J. Corey, *Tetrahedron*, **18**, 791 (1962).
10) I. Kitagawa, Y. Ikenishi, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 2470 (1975).
11) The low yield was due to instability of the dienic compound (**21**).



i : *m/e* 169 (5%) ii : *m/e* 127 (51%) iii : *m/e* 126 (100%)

Chart 2

The dienic compound (**21**) is fairly unstable. It possesses a molecular composition $C_{10}H_{12}O_6$ (high mass). The IR spectrum of **21** shows the presence of acetoxyls (1765, 1738, 1235, and 1217 cm^{-1}) and double bonds (1615 cm^{-1}) which are defined as a heteroannular diene by its ultraviolet (UV) spectrum (λ_{max} 238.5 nm, ε=12600). In the PMR spectrum of **21**, are observed signals due to two acetoxyls (δ 2.14, 6H, s), one methoxyl (δ 3.91, 3H, s), two olefinic protons (δ 5.24, 1H, d, J=3 Hz, assigned to H_B; δ 5.46, 1H, s, assigned to H_C), and one methine proton (δ 5.88, 1H, d, J=3 Hz, H_A) which is geminal to an acetoxyl. Among three low field protons, adjacency of H_A and H_B has been corroborated by decoupling experiments and spacial proximity of H_C and a methoxyl group has been shown by the nuclear Overhauser effect (NOE) observed with 20% signal enhancement. In addition to this spectroscopic evidence, examination of the mass and high resolution mass spectra (giving the fragment ions **i**, **ii**, and **iii**) has led us to formulate the dienic compound as **21**.

The structure **21** has further been supported by spectroscopic evidence from its desacetyl derivative **22**. Thus, the IR spectrum lacks the acetoxyl absorption band but shows absorption

bands of hydroxyl (3350 cm^{-1}) and the diene chromophore (1683, 1592 cm^{-1}), the presence of the latter being supported by the UV spectrum (λ_{max} 237.0 nm, ε=14600). In the PMR spectrum of **22**, signals due to one methoxyl (δ 3.92, 3H, s), two olefinic protons (δ 4.49, 1H, d, J=3 Hz, H_B; δ 5.31, 1H, s, H_C), and one methine proton (δ 4.08, 1H, d, J=3 Hz, H_A) are observed.

It has been demonstrated that, by the present lead tetraacetate degradation method, a permethylated derivative of a glucuronide-saponin preserving its free carboxyl function is readily cleaved to furnish the methylated sapogenol and methylated carbohydrate moieties along with a dienic compound which is derived from the glucuronic acid portion. Furthermore, it should be noted here that an acid labile 13β,28-oxide moiety as found in protoprimulagenin A (**23**)[10] has been kept intact during the degradation procedure.

As for the reaction mechanism, a scheme as shown in Chart 3 seems to be likely. It is assumed that the dienic compound (**21**) and methylated carbohydrate derivatives such as **18**, **19**, and **20** are derived *via* a hypothetical intermediate dialdehyde (**iv**). More detailed investigations on the reaction pathway are in progress in our laboratory.

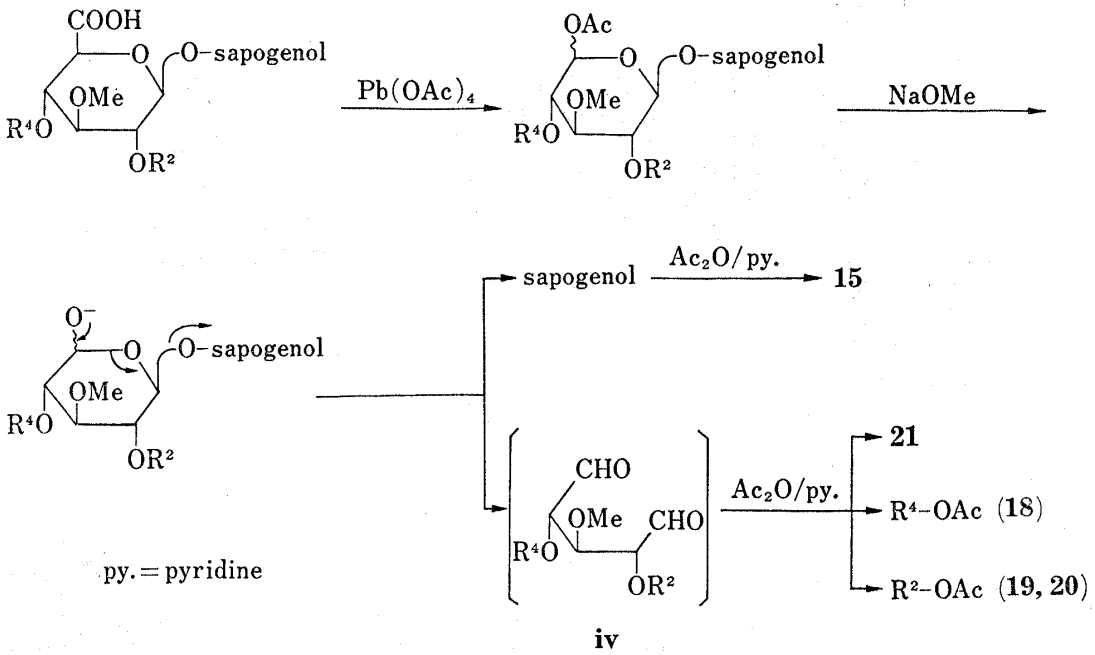


Chart 3

Application to Other Glucuronide-saponins

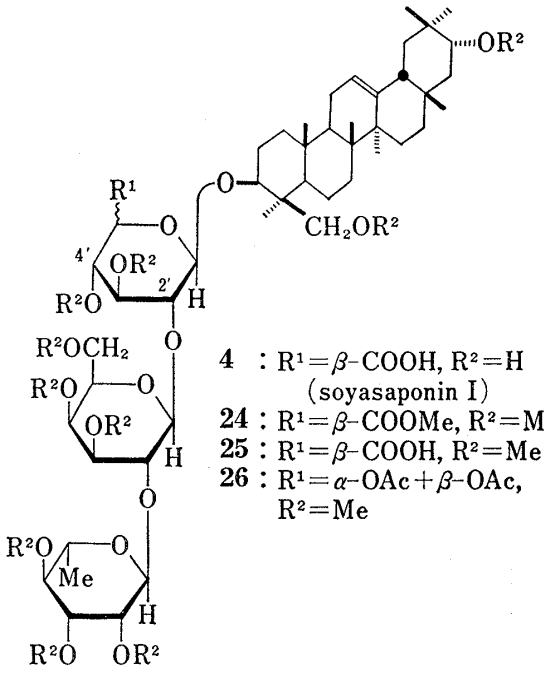
In order to determine the scope of the present degradation method, the method was applied to some other glucuronide-saponins such as soyasaponin I (**4**),[7] desacyl-jegosaponin (**30**),[12] and desacyl-boninsaponin A (**35**).[13]

A methylated carboxylic acid derivative (**25**) (IR: 1749, 1732 cm^{-1}), which was prepared from a permethylated derivative (**24**) of soyasaponin I (**4**), was oxidized with lead tetraacetate to yield a mixture of two epimeric acetates (**26**, IR: 1760, 1215 cm^{-1}). **26** was then treated with sodium methoxide and the product was acetylated as for **14**. A product derived from the sapogenol portion has been identified as 3-O-acetyl-21,24-di-O-methyl-soyasapogenol B (**27**)[7] (83%) while the products originating from the carbohydrate portion have been revealed to comprise two disaccharides, **28** and **29** (in 86% combined yield), and the diene **21** (20%).

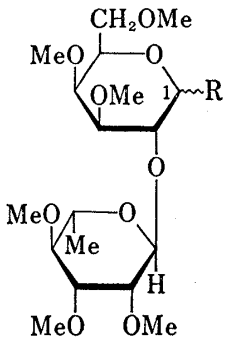
12) I. Kitagawa, Y. Imakura, T. Hayashi, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1520 (1975).
13) I. Kitagawa, K.S. Im, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 1260 (1976).

The IR and PMR spectra of one disaccharide (**28**) indicate the presence of one acetoxyl (IR: 1758, 1215 cm^{-1}; PMR: δ 2.10, 3H, s) and two anomeric protons (δ 5.22, 1H, br. s, $W_{h/2}$=6 Hz, in rhamnoside moiety; δ 5.45, 1H, d, J=8 Hz, in galactoside moiety). On methanolysis, **28** afforded methyl 2,3,4-tri-O-methyl-rhamnopyranoside and methyl 3,4,6-tri-O-methyl-galactopyranoside, the structure **28** with 1β-OAc being thus assured.

The other disaccharide has been assigned as **29** on a similar basis. In this case, the signal due to one anomeric proton on the galactoside moiety is observed at δ 6.18 (1H, d, J=4 Hz).

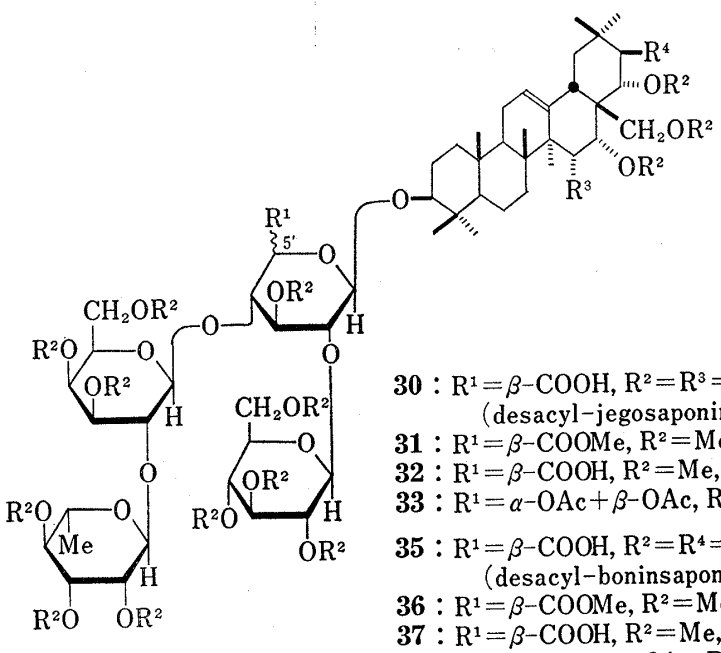


4 : R^1=β-COOH, R^2=H (soyasaponin I)
24 : R^1=β-COOMe, R^2=Me
25 : R^1=β-COOH, R^2=Me
26 : R^1=α-OAc+β-OAc, R^2=Me

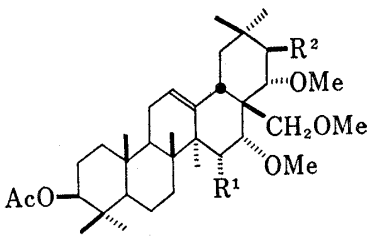


28 : R=β-OAc
29 : R=α-OAc

Chart 4



30 : R^1=β-COOH, R^2=R^3=H, R^4=OH (desacyl-jegosaponin)
31 : R^1=β-COOMe, R^2=Me, R^3=H, R^4=OMe
32 : R^1=β-COOH, R^2=Me, R^3=H, R^4=OMe
33 : R^1=α-OAc+β-OAc, R^2=Me, R^3=H, R^4=OMe
35 : R^1=β-COOH, R^2=R^4=H, R^3=OH (desacyl-boninsaponin A)
36 : R^1=β-COOMe, R^2=Me, R^3=OMe, R^4=H
37 : R^1=β-COOH, R^2=Me, R^3=OMe, R^4=H
38 : R^1=α-OAc+β-OAc, R^2=Me, R^3=OMe, R^4=H



34 : R^1=H, R^2=OMe
39 : R^1=OMe, R^2=H

Chart 5

In the case of the successful degradation of soyasaponin I (**4**), it is noteworthy that **4** carries only one carbohydrate residue at C-2′ of its glucuronide moiety and yet it is readily decomposed at the glucuronide moiety giving **21**, **28**, and **29**. Therefore, it is presumed that the reaction pathway involves an intermediate in which the substituents at C-2′ and/or C-4′ of the glucuronide moiety are readily eliminated during reaction.

Next, desacyl-jegosaponin (**30**), which was obtained from the pericarps of *Styrax japonica* Sieb. et Zucc.,[12] and desacyl-boninsaponin A (**35**) from the bark of *Schima mertensiana* Koidz.,[13] were subjected to the present degradation. The degradation of **32** and **37** *via* **33** and **38** respectively furnished the anticipated products: **21** (39%), **19**, **20** (in 83% combined yield), **28**, **29** (in 85% combined yield), and 3-O-acetyl-16,21,22,28-tetra-O-methyl-barringtogenol C (**34**, 89%) from **33**, and **21** (32%), **19**, **20** (in 80% combined yield), **28**, **29** (in 75% combined yield), and 3-O-acetyl-15,16,22,28-tetra-O-methyl-A_1-barrigenol (**39**, 84%) from **38**.

In conclusion, based on considerations of the reaction pathway, the present degradation method seems to be applicable also for the cleavage of other kinds of uronide linkages. Previously, for selective degradation at the glucuronide portion in polysaccharides, the β-elimination reaction has generally been utilized,[14] but since, by the present degradation method, all the constituents of the starting oligoglycosides are obtained in excellent yields except the dienic compound from the glucuronide moiety, this method may be preferable for selective degradation at the glucuronide moiety in polysaccharides and will be useful for structural studies.

Experimental[15]

Methylation of Prosapogenol (5) giving 6——i) Dimsyl Carbanion: A mixture of NaH (1 g, a commercial sample of NaH was defatted with dry *n*-hexane beforehand) in DMSO (20 ml) was heated under a N_2 atmosphere at 70—80° for 2 hr to yield slightly greenish dimsyl carbanion. ii) To a solution of prosapogenol (**5**, 300 mg)[7] in DMSO (5 ml) was added dimsyl carbanion (10 ml, prepared as above) and the total mixture was kept stirring at room temperature for one hour, treated with CH_3I (5 ml), kept stirring in the dark overnight, poured into ice-water, and extracted with ether. The ether extract was washed with aq. 10% $Na_2S_2O_3$ and water successively and dried over $MgSO_4$. A syrup obtained by evaporation of the solvent was purified by preparative TLC (benzene–acetone=5: 1) to give **6** (220 mg), amorphous, $[\alpha]_D^{18}$ +30.6° (c=1.1, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{70}O_9$: C, 70.16; H, 9.81. Found: C, 70.42; H, 10.01. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1759 (COOMe). PMR ($CDCl_3$) δ: 0.86, 0.89, 0.95 (3H each), 0.99 (6H), 1.09, 1.15 (3H each) (all s, *tert.* C$\underline{H}_3\times$7), 3.23, 3.26, 3.58 (3H each), 3.61 (6H), 3.78 (3H) (all s, OC$\underline{H}_3\times$6), 4.32 (1H, d, J=7 Hz, anomeric H), 5.20 (1H, br. s, $W_{h/2}$=6 Hz, 12-H).

Alkaline Hydrolysis of 6 giving 7——A solution of **6** (220 mg) in acetone (10 ml) was treated with aq. 5% K_2CO_3 (5 ml) and heated under reflux for 2 hr. The reaction mixture was acidified with aq. 10% HCl, concentrated under reduced pressure to remove acetone, and extracted with ether. After the usual work-up, evaporation of the solvent gave a pure sample of **7** (210 mg), amorphous $[\alpha]_D^{18}$ +30.4° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{68}O_9$: C, 69.85; H, 9.72. Found: C, 69.89; H, 9.70. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1735 (COOH). PMR ($CDCl_3$) δ: 0.86, 0.89, 0.95 (3H each), 0.98 (6H), 1.07, 1.14 (3H each) (all s, *tert.* C$\underline{H}_3\times$7), 3.22, 3.26, 3.51 (3H each), 3.60 (6H) (all s, OC$\underline{H}_3\times$5), 4.36 (1H, d, J=7 Hz, anomeric H), 5.19 (1H, br. s, $W_{h/2}$=7 Hz, 12-H), 6.95 (1H, br. s, $W_{h/2}$=18 Hz, exchangeable with D_2O, COO$\underline{H}$).

Lead Tetraacetate Oxidation of 7 giving 8 and 9——To a solution of **7** (150 mg) in benzene (10 ml) was added $Pb(OAc)_4$ (300 mg) and the total mixture was heated under reflux for one hour, diluted with ether (100 ml), and washed with water. The organic layer was taken and concentrated to give a product which was purified by preparative TLC (benzene–acetone=10: 1) to furnish **8** (69 mg, 45%) and **9** (65 mg, 42%). **8**, amorphous, $[\alpha]_D^{18}$ +22.3° (c=1.1, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{70}O_9$: C, 70.16; H, 9.81. Found: C, 70.32;

14) *a*) J. Kiss, *Advan. Carbohyd. Chem. & Biochem.*, **29**, 229 (1974); *b*) B. Lindberg, J. Lönngren, and S. Sevensson, *Advan. Carbohyd. Chem. & Biochem.*, **31**, 185 (1975).

15) The instruments used in the experimental section and experimental conditions for chromatography were same as in our previous paper[13] unless specified otherwise. The UV spectra were taken with the Shimadzu MPS-50L Spectrophotometer and the high resolution mass spectra with the JEOL JMS-O1SG Mass Spectrometer. The detections on preparative TLC were made by keeping the developed plates in the I_2 chamber. NOE experiments were undertaken in degassed $CDCl_3$ solution with the Hitachi R-22 NMR Spectrometer (90 MHz, frequency-swept, external CF_3COOH-locked mode). The NOE values were obtained as percentage increases in the integrated signal intensities (accuracies within ± 2%).

H, 9.83. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1762, 1226 (OAc). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.96 (3H each), 0.99 (6H), 1.09, 1.15 (3H each) (all s, *tert.* C$\underline{H}_3\times7$), 2.11 (3H, s, OAc), 3.24 (6H), 3.42 (3H), 3.60 (6H) (all s, OC$\underline{H}_3\times5$), 4.65 (1H, d, $J=7$ Hz, anomeric H), 5.19 (1H, br. s, $W_{h/2}=9$ Hz, 12-H), 6.21 (1H, d, $J=3.5$ Hz, 5′-H). **9**, amorphous, $[\alpha]_D^{18}$ +48.7° ($c=1.1$, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{70}O_9$: C, 70.16; H, 9.81. Found: C, 70.03; H, 9.58. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1768, 1222 (OAc). PMR ($CDCl_3$) δ: 0.84, 0.88, 0.94 (3H each), 0.98 (6H), 1.08, 1.14 (3H each) (all s, *tert.* C$\underline{H}_3\times7$), 2.10 (3H, s, OAc), 3.22, 3.24, 3.50 (3H each), 3.57 (6H) (all s, OC$\underline{H}_3\times5$), 4.45 (1H, d-like, signal width=7 Hz, anomeric H), 5.18 (1H, br. s, $W_{h/2}=7$ Hz, 12-H), 5.43 (1H, d-like, signal width=7 Hz, 5′-H). Anomeric H: δ 4.56 (d, $J=7$ Hz) in d_6-acetone; δ 4.51 (d.d, signal width=7 Hz) in d_6-benzene; δ 4.57 (d-like, signal width=7 Hz) in d_5-pyridine; δ 4.35 (m, signal width=7 Hz) in CCl_4. 5′-H: δ 5.44 (d.d, signal width=7 Hz) in d_6-acetone; δ 5.74 (t-like, signal width =7 Hz) in d_6-benzene; δ 5.51 (d.d, signal width=7 Hz) in d_5-pyridine; δ 5.27 (m, signal width=7 Hz) in CCl_4.

Alkaline Degradation of 8 and 9 giving 10——The respective solutions of **8** and **9** (50 mg each) in 0.1% NaOMe–MeOH (2 ml each) were kept stirring at room temperature for 30 min. During this period, the reaction mixture turned yellow and precipitated white **10**, which was collected by filtration (31 mg, 91% from **8**; 33 mg, 95% from **9**). Recrystallization from MeOH gave 21,24-di-O-methyl-soyasapogenol B (**10**)[7] as identified by IR (CCl_4), mixed mp, and TLC.

Alkaline Hydrolysis of 12 giving 13——A solution of the pentadeca-O-methyl derivative (**12**, 230 mg)[10] in MeOH (10 ml) was treated with aq. 5% K_2CO_3 (5 ml) and heated under reflux for 3 hr. After cooling, the reaction mixture was acidified with aq. 10% HCl and extracted with ether. The usual work-up of the extract gave **13** (211 mg), amorphous, $[\alpha]_D^{18}$ −48.0° ($c=1.1$, $CHCl_3$). *Anal.* Calcd. for $C_{74}H_{126}O_{27}$: C, 61.39; H, 8.77. Found: C, 60.93; H, 8.77. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750, 1740 (COOH). PMR ($CDCl_3$) δ: 0.87 (9H), 0.93, 1.00 (3H each), 1.10 (6H) (all s, *tert.* C$\underline{H}_3\times7$), 1.30 (6H, d, $J=6$ Hz, *sec.* C$\underline{H}_3\times2$ in two rhammosides), 3.23—3.59 (42H, OC$\underline{H}_3\times14$), 4.45 (2H, m), 4.82 (1H, d, $J=8$ Hz), 5.05 (2H, br. s, $W_{h/2}=7$ Hz) (anomeric H×5).

Lead Tetraacetate Oxidation of 13 giving 14——A solution of **13** (150 mg) in benzene (10 ml) was treated with Pb $(OAc)_4$ (300 mg) and heated under reflux for 4 hr. After cooling, the reaction mixture was diluted with ether and washed with water. The organic layer was treated as for **7** to give **14** (142 mg, 94%). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1762, 1228 (OAc). PMR (CCl_4)δ: 1.99, 2.04 (totally 3H, both s, OAc), 5.52 (1/2H, d, $J=8$ Hz, 5′ α-H), 6.05 (1/2H, d, $J=4$ Hz, 5′β-H).

Alkaline Degradation followed by Acetylation of 14 giving 15, 18, 19, 20, and 21——A solution of **14** (350 mg) in 0.1% NaOMe–MeOH (8 ml) was kept stirring at room temperature for 30 min until the solution color turned yellow. After neutralization with anhydrous 10% HCl–MeOH, the reaction mixture was evaporated to dryness under reduced pressure and the residue was then acetylated with Ac_2O–pyridine (1: 1, 4 ml) at room temperature overnight, poured into cold water, and extracted with ether. The usual work-up followed by preparative TLC separation (benzene–acetone=3: 1) of the extract furnished 3-O-acetyl-16-O-methyl-protoprimulagenin A (**15**, 110 mg, 90%), trisaccharide (**18**, 138 mg, 92%), two monosaccharides (**19**, 24 mg and **20**, 22 mg, totally 70%), and a diene (**21**, 16 mg, 32%).

15, colorless needles from acetone, mp 185—188°, $[\alpha]_D^{13}$ +1.0° ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 76.92; H, 10.60. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1730, 1238 (OAc). PMR ($CDCl_3$) δ: 0.83, 0.88 (6H each), 0.93, 1.03, 1.11 (3H each) (all s, *tert.* C$\underline{H}_3\times7$), 1.94 (3H, s, OAc), 3.21 (3H, s, OC$\underline{H}_3$), 4.39 (1H, t-like, 3α-H). A solution of **15** (20 mg) in 0.1% NaOMe–MeOH (5 ml) was kept stirring at room temperature for 30 min, treated with Dowex 50w×8 (H^+, 5 g), and kept stirring again for one hour. After removing the resin by filtration, the solution was evaporated under reduced pressure to give **16** (15 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), PMR ($CDCl_3$) δ: 0.75 (3H), 0.86 (6H), 0.92, 0.96, 1.09, 1.15 (3H each) (all s, *tert.* C$\underline{H}_3\times7$), 3.21 (3H, s, OC$\underline{H}_3$). **16** (15 mg) was dissolved in aq. 20% H_2SO_4–acetone (1: 2, 12 ml) and the solution was heated under reflux for 30 min and concentrated under reduced pressure to remove acetone. The precipitate (12 mg) was collected by filtration and identified as 3-O-methyl-primulagenin A (**17**)[10] by IR (CCl_4) and TLC.

18, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1767, 1225 (OAc), PMR ($CDCl_3$) δ: 1.21, 1.25 (3H each, both d, $J=6$ Hz, *sec.* C$\underline{H}_3\times2$ in two rhamnosides), 2.10 (3H, s, OAc), 5.44 (1/3H, d, $J=7$ Hz, 5′α-H), 6.15 (2/3H, d, $J=4$ Hz, 5′β-H). A solution of **18** (50 mg) in anhydrous 6% HCl–MeOH (5 ml) was heated under reflux for 2 hr, neutralized with Ag_2CO_3, and filtered. The filtrate was concentrated and the products were 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) by TLC (using benzene–acetone=1: 1 and benzene–MeOH=5: 1) and by GLC (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′07″ (major), 2′46″; b=3′41″ (major), 5′47″; c=11′04″ (major), 16′04″.

19, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770, 1224 (OAc), PMR ($CDCl_3$) δ: 2.11 (3H, s, OAc), 3.38 (3H), 3.52 (6H), 3.62 (3H) (all s, OC$\underline{H}_3\times4$), 5.42 (1H, d, $J=7$ Hz, anomeric H). **20**, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1764, 1227 (OAc), PMR ($CDCl_3$) δ: 2.07 (3H, s, OAc), 3.31, 3.39, 3.46, 3.53 (3H each, all s, OC$\underline{H}_3\times4$), 6.14 (1H, d, $J=3$ Hz, anomeric H).

Each solution of **19** and **20** (10 mg each) in anhydrous 6% HCl–MeOH (2 ml) was heated under reflux for 30 min, neutralized with Ag_2CO_3, and treated as for **18**. The product thus obtained was identified as methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (d) by TLC (benzene–MeOH=10: 1) and GLC (as for **18**, t_R: d=3′51″, 4′56″ (major).

21, colorless prisms from ether–CCl_4, mp 129—130°.[16] *Anal.* Calcd. for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 52.63; H, 5.28. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1765, 1738, 1615, 1235, 1217. UV λ_{max}^{EtOH} nm: 238.5 (ε=12600). PMR ($CDCl_3$) δ: 2.14 (6H, s, OAc×2), 3.91 (3H, s, $OC\underline{H}_3$), 5.24 (1H, d, J=3 Hz, varied to s on irradiation at δ 5.88, H_B), 5.46 (1H, s, H_C), 5.88 (1H, d, J=3 Hz, varied to s on irradiation at δ 5.24, H_A). Mass Spectrum *m/e* (%): 228 (2.6, M^+), 169 (5.3, **i**), 168 (17.5), 144 (21.1), 127 (50.9, **ii**), 126 (100, **iii**). High resolution mass spectrum *m/e*: Calcd. for $C_{10}H_{12}O_6$ (M^+): 228.063, $C_8H_9O_4$ (**i**): 169.050, $C_6H_7O_3$ (**ii**): 127.040, $C_6H_6O_3$ (**iii**): 126.032. Found: 228.064, 169.050, 127.040, 126.033.

Alkaline Hydrolysis of 21 giving 22——A solution of **21** (100 mg) in 0.1% NaOMe–MeOH (2 ml) was kept stirring at room temperature for 30 min, neutralized with anhydrous HCl–MeOH, and evaporated under reduced pressure to give a product which was purified by preparative TLC ($CHCl_3$–MeOH=5: 1). **22** (20 mg), amorphous. High resolution mass spectrum *m/e*: Calcd. for $C_6H_8O_4$ (M^+): 144.042. Found: 144.043. IR ν_{max}^{KBr} cm^{-1}: 3350, 1683, 1592. UV λ_{max}^{EtOH} nm: 237.0 (ε=14600). PMR (d_6-acetone+D_2O (one drop)) δ: 3.92 (3H, s, $OC\underline{H}_3$), 4.08 (1H, d, J=3 Hz, H_A), 4.49 (1H, d, J=3 Hz, H_B), 5.31 (1H, s, H_C). Mass Spectrum *m/e* (%): 144 (M^+, 4.5), 127 (26, **ii**), 126 (8, **iii**), 98 (60), 69 (100).

21-b

Alkaline Hydrolysis of 24 giving 25——A solution of a permethylate (**24**, 210 mg) of soyasaponin I (**4**)[7] in MeOH (30 ml) was treated with aq. 5% K_2CO_3 (5 ml) and the total mixture was heated under reflux for 5 hr, made weakly acidic with aq. 10% H_2SO_4, and extracted with ether. After the usual work-up, a syrupy product obtained from the extract was crystallized from acetone–MeOH to give **25** (190 mg) as colorless needles of mp 153—155°, $[\alpha]_D^{13}$ +5.8° (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{58}H_{98}O_{18}$: C, 64.30; H, 9.12. Found: C, 64.18; H, 9.12. IR ν_{max}^{Nujol} cm^{-1}: 1749, 1732 (COOH). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.95 (3H each), 1.00 (6H), 1.08, 1.16 (3H each) (all s, *tert.* $C\underline{H}_3$×7), 1.23 (3H, d, J=6 Hz, *sec.* $C\underline{H}_3$ in rhamnoside), 3.27, 3.37 (3H each), 3.48 (9H), 3.44, 3.52 (6H each), 3.63 (3H) (all s, $OC\underline{H}_3$×10), 4.41, 4.51 (1H each, both d, J=7 Hz, anomeric protons of glucuronide and galactoside), 5.23 (2H, br. s, $W_{h/2}$=7 Hz, anomeric H of rhamnoside and 12-H), 6.50 (1H, br. s, $W_{h/2}$=12.5 Hz, exchangeable with D_2O, $COO\underline{H}$).

Lead Tetraacetate Oxidation of 25 giving 26——A solution of **25** (150 mg) in benzene (7 ml) was treated with $Pb(OAc)_4$ (300 mg) and the total mixture was heated under reflux for 3 hr and was worked up as for **13** to give **26** (130 mg), amorphous, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1766, 1215 (OAc). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.95 (3H each), 1.00 (6H), 1.08, 1.16 (3H each) (all s, *tert.* $C\underline{H}_3$×7), 1.23 (3H, d, J=6 Hz, *sec.* $C\underline{H}_3$ in rhamnoside), 2.10, 2.13 (totally 3H, both s, OAc), 5.56 (2/3H, m, signal width=7 Hz, 5′α-H),[17] 6.22 (1/3 H, d, J=4 Hz, 5′β-H).

Alkaline Degradation of 26 giving 27, 28, 29, and 21——A solution of **26** (200 mg) in 0.5% NaOMe–MeOH (4 ml) was kept stirring at room temperature for 30 min until the reaction mixture became yellow and turbid. Neutralization with anhydrous 10% HCl–MeOH followed by evaporation of the solvent under reduced pressure furnished a product which was acetylated overnight with Ac_2O–pyridine (1: 2, 3 ml) and treated as for **14**. The products were purified by preparative TLC (benzene–MeOH=15: 1) to give **27** (80 mg, 83%),[7] two disaccharides (**28**, 22 mg and **29**, 49 mg) (86% combined yield), and the diene (**21**, 8 mg, 20%). **27** and **21** were identical to their respective authentic samples by IR (CCl_4), TLC, and mixed mp. **28**, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1758, 1215 (OAc), PMR ($CDCl_3$) δ: 1.25 (3H, d, J=6 Hz, *sec.* $C\underline{H}_3$ in rhamnoside), 2.10 (3H, s, OAc), 3.38 (3H), 3.44, 3.51 (6H each), 3.56 (3H) (all s, $OC\underline{H}_3$×6), 5.22 (1H, br. s, $W_{h/2}$=6 Hz, anomeric H of rhamnoside), 5.45 (1H, d, J=8 Hz, anomeric H of galactoside). **29**, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1767, 1221 (OAc), PMR ($CDCl_3$) δ: 1.23 (3H, d, J=6 Hz, *sec.* $C\underline{H}_3$ in rhamnoside), 2.11 (3H, s, OAc), 3.37 (3H), 3.47 (6H), 3.50, 3.53, 3.56 (3H each) (all s, $OC\underline{H}_3$×6), 5.05 (1H, d, J=2 Hz, anomeric H of rhamnoside), 6.18 (1H, d, J=3.5 Hz, anomeric H of galactoside).

A solution of each of **28** (22 mg) and **29** (54 mg) in anhydrous 6% HCl–MeOH (2 ml) was heated under reflux for 30 min, neutralized with Ag_2CO_3, and treated as for **18**. The products thus obtained were identified as methyl 2,3,4-tri-O-methyl-rhamnopyranoside (a) and methyl 3,4,6-tri-O-methyl-galactopyranoside (c) by TLC (benzene–acetone=1: 1) and GLC (as for **18**, t_R: a=2′07″ (major), 2′46″; c=11′05″ (major), 16′00″.

Alkaline Hydrolysis of 31 giving 32——A solution of **31** (300 mg), a permethylated derivative of desacyljegosaponin,[12] in MeOH (12 ml) was treated with aq. 10% K_2CO_3 (5 ml) and heated under reflux for 2 hr. A syrupy product obtained after treatment as for **24** was crystallized from *n*-hexane–acetone to furnish **32** as colorless needles of mp 209—210°, $[\alpha]_D^{16}$ −19.3° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{69}H_{118}O_{25}$: C, 61.49; H, 8.83. Found: C, 61.61; H, 8.81. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1751, 1723 (COOH).

Lead Tetraacetate Oxidation of 32 giving 33——To a solution of **32** (200 mg) in benzene (10 ml) was added $Pb(OAc)_4$ (350 mg) and the total mixture was heated under reflux for 3 hr and worked up as for **13** to give **33** (195 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1757, 1224 (OAc). PMR ($CDCl_3$) δ: 2.06, 2.09 (totally 3H, each s, OAc), 5.68 (1/2H, d, J=7 Hz, 5′α-H), 6.18 (1/2H, d, J=4 Hz, 5′β-H).

Alkaline Degradation of 33 giving 19, 20, 21, 28, 29, and 34——A solution of **33** (120 mg) in 0.5% NaOMe–MeOH (3 ml) was left standing at room temperature for 5 min, neutralized with anhydrous 6% HCl–MeOH, and evaporated under reduced pressure to give a residue which was acetylated overnight with Ac_2O–pyridine

16) Optically inactive.
17) The multiplicity is due to virtual long-range coupling.

(2: 1, 3 ml). Preparative TLC (benzene–acetone=5: 1) of the product funished **34** (43 mg, 89%), two monosaccharides (**19** and **20**, totally 19 mg, 83%), the diene (**21**, 7 mg, 39%), and two disaccharides (**28** and **29**, totally 32 mg, 85%). **34** and **21** were identical to the respective authentic samples by IR (CCl_4) and TLC. **19** and **20** were identical to authentic samples by TLC (benzene–acetone=3: 1) and were also respectively subjected to methanolysis by heating for 30 min with anhydrous 10% HCl–MeOH (1 ml) to furnish methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (d) (identified by TLC and GLC as above). **28** and **29** were identical to the authentic samples obtained above by TLC (benzene–acetone=3: 1) and were also respectively subjected to methanolysis by refluxing for 50 min in anhydrous 10% HCl–MeOH (1.5 ml). The products were identical to methyl 2,3,4-tri-O-methyl-rhamnopyranoside (a) and methyl 3,4,6-tri-O-methyl-galactopyranoside (c) by TLC and GLC as above.

Alkaline Hydrolysis of 36 giving 37——A solution of **36** (145 mg)[13] in MeOH (20 ml) was treated with aq. 5% K_2CO_3 (2.5 ml) and the total mixture was heated under reflux for 3 hr and worked up as for **24** to give **37** (135 mg), amorphous, $[\alpha]_D^{16}$ −10.9° (c=0.66, $CHCl_3$). *Anal.* Calcd. for $C_{69}H_{118}O_{25}$: C, 61.49; H, 8.83. Found: C, 61.70; H, 8.82. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1743, 1723 (COOH).

Lead Tetraacetate Oxidation followed by Alkali Treatment of 37 giving 19, 20, 21, 28, 29, and 39——To a solution of **37** (110 mg) in benzene (5 ml) was added $Pb(OAc)_4$ (160 mg) and the total mixture was heated under reflux for 2 hr and worked up as for **13** to furnish **38** (80 mg), amorphous, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1749, 1226 (OAc). **38** (70 mg) was then dissolved in 1% NaOMe–MeOH (2 ml) and left standing at room temperature for 5 min. The reaction mixture was neutralized with anhydrous 10% HCl–MeOH and evaporated under reduced pressure to give a residue which was acetylated overnight with Ac_2O–pyridine (2: 1, 1.5 ml). Preparative TLC (benzene–acetone=4: 1) of the product furnished **39** (24 mg, 84%), two monosaccharides (**19** and **20**, totally 11 mg, 80%), the diene (**21**, 4 mg, 32%), and two disaccharides (**28** and **29**, 16 mg, 75%). **39** and **21** were identical to authentic samples by IR (CCl_4) and TLC. The identification of **19**, **20**, **28**, and **29** was made by TLC and GLC as above.

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