[Chem. Pharm. Bull.
23(7)1520—1531(1975)]

UDC 547.597.02 : 581.192

Saponin and Sapogenol. X.[1] Structures of Jegosapogenin and Desacyljegosaponin obtained from Pericarps of *Styrax japonica* Sieb. et Zucc.

Isao Kitagawa, Yasuhiro Imakura, Teruaki Hayashi, and Itiro Yosioka

Faculty of Pharmaceutical Sciences, Osaka University[2]

(Received December 2, 1974)

The full detail of the chemical evidence on the structure of jegosapogenin (=21-O-tigloyl-barringtogenol C (Ia)), which was obtained along with barringtogenol C (I) and barringtogenol D (II) by acid hydrolysis of the pericarps saponin (=jegosaponin) of *Styrax japonica* Sieb. et Zucc. (Styracaceae), is described.

The structure of desacyl-jegosaponin, which is a common desacylated product of the saponin constituents of jegosaponin, and was prepared in a high yield by alkaline treatment of jegosaponin, has been established as barringtogenol C(3)-[β-D-glucopyranosyl-($1_{glu} \rightarrow 2_{glr}$)][$\alpha$-L-rhamnopyranosyl ($1_{rham} \rightarrow 2_{gal}$)-$\beta$-D-galactropyranosyl ($1_{gal} \rightarrow 4_{glr}$)]-$\beta$-D-glucuronopyranoside (VIf) on the basis of the chemical and physicochemical evidence.

The pericarps of *Styrax japonica* Sieb. et Zucc. (Styracaceae) have long been known as a rich saponin source. In the early years, several attempts for the chemical elucidation of the saponin named jegosaponin were made,[3] however no conclusive evidence on the structure of jegosaponin was provided, but it was clarified that the acid hydrolysis of jegosaponin furnished jegosapogenin as an aglycone which was further hydrolyzed with alkali to afford jegosapogenol and tiglic acid. Afterwards, some chemical investigations on jegosapogenin and jegosapogenol were reported,[4] and it was rather recently when jegosapogenol was determined to be identical with barringtogenol C (I)[5] by several independent works.[6,7]

As a continuative chemical study on the naturally occurring saponins, we have been working on the structure of jegosaponin for these years. As reported in the preliminary communications, we first elucidated the structure of jegosapogenin, which was obtained by acid hydrolysis of jegosaponin, to be 21-O-tigloyl-barringtogenol C (Ia)[7] and then, after clarifying that jegosaponin is a mixture of several acylated saponins by virtue of the soil bacterial hydrolysis,[8] we have recently put forward the structure of desacyl-jegosaponin (VIf) which is a common desacylated product of the saponin constituents of jegosaponin.[9] In the present paper, we wish to describe the full accounts of these chemical studies which are consistent with the structure assignments of jegosapogenin (Ia) and desacyl-jegosaponin (VIf), respectively.

---

1) Part IX: I. Kitagawa, H. Suzuki, K. Kitazawa, N. Yamao, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 355 (1975).

2) Location: *133-1, Yamada-Kami, Suita, Osaka 565, Japan.*

3) *a*) Y. Asahina and K. Momotani, *Yakugaku Zasshi*, **34**, 105 (1914); **35**, 1 (1915); *b*) C. Matsunami, *Yakugaku Zasshi*, **47**, 557 (1927).

4) *a*) C. Sone, *Acta Phytochim.*, **8**, 23 (1934); **9**, 83 (1935); *b*) S. Tobinaga, *Yakugaku Zasshi*, **78**, 526, 529 (1958).

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

6) *a*) T. Nakano, M. Hasegawa, T. Fukumaru. L.J. Durham, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **34**, 3135 (1969); *b*) H. Sugiyama, H. Aoyama, and K. Yamada, *Nippon Kagaku Zasshi*, **88**, 1316 (1967).

7) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353 (Preliminary report).

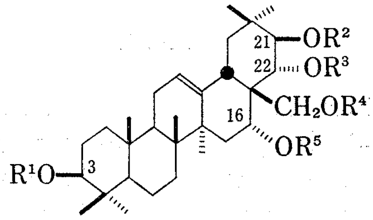
8) I. Yosioka, S. Saijoh, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 564 (1972).

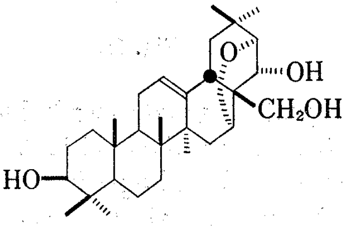
9) I. Kitagawa, Y. Imakura, T. Hayashi, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1675, 3009 (1974) (Preliminary reports).

Jegosapogenin (Ia)[7]

Acid hydrolysis of jegosaponin, which was isolated from the pericarps of *Styrax japonica* as described before,[8] afforded jegosapogenin (Ia) and two sapogenols being identical with barringtogenol C (I)[5] and barringtogenol D (II),[5] respectively. As elucidated previously,[5,8] I and II were secondarily formed during the acid hydrolysis of jegosaponin.

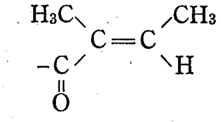
Jegosapogenin (Ia), $C_{35}H_{56}O_6$, mp 263—265°, shows the hydroxyl (3390 cm^{-1}), conjugated ester carbonyl (1690 cm^{-1}) and double bond (1648 cm^{-1}) absorption bands in its infrared (IR) spectrum. On alkaline treatment, Ia afforded barringtogenol C(I) and tiglic acid, thus indicating Ia to be a tigloyl derivative of I. Ordinary acetylation of Ia with acetic anhydride and pyridine yielded a triacetate (Ib) which preserves free 16α-OH as shown by its IR (3460 cm^{-1}) and proton magnetic resonance (PMR) spectrum (1H, m, δ 4.18: 16β-H[5]), and therefore 16α-OH in Ia is not esterified with tiglic acid. On treatment with anhydrous cupric sulfate and acetone, Ia was converted to a monoacetonide (Ic), which was further acetylated to give a monoacetylmonoacetonide (Id) with acetic anhydride and pyridine at room temperature. Here again, 16α-OH in Id was not acetylated. As is apparent from the PMR data given in Table I, 3β-OH in Ic is not acylated (3α-H at δ 3.26) whereas 3α-H geminal to an acetoxy function in Id is observed at δ 4.48 (t-like), thus 3β-OH being excluded from the possible location of the tigloyl moiety in Ia.





II barringtogenol D

	R¹	R²	R³	R⁴	R⁵	
I	H	H	H	H	H	=barringtogenol C
Ia	H	tigloyl	H	H	H	=jegosapogenin
Ib	Ac	tigloyl	Ac	Ac	H	
Ic	H	tigloyl	-C(CH₃)₂-		H	
Id	Ac	tigloyl	-C(CH₃)₂-		H	
Ie	Ac	Ac	Ac	Ac	H	
If	H	Me	Me	Me	Me	
Ig	Ac	Me	Me	Me	Me	



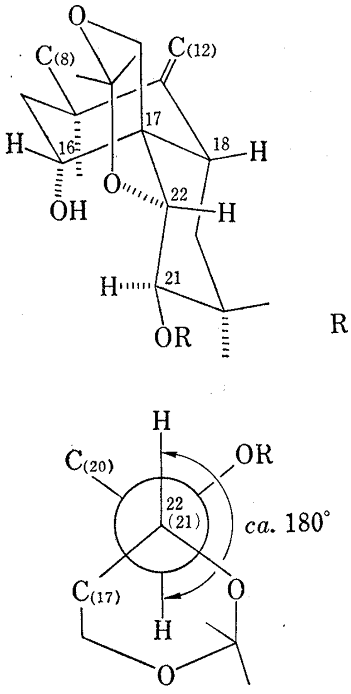
tigloyl

Chart 1

Table I. The PMR Data of Ib, Ic, Id, and Ie (in $CDCl_3$, 60 MHz) (Chemical shifts: δ; *J* Values in the parentheses: Hz)[a]

	3α-H	16β-H	21α-H 22β-H	28-H_2	tigloyl		
					β-H	β-CH_3	α-CH_3
Ib	4.51 (t-like)	4.18 (m)	5.62, 5.43 (AB q, 11)	3.68 (br. s)	6.83 (m)	1.80 (d, 6)	1.83 br. s)
Ic	3.26 (m)	4.82 (m)	5.65 3.90 (each d, 11)	3.59, 3.38 (AB q, 11)	6.84 (m)	1.92 (d, 6)	1.84 (br. s)
Id	4.48 (t-like)	4.80 (m)	5.64 3.87 (each d, 11)	3.55, 3.35 (AB q, 13)	6.80 (m)	1.80 (d, 6)	1.82 (br. s)
Ie	4.50 (t-like)	4.18 (m)	5.52, 5.40 (AB q, 11)	3.68 (br. s)	···	···	···

a) abbreviations: br=broad, d=doublet, m=multiplet, t=triplet, q=quartet



R=tigloyl

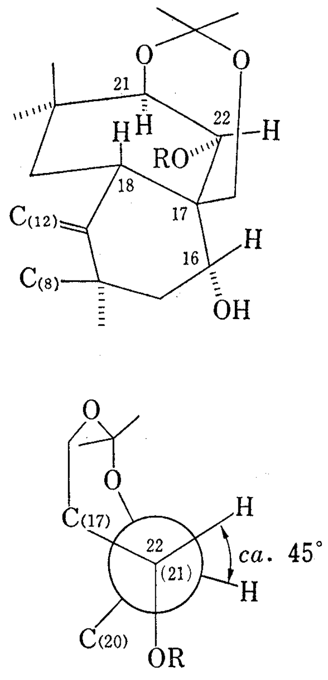


Fig. 1a

Fig. 1b

Since two proton signals ascribable to 21α-H and 22β-H in Ic and Id are observed respectively at δ 5.65 & 3.90 and δ 5.64 & 3.87 with J=11 Hz (*trans* diaxial coupling), it has become clear that the acetonide linkages in Ic and Id are formed between 17-CH_2OH and either 21β-OH or 22α-OH and consequently that the tigloyl function attaches to either 22α-OH or 21β-OH. As discussed before in case of the sapogenols of *Thea sinensis* L.[10] and 21-O-angeloyl-R_1-barrigenol,[11] the ready formation of acetonide linkage and the J values of 21α-H and 22β-H in Ic and Id, the latter assuring the chair conformation of the ring E,[12] demonstrate that the acetonide linkage in Ic is formed between 17-CH_2OH and 22α-OH rather than 21β-OH (Fig. 1a). To constitute the latter acetonide, the less favored boat-like or twist-boat conformation of the ring E is required and the probable dihedral angle between 21α-H and 22β-H would result in about 45° and such a large coupling constant (11 Hz) would not be observed (Fig. 1b). Moreover, the deshielded chemical shifts of 16β-H in Ic and Id (δ 4.82 and 4.80) as compared with those in Ib and Ie support the assignment, because, as shown in Fig. 1a, 16β-H in Ic and Id is considered to locate in the *peri* position toward the 22α- and 28-oxygens and will suffer the anisotropic effect.

Consequently, the structure of jegosapogenin has been established as 21-O-tigloyl-barringtogenol C (Ia).

Desacyl-jegosaponin (VIf)[9]

Although the chemical degradation disclosed that jegosapogenin (Ia) was an aglycone of jegosaponin, in order to assure its genuineness, the soil bacterial hydrolysis[13] of jegosaponin was undertaken prior to the structure study of jegosaponin. As reported previously,[8] it has become apparent that jegosaponin, although it showed a single spot on the thin-layer

---

[10] I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971).

[11] I. Yosioka, K. Hino, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 1499 (1972).

[12] I. Yosioka, A. Matsuda, K. Imai, T. Nishimura, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1200 (1971).

[13] *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 cited therein.

chromatogram (TLC), is a mixture of several saponins in which the triterpenoid parts at least comprise of the several acylated barringtogenol C derivatives. However, since every effort for the separation of the saponin component has been without success, we have initiated the structure elucidation of the desacylated derivative of jegosaponin (named desacyl-jegosaponin[14)]), which is obtained by alkaline treatment of jegosaponin as a sole product in a high yield and is considered to be a common desacylated product of the saponin constituents of jegosaponin.

Desacyl-jegosaponin (VIf), $C_{54}H_{88}O_{25} \cdot H_2O$, mp 248—251°, shows the presence of hydroxyl (br, 3400 cm^{-1}) and carboxyl (1730 cm^{-1}) in its IR spectrum. Acid hydrolysis of VIf yielded barringtogenol C (I), D-glucuronic acid (*vide infra*), D-glucose, D-galactose, and L-rhamnose, while mild acid treatment of VIf with 1 N H_2SO_4–EtOH followed by silica gel column chromatography and TLC separation furnished three prosapogenols designated as DJ-1 (IIIa), DJ-2 (IVa), and DJ-3 (Va) in which barringtogenol C (I) is the common aglycone. It should be pointed out here that since three monosaccharides of D-series mentioned above are known to take the Cl form as the preferred conformation, the coupling patterns of the respective anomeric protons are expected to reveal the mode of the glycoside linkages.

DJ-1 (IIIa), mp 199—201°, is an uronide of barringtogenol C as revealed by acid hydrolysis and possesses an ethoxycarbonyl function as shown by its IR absorption band at 1730 cm^{-1} and by the proton signals at δ 1.25 (3H, t, J=7 Hz) and δ 4.20 (2H, q, J=7 Hz) observed in the PMR spectrum of its hexaacetate (IIIb). The ethoxyl group in DJ-1 is considered to be introduced during the acid treatment in ethanol of desacyl-jegosaponin and the same group presents also in the other two prosapogenols DJ-2 and DJ-3 (*vide infra*). In order to confirm the presence of glucuronide moiety, DJ-1 was treated with $NaBH_4$ to give a reduction product (IIId), which possesses the hydroxyls but lacks the ethoxycarbonyl function as revealed by its IR spectrum. Acid hydrolysis of IIId afforded barringtogenol C and D-glucose. Methylation of DJ-1 with CH_3I/NaH/dimethyl sulfoxide (DMSO)[15)] furnished an octa-O-methyl derivative (IIIc), which does not preserve the free hydroxyl as shown by its IR spectrum (CCl_4). The PMR spectrum of IIIc exhibits the signals due to seven methoxyls, one methoxycarbonyl, and one anomeric proton at δ 4.32 (d, J=8 Hz) which suggests the presence of the β-glucuronopyranoside linkage. In the mass spectrum of IIIc, the prominent fragment ion peaks are observed at *m/e* 197 (**i**-CH_2OMe-3MeOH, derived *via* the reverse Diels-Alder type fragmentation of ring C[5,16a)] and at *m/e* 233 (**iii**), 201 (**iii**-MeOH), and 169 (**iii**-2MeOH) (originated from the glucuronopyranoside moiety[16b)]).

DJ-2 (IVa) (amorphous) is a glucoside of DJ-1. On acetylation with acetic anhydride and pyridine, DJ-2 was converted to a crystalline nonaacetate (IVb), mp 159—161°, whose IR spectrum exhibits the hydroxyl (3610, 3490 cm^{-1} (w)), ethoxycarbonyl and acetoxyl (1765, 1756 cm^{-1}) absorption bands. The PMR spectrum of IVb shows the signals assignable to an ethoxycarbonyl (δ 1.26, 3H, t, J=8 Hz; δ 4.19, 2H, q, J=8 Hz), nine acetoxyls, and two anomeric protons at δ 4.51 (1H, d, J=8 Hz) and δ 4.69 (1H, d, J=7.5 Hz) being indicative of the presence of two β-linkages. Complete methylation of DJ-2 with CH_3I/NaH/DMSO furnished an undeca-O-methyl derivative (IVc) (no OH in its IR spectrum), which on subsequent treatment with $LiAlH_4$ was converted to a reduction product (IVe) (IR: 3400 cm^{-1} (w)). The two anomeric proton signals in the PMR spectrum of IVe are observed at δ 4.38 and δ 4.67 (1H each, d, J=7 Hz), which suggests that glucuronic acid and glucose moieties in DJ-2 are connected with β-linkage.

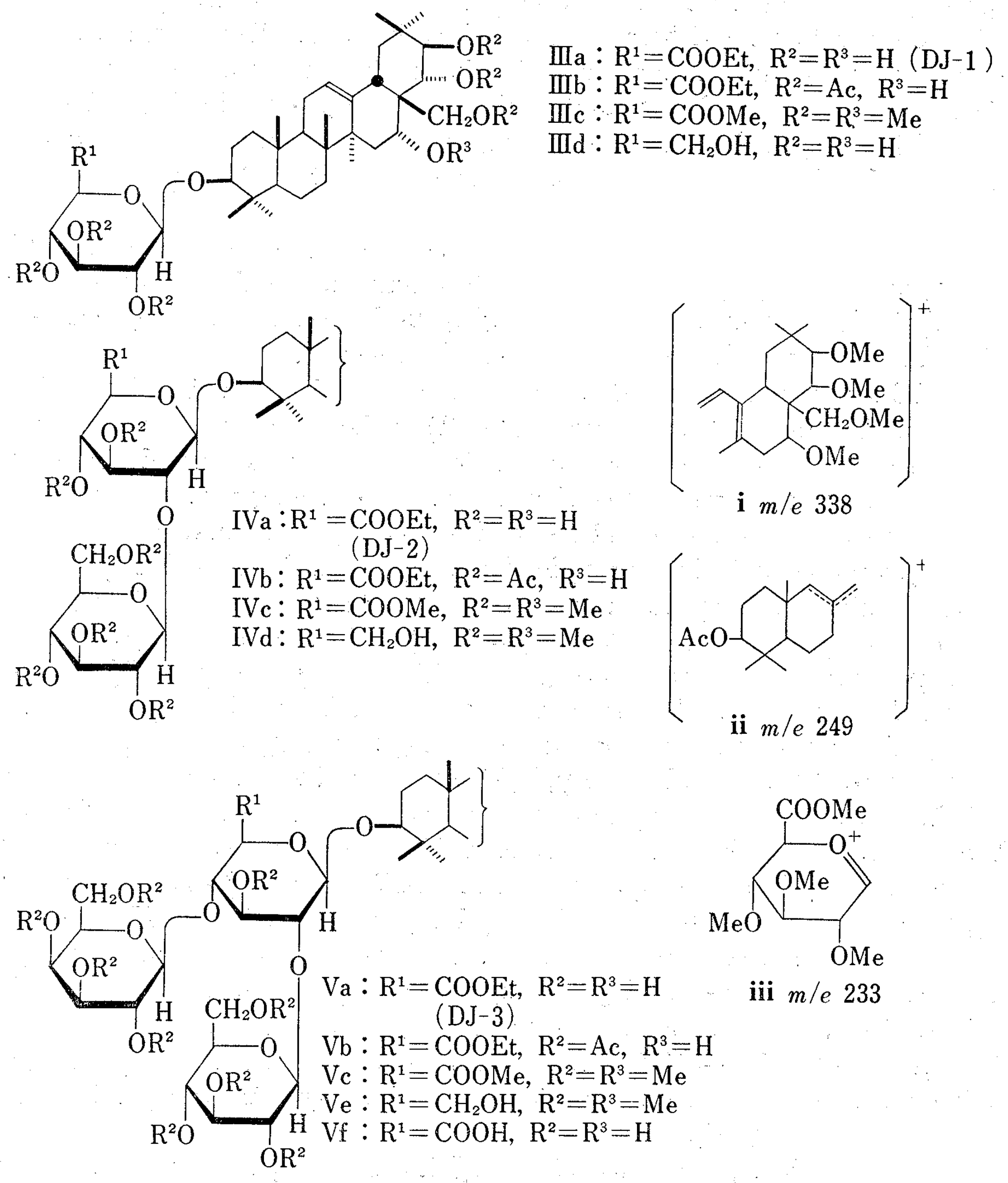
DJ-3 (Va), mp 257—261°, a galactoside of DJ-2, afforded on ordinary acetylation a dodecaacetate (Vb), whose PMR spectrum shows the proton signals due to an ethoxycarbonyl

---

14) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1339 (1974).

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

16) *a*) 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; *b*) *Idem, ibid.*, p. 203.



IIIa : R^1=COOEt, R^2=R^3=H (DJ-1)
IIIb : R^1=COOEt, R^2=Ac, R^3=H
IIIc : R^1=COOMe, R^2=R^3=Me
IIId : R^1=CH_2OH, R^2=R^3=H

IVa : R^1=COOEt, R^2=R^3=H (DJ-2)
IVb : R^1=COOEt, R^2=Ac, R^3=H
IVc : R^1=COOMe, R^2=R^3=Me
IVd : R^1=CH_2OH, R^2=R^3=Me

i *m/e* 338

ii *m/e* 249

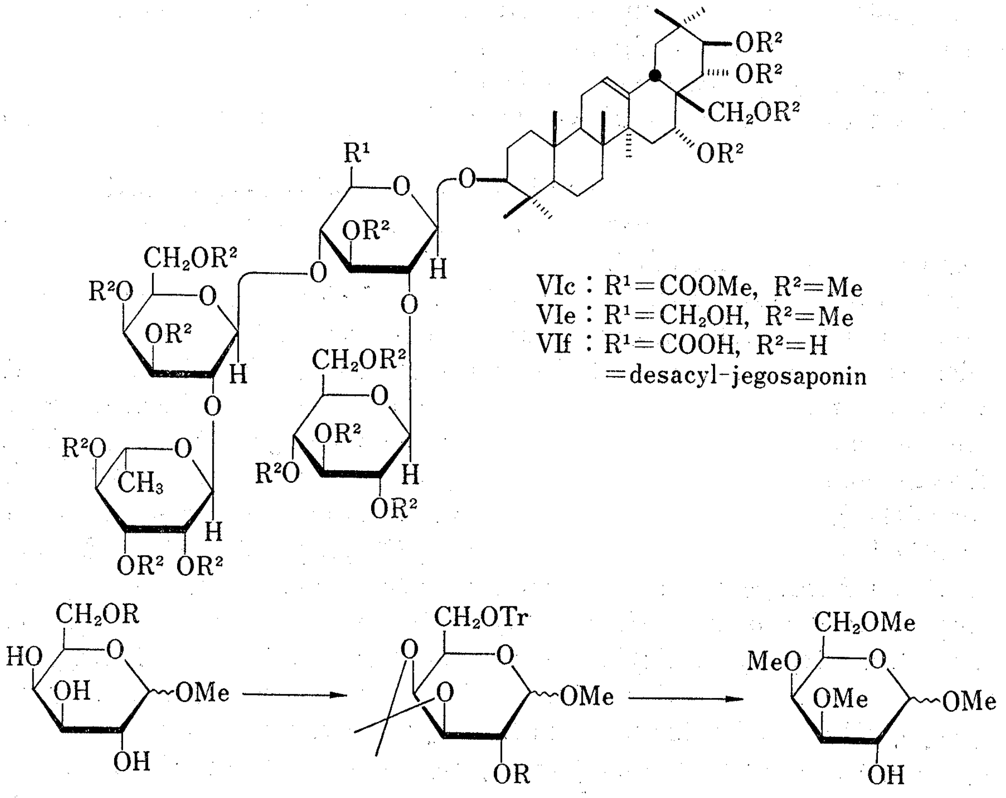
iii *m/e* 233

Va : R^1=COOEt, R^2=R^3=H (DJ-3)
Vb : R^1=COOEt, R^2=Ac, R^3=H
Vc : R^1=COOMe, R^2=R^3=Me
Ve : R^1=CH_2OH, R^2=R^3=Me
Vf : R^1=COOH, R^2=R^3=H

Chart 2

function as above. On methylation with CH_3I/NaH/DMSO, DJ-3 afforded a tetradeca-O-methyl derivative (Vc), which possesses no hydroxyl (IR) and was subjected to $LiAlH_4$ reduction to give a product (Ve) (IR: 3600 cm^{-1} (w)). The PMR spectrum of Ve shows three anomeric proton signals at δ 4.41, 4.88, and 4.96 (1H each, d, J=7 Hz) being indicative of the presence of three β-linkages in DJ-3.

Methanolysis of IVe and Ve with 9% HCl-MeOH furnished, as the methylated carbohydrate ingredients, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside and methyl 3,4-di-O-methyl-glucopyranoside from the former and methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, and methyl 3-O-methyl-glucopyranoside from the latter. A methylated aglycone (If) commonly obtained from both IVe and Ve was characterized as the monoacetate (Ig), which possesses an acetoxyl (IR: 1737, 1246 cm^{-1}; PMR: δ 2.04, 3H, s) and four methoxyls (PMR) but no hydroxyl (IR). The location of the acetoxyl has been determined to be at C-3 on the basis of a characteristic proton signal observed at δ 4.53 (1H, t-like) assignable to 3α-H geminal to 3β-OAc.[5] In addition, the fragment ion peaks at *m/e* 338 (**i**), 249 (**ii**), 197 (**i**-CH_2OMe-3MeOH), and 189 (**ii**-AcOH) observed in the



VIc : R^1=COOMe, R^2=Me
VIe : R^1=CH_2OH, R^2=Me
VIf : R^1=COOH, R^2=H
=desacyl-jegosaponin

VIIa : R=H
VIIb : R=Tr

VIIIa : R=H, α-anomer
VIIIb : R=H, β-anomer
IXa : R=CONH-C_6H_5
α-anomer
IXb : R=CONH-C_6H_5
β-anomer

Xa : α-anomer
Xb : β-anomer

Chart 3

mass spectrum of Ig also support the assignment. Therefore, the methylated aglycone has been determined to be 16,21,22,28-tetra-O-methyl-barringtogenol C (If) and it follows that the carbohydrate moieties in the above mentioned prosapogenols (DJ-1, DJ-2, and DJ-3) are connected at 3β-OH of barringtogenol C.

The accumulated evidence so far has led to the formulations of DJ-1, DJ-2, and DJ-3 as IIIa, IVa, and Va, respectively.

Finally, the structure of desacyl-jegosaponin (VIf) has been elucidated on the basis of the following evidence. Complete methylation of desacyl-jegosaponin as above afforded a crystalline hexadeca-O-methyl derivative (VIc), mp 219—221°, which exhibits no hydroxyl but the ester absorption band (1762 cm^{-1}) in its IR spectrum (CCl_4). The PMR spectrum of VIc (in $CDCl_3+C_6D_6$) shows four anomeric proton signals at δ 4.47, 4.96, 5.07 (1H each, d, $J=7$ Hz) and δ 5.29 (2H, br.s, overlapped with the signal due to 12-H). Reduction of VIc with $LiAlH_4$ furnished a pentadeca-O-methyl derivative (VIe), mp 210—212° (IR: 3592 cm^{-1} (w)), whose PMR spectrum (in $CDCl_3$) shows a three-proton doublet at δ 1.23 assignable to the rhamnose-methyl and four anomeric proton signals at δ 4.38 (1H, d, $J=7$ Hz, glucuronopyranoside), 4.59 (1H, d, $J=7$ Hz, glucopyranoside), 4.96 (1H, d, $J=7$ Hz, galactopyranoside), and δ 5.19 (1H, br.s, rhamnopyranoside). Methanolysis of VIe yielded, as the methylated carbohydrate components, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside (*vide infra*), and methyl 3-O-methyl-glucopyranoside, and If as the methylated aglycone which was identified as its monoacetate (Ig) as described above. The β-linkage of three monosaccharides of D-series has been substantiated by the coupling patterns of the respective anomeric protons. On the other hand, the anomeric configuration of the terminal rhamnopyranoside linkage has been determined as α by application of the Klyne's rule[17]: $[M]_D$(VIf)$-[M]_D$(Vf)[18]$=-120.5°$; $[M]_D$ (methyl α-L-rhamnopyranoside)$=-109°$; $[M]_D$ (methyl β-L-rhamnopyranoside)$=+169°$.[19]

Consequently, the structure of desacyl-jegosaponin has been established as barringtogenol C(3)-[β-D-glucopyranosyl($1_{glu}\rightarrow 2_{glr}$)][α-L-rhamnopyranosyl($1_{rham}\rightarrow 2_{gal}$)-β-D-galactopyranosyl($1_{gal}\rightarrow 4_{glr}$)]-β-D-glucuronopyranoside (VIf).

Since methyl 3,4,6-tri-O-methyl-D-galactopyranoside[20] was unavailable for comparison, we prepared it through the following procedure. Thus, methyl 6-O-trityl-D-galactopyranoside (VIIb), which was prepared from methyl D-galactopyranoside (VIIa), was treated with 2,2-dimethoxypropane and *p*-toluenesulfonic acid to afford two isopropylidene derivative (VIIIa, b). The one showing the anomeric proton signal at δ 4.63 (d, $J=4$ Hz) in its PMR spectrum is assigned as α-anomer (VIIIa), while the other (the anomeric proton at δ 4.13, d, $J=8$ Hz) as β-anomer (VIIIb). Respective treatment of the both anomers with phenylisocyanate in pyridine furnished two crystalline phenylcarbamate, IXa, mp 190—191°, and IXb, mp 148—150°. The IR and PMR spectra of both agree with their formulations: especially, the anomeric protons being observed at δ 4.80 (2H, br.s, $W_{h/2}=6$ Hz, overlapped with the signal due to 2-H) in IXa and at δ 4.27 (1H, d, $J=8$ Hz) in IXb (the signal due to 2-H at δ 4.77, d.d, $J=6$ & 8 Hz). Treatment of IXa and IXb with methanolic hydrogen chloride followed by methylation with $CH_3I/Ag_2O/DMF$[21] and reduction with $LiAlH_4$ resulted in the formation of methyl 3,4,6-tri-O-methyl-α-D-galactopyranoside (Xa) and -β-D-galactopyranoside (Xb). The assignment of the anomeric configurations was based on the anomeric proton signal patterns: δ 4.58 (1H, d, $J=4$ Hz) in Xa and δ 4.12 (1H, d, $J=8$ Hz) in Xb. Xa and Xb thus prepared were identical respectively with the partially methylated α- and β-D-galactopyranoside specimens obtained by methanolysis of VIe (*vide supra*).

Desacyl-jegosaponin (VIf) possesses the glucuronide moiety directly attached to the aglycone and was already shown to be cleaved on ultraviolet irradiation to afford the aglycone.[14,22] The present structure elucidation provides with the further chemical support for the previously reported photochemical cleavage of the uronide linkage in saponin.[14,23]

Experimental[24]

Acid Hydrolysis of Jegosaponin——A solution of jegosaponin (30 g)[8] in 10% H_2SO_4–EtOH (150 ml–150 ml) was refluxed for 4 hr. After cooling, the resulting precipitates were collected by filtration and the filtrate was concentrated under reduced pressure to remove EtOH and the precipitates were again collected by filtration. The combined precipitates were washed with water and dried *in vacuo* to give the crude sapogenol

---

[17] W. Klyne, *Biochem. J.*, **47**, xli (1950).

[18] Prepared by alkaline treatment of DJ-3 (Va) for the comparison of the optical data.

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

[20] E.B. Rathbone, A.M. Stephen, and K.G.R. Pachler, *Carbohyd. Res.*, **21**, 83 (1972).

[21] R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

[22] I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem.* & *Ind.*, **1973**, 276.

[23] I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, **1973**, 3997.

[24] The following instruments were used for obtaining the physical data. Melting points: Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type), and recorded uncorrected; Specific rotations: Rex Photoelectric Polarimeter NEP-2 (l=1 dm); IR spectra: Hitachi IR Spectrometer EPI-S2 or EPI-G3; PMR spectra (tetramethylsilane as an internal standard): Hitachi R-20A (60 MHz) or R-22 (90 MHz) NMR Spectrometer; Mass spectra: Hitachi RMU-6D Mass Spectrometer (direct inlet, at 70 eV). For chromatography, silica gel (Merck, 70—230 mesh) or alumina (Merck or, Woelm neutral) was used for column and silica gel (Camag D-5) for TLC on which detection was made by spraying 1% $Ce(SO_4)_2$–10% H_2SO_4 solution followed by heating. For gas-liquid chromatography (GLC), Hitachi Gas Chromatograph Model 063 with FID was used, and Toyo Filter Paper No. 50 was used for paper partition chromatography (PPC).

(9 g), of which 1 g was chromatographed on alumina (50 g) eluting with $CHCl_3$ to afford jegosapogenin (Ia, 408 mg), barringtogenol D (II, 42 mg), and barringtogenol C (I, 54 mg). I and II were identified with the authentic specimens5) by mixed mp, IR (KBr), and TLC. The analytical sample of jegosapogenin (Ia) was obtained by recrystallization from aq. EtOH as colorless needles of mp 245—247° (mp 263—265° from acetone–benzene), $[\alpha]_D^{18}+10.5°$ ($c=1.0$, pyridine). *Anal.* Calcd. for $C_{35}H_{56}O_6$: C, 73.39; H, 9.85. Found: C, 72.82; H, 9.83. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1690 (tiglate CO), 1648 (C=C), 1265.

Alkaline Treatment of Jegosapogenin (Ia)——A mixture of Ia (3 g) in aq. 80% EtOH–KOH (100 ml-6 g) was heated under reflux for 1 hr and diluted with water. The precipitates collected by filtration were washed with a small amount of EtOH and chromatographed on alumina (80 g) as above and crystallized from aq. EtOH to give colorless needles (450 mg) which were identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. The identity was also assured by preparing the tetraacetate (Ie).5) The aqueous filtrate was extracted with ether and the extractive obtained by evaporation of the solvent was treated with ethereal diazomethane to give a methyl ester which was identified with methyl tiglate by GLC: 1.5% SE-30 on chromosorb W, 3 mm × 2 m column, temp. 50°, N_2 70 ml/min, retention time (t_R) 3.2 min.

Jegosapogenin Triacetate (Ib)——Acetylation of Ia (120 mg) with Ac_2O (3 ml)-pyridine (5 ml) by keeping at room temperature for three days afforded a product, which, after ordinary work-up, was chromatographed on silica gel (5 g) eluting with benzene–$CHCl_3$ (2:1) to give Ib, colorless needles (85 mg) of mp 280—283° (from aq. EtOH), $[\alpha]_D^{18}+19.6°$ ($c=0.8$, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{62}O_9$: C, 70.45; H, 8.94. Found: C, 70.23; H, 9.10. IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1739 (OAc), 1726 (tiglate CO), 1654 (C=C). PMR: as given in Table I.

Jegosapogenin Monoacetonide (Ic)——To a solution of Ia (120 mg) in dry acetone (50 ml) was added anhydrous $CuSO_4$ (3 g) and the total mixture was kept stirring for 8 hr at room temperature and left standing overnight. After removing the precipitates by filtration, the filtrate was evaporated to dryness and the residue was chromatographed on alumina (5 g) eluting with benzene–$CHCl_3$ mixture (7:3—4:6) to give Ic, colorless plates (47 mg) of mp 175—177° (from aq. EtOH), $[\alpha]_D^{18}+16.6°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{60}O_6$: C, 74.47; H, 9.87. Found: C, 74.50; H, 10.03. IR ν_{max}^{KBr} cm^{-1}: 3550(OH), 1726, 1717(tiglate CO), 1645(C=C). PMR: as given in Table I.

Acetylation of Ic——Treatment of Ic (80 mg) with Ac_2O (3 ml) and pyridine (5 ml) at room temperature for 24 hr followed by usual work-up yielded a product, which was purified by passing through an alumina column (5 g) with the aid of $CHCl_3$–EtOAc (9:1). The acetate (Id, 61 mg) was obtained by recrystallization from aq. EtOH as colorless rods of mp 232—234°, $[\alpha]_D^{18}+26.8°$ ($c=0.47$, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{62}O_7$: C, 73.35; H, 9.54. Found: C, 73.09; H, 9.60. IR ν_{max}^{KBr} cm^{-1}: 3510 (OH), 1742 (OAc), 1725 (tiglate CO), 1652 (C=C). PMR: as given in Table I.

Desacyl-jegosaponin (VIf)——Recrystallization from aq. EtOH of desacyl-jegosaponin, which was prepared by alkaline treatment of jegosaponin as described before,14) afforded colorless needles of mp 248—251°, $[\alpha]_D^{10}-12.0°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{54}H_{88}O_{25}\cdot H_2O$: C, 56.14; H, 7.85. Found: C, 56.10; H, 7.89. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1730 (COOH).

Complete Acid Hydrolysis of Desacyl-jegosaponin (VIf)——a) A solution of VIf (30 mg) in 6 N H_2SO_4–EtOH (1:1, 10 ml) was heated under reflux for 3 hr and poured into cold water. The precipitates (9 mg) were collected by filtration, recrystallized from aq. EtOH, and identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. The aqueous filtrate was neutralized with aq. saturated $Ba(OH)_2$ and the precipitates were removed by a centrifuge. The syrup obtained after concentration of the aqueous layer under reduced pressure was subjected to PPC developing with iso-PrOH–*n*-BuOH–water (7:1:2) for 24 hr. The spots were detected by the naphthoresorcinol-phosphoric acid reagent and identified with glucuronic acid, glucose, galactose, and rhamnose, respectively. b) In order to examine the optical properties of the monosaccharide ingredients, VIf (500 mg) was hydrolyzed with acid and the monosaccharides were isolated by preparative PPC (developed as above): L-rhamnose: $Rf=0.66$, $[\alpha]_D^{20}+21.0°$ ($c=0.8$, water, 90 min after making solution)(authentic specimen: $[\alpha]_D^{20}+17.5°$); D-glucose: $Rf=0.50$, $[\alpha]_D^{20}+39.7°$ ($c=1.3$, water, 19 hr after making solution)(authentic specimen: $[\alpha]_D^{20}+28.5°$); D-galactose: $Rf=0.44$, $[\alpha]_D^{20}+65.6°$ ($c=0.5$, water, 12 hr after making solution)(authentic specimen: $[\alpha]_D^{20}+55.8°$); glucuronic acid: $Rf=0.10$. Three monosaccharides were also identified as their trimethylsilyl derivatives by GLC: 3% SE-30 on chromosorb W (80—100 mesh), 3 mm × 2 m column, temp. 220°, carrier gas N_2 20 ml/min, t_R (min): L-rhamnose (2.0, 2.2, 2.8); D-glucose (3.9, 4.5, 5.1); D-galactose (5.0, 6.5).

Partial Acid Hydrolysis of Desacyl-jegosaponin (VIf) giving DJ-1 (IIIa), DJ-2 (IVa), and DJ-3 (Va)——A solution of VIf (5.5 g) in 1 N H_2SO_4–EtOH (1:2, 270 ml) was refluxed for 2 hr and concentrated under reduced pressure to remove EtOH while neutralizing with aq. 10% NaOH. The resulting aqueous mixture was extracted several times with *n*-BuOH saturated with water and the organic layer was taken, washed with water, and evaporated to dryness *in vacuo* to give the residue (3.3 g). The residue was then chromatographed on silica gel (495 g) eluting with $CHCl_3$–MeOH–H_2O (50:12:1) to give barringtogenol C (I, 420 mg), DJ-1 (IIIa, 146 mg), DJ-2 (IVa, 152 mg), DJ-3 (Va, 289 mg), and the mixtures (combined weight 1.5 g).

DJ-1 (IIIa), mp 199—201° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{9}-12.0°$ ($c=1.0$. MeOH). *Anal.* Calcd. for $C_{38}H_{62}O_{11}\cdot 2H_2O$: C, 62.44; H, 9.10. Found: C, 62.79; H, 8.67. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1730 (COOEt).

DJ-2 (IVa), white powder (precipitated with ether), $[\alpha]_D^{10}-14.0°$ ($c=1.0$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1740 (COOEt).

DJ-3 (Va), mp 265—269° (colorless needles from EtOH), mp 279—282° (from aq. EtOH), $[\alpha]_D^{11}-4.7°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{50}H_{82}O_{21}\cdot\frac{1}{2}H_2O$: C, 58.42; H, 8.08. Found: C, 58.34; H, 8.14. IR ν_{max}^{KBr} cm^{-1}: 3460—3360 (br, OH), 1736 (COOEt).

Acid Hydrolysis of DJ-1 (IIIa)——A solution of IIIa (10 mg) in 1 N H_2SO_4–EtOH (2:1, 12 ml) was refluxed for 8 hr, diluted with water, and concentrated under reduced pressure to remove EtOH. The precipitates collected by filtration were crystallized from MeOH and identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. After treating the aqueous filtrate as in case of the acid hydrolysis of VIf, the residue was identified with glucuronic acid by PPC (iso-PrOH–*n*-BuOH–H_2O=7:1:2, developed for 24 hr).

Acetylation of DJ-1 (IIIa) giving Hexaacetate (IIIb)——Acetylation of IIIa (40 mg) with Ac_2O (2 ml) and pyridine (2 ml) at 28° for 24 hr followed by usual work-up yielded a product (45 mg) which was purified by preparative TLC (benzene–EtOAc=1:1, detection with water) to give IIIb (30 mg, amorphous), $[\alpha]_D^{24}+0.71°$ ($c=0.56$, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3618, 3448 (w, OH), 1766, 1754 (OAc, COOEt). Since the purity of IIIb was assured by TLC, the PMR analysis was made. PMR ($CDCl_3$, 90 MHz) δ: 0.71 ,0.89 (3H each), 0.92 (6H), 1.03, 1.25, 1.43 (3H each) (all s, $CH_3\times7$), 1.25 (3H, t, $J=7$ Hz), 4.20 (2H, q, $J=7$ Hz), (OCH_2-CH_3), 2.00, 2.04 (18H, OAc×6), 3.11 (1H, m, 3α–H), 4.59 (1H, d, $J=8$ Hz, anomeric H), 5.37, 5.52 (2H, ABq, $J=10$ Hz, 21α–H, 22β–H).

$NaBH_4$ Reduction of DJ-1 (IIIa) giving IIId——To a solution of IIIa (50 mg) in EtOH (2 ml) was added $NaBH_4$ (15 mg) in EtOH (1.5 ml) and the total mixture was kept stirring at room temperature for 30 min, treated with acetone (6 ml), stirred further for 10 min, and treated with EtOH (6 ml). The reaction mixture was then passed through the columns of Amberlite IRA-400 (OH^-)(1 g) and Dowex 50W×8 (H^+)(1 g) successively and concentrated under reduced pressure to give a reaction product (45 mg). Crystallization of the product from MeOH gave a pure sample of IIId, mp 247—249° (colorless needles), $[\alpha]_D^{20}-5.5°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{36}H_{60}O_{10}\cdot4H_2O$: C, 59.64; H, 9.46. Found: C, 60.18; H, 8.95. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1625 (C=C).

Acid Hydrolysis of IIId——A mixture of IIId (10 mg) in 1 N H_2SO_4–EtOH (2:1, 12 ml) was heated under reflux for 8 hr, diluted with water, and concentrated under reduced pressure to remove EtOH. The precipitates collected by filtration were crystallized from MeOH and identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. The aqueous filtrate was passed through a column of Amberlite IR 45 (OH^-) (5 g) and concentrated under reduced pressure to give a residue which was identified with glucose by PPC (detection by aniline hydrogen phthalate, *Rf*=0.50) and by GLC (as trimethylsilyl derivative). In order to examine the optical property, acid hydrolysis of IIId (40 mg) was undertaken and glucose isolated by preparative PPC showed $[\alpha]_D^{20}+30.3°$ ($c=1.14$, water), thus being confirmed as D-glucose.

Methylation of DJ-1 (IIIa) giving IIIc——a) DMSO Carbanion: A mixture of NaH (110 mg, commercial NaH was washed with *n*-hexane beforehand) in DMSO (2 ml) was heated at 60—65° for 1.5 hr under N_2 atmosphere. b) To a solution of IIIa (40 mg) in DMSO (2 ml) was added the above prepared DMSO carbanion and the total mixture was kept stirring at room temperature for 1 hr, treated with CH_3I (2 ml), kept stirring for 19 hr in the dark, and poured into ice-water. The aqueous mixture was extracted with $CHCl_3$ several times and the $CHCl_3$ extract was washed with aq. 5% $Na_2S_2O_3$ and water successively and treated in the usual manner. The product was purified by preparative TLC (benzene–acetone=10:1, detection with I_2) and IIIc (14 mg) was obtained as white powder, $[\alpha]_D^{26}-6.0°$ ($c=0.5$, $CHCl_3$). *Anal.* Calcd. for $C_{44}H_{74}O_{11}$: C, 67.83; H, 9.57. Found: C, 67.59; H, 9.36. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1753 (COOMe). PMR ($CDCl_3$, 90 MHz) δ: 0.84, 0.87 (3H each), 0.91 (9H), 1.02, 1.31 (3H each) (all s, $CH_3\times7$), 3.27, 3.28 (3H each), 3.50 (6H), 3.56, 3.62, 3.63 (3H each) (all s, $OCH_3\times7$), 3.80 (3H, s, $COOCH_3$), 4.34 (1H, d, $J=8$ Hz, anomeric H), 5.27 (1H, m, 12–H). Mass Spectrum *m/e* (%): 778 (M^+, <2), 746 (M^+–MeOH, 3), 714 (M^+–2MeOH, 4), 669 (M^+–2MeOH–CH_2OMe, 5), 306 (**i**-MeOH, 3), 274 (**i**-2MeOH, 4), 261 (**i**-MeOH–CH_2OMe, 8), 242 (**i**–3MeOH, 3), 233 (**iii**, 4), 229 (**i**–2MeOH–CH_2OMe, 34), 201 (**iii**–MeOH, 33), 197 (**i**–3MeOH–CH_2OMe, 19), 169 (**iii**–2MeOH, 8), 75 (100).

Acid Hydrolysis of DJ-2 (IVa)——A solution of IVa (10 mg) in 1N H_2SO_4–EtOH (3:1, 8 ml) was heated under reflux for 5 hr, diluted with water, and concentrated under reduced pressure to remove EtOH. The precipitates were collected by filtration, crystallized from MeOH, and identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. The aqueous filtrate was neutralized with aq. saturated $Ba(OH)_2$, centrifuged to remove the precipitates, evaporated to dryness *in vacuo*, and identified with glucuronic acid (*Rf*=0.10) and glucose (*Rf*=0.52) by PPC as above.

Acetylation of DJ-2 (IVa) giving Nonaacetate (IVb)——Acetylation of IVa (100 mg) with Ac_2O (5 ml) and pyridine (4 ml) at 28° for 24 hr followed by usual work-up gave a product (134 mg) which was purified by preparative TLC (benzene–EtOAc=1:1, detection by spraying water). Crystallization of the product from MeOH furnished IVb (96 mg) of mp 159—161°, $[\alpha]_D^{26}-4.9°$ ($c=0.7$, $CHCl_3$). *Anal.* Calcd. for $C_{62}H_{90}O_{25}$: C, 60.28; H, 7.34. Found: C, 59.80; H, 7.32. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3490 (w, OH), 1765, 1756 (OAc, COOEt). PMR ($CDCl_3$, 90 MHz) δ: 1.26 (3H, t, $J=8$ Hz), 4.19 (2H, q, $J=8$ Hz) (OCH_2CH_3), 1.98 (6H, s), 2.00 (12H, s), 2.02, 2.04, 2.10 (3H each, s) (OAc×9), 3.67 (2H, br. s, $W_{h/2}=6$ Hz, 28-H_2), 4.51 (1H, d, $J=8$ Hz), 4.69 (1H, d, $J=7.5$ Hz) (anomeric H×2), 5.38, 5.51 (2H, ABq, $J=10$ Hz, 21α-H, 22β-H).

Methylation of DJ-2 (giving IVc) followed by $LiAlH_4$ Reduction giving IVe——A solution of IVa (100 mg) in DMSO (5 ml) was treated with DMSO carbanion (prepared from 400 mg of NaH and 7 ml of DMSO as above) at room temperature for 1 hr under N_2 atmosphere, added with CH_3I (8 ml) and kept stirring for further 19 hr in the dark. After pouring into ice-water, the total reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was treated as above and subjected to preparative TLC (benzene–acetone=20:2, detection by spraying water) to give IVc (45 mg) which showed no OH absorption band in the IR spectrum. IVc thus obtained was dissolved in dry ether (20 ml), treated with $LiAlH_4$ (30 mg), and heated under reflux for 2 hr. The reaction mixture was then treated with aqueous ether, poured into diluted H_2SO_4, and extracted with ether. The crude product obtained after usual work-up was purified by preparative TLC (benzene–acetone=7:1, detection with water) and treated with acetone to give IVe (38 mg, amorphous), $[\alpha]^{8}_{D}+4.4°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{52}H_{90}O_{15}$: C, 65.38; H, 9.49. Found: C, 65.59; H, 9.25. IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3400 (w, OH), no COOMe. PMR ($CDCl_3$, 90 MHz) δ: 3.28 (6H, s), 3.36 (3H, s), 3.51, 3.54 (6H each, s), 3.58 (3H, s), 3.63 (6H, s) ($OCH_3 \times 10$), 4.38 (1H, d, $J=7$ Hz), 4.67 (1H, d, $J=7$ Hz) (anomeric $H \times 2$), 5.28 (1H, m, 12-H).

Acid Hydrolysis of DJ-3 (Va)——A solution of Va (10 mg) in 1 N H_2SO_4–EtOH (3:1, 8 ml) was refluxed for 6 hr, diluted with water, concentrated under reduced pressure to remove EtOH. The precipitates were collected by filtration, crystallized from MeOH, and identified with barringtogenol C (I) by mixed mp, IR (KBr), and TLC. The aqueous filtrate was treated as above and glucose ($Rf=0.51$), galactose ($Rf=0.40$), and glucuronic acid ($Rf=0.10$) were identified by PPC.

Acetylation of DJ-3 (Va) giving Dodecaacetate (Vb)——Acetylation of Va (45 mg) with Ac_2O (4 ml) and pyridine (3 ml) at 30° for 45 hr followed by usual work-up gave a product (54 mg) which was purified by preparative TLC (benzene–EtOAc=1:2, detection with water) to give Vb (34 mg, amorphous), $[\alpha]^{18}_{D}-22.5°$ ($c=0.5$, $CHCl_3$), IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3475 (w, OH), 1750 (br, OAc, COOEt). Although the purity of Vb was secured by TLC, the crystallization was unsuccessful. PMR ($CDCl_3$, 90 MHz) δ: 0.81 (3H, s), 0.88 (6H, s), 0.91 (3H, s), 1.04 (6H, s), 1.42 (3H, s) ($CH_3 \times 7$), 1.26 (3H, t, $J=7.5$ Hz), 4.18 (2H, q, $J=7.5$ Hz) (OCH_2CH_3), 1.88 (15H, s), 1.93 (18H, s), 2.22 (3H, s) ($OAc \times 12$), 3.04 (1H, m, 3α-H).

Methylation of DJ-3 (Va) giving Tetradeca-O-methyl Derivative (Vc)——A solution of Va (100 mg) in DMSO (7 ml) was treated with DMSO carbanion (prepared from 500 mg of NaH and 8 ml of DMSO at 60—80° as above) and kept stirring at room temperature for 1 hr under N_2 atmosphere. The reaction mixture was added with CH_3I (7 ml), kept stirring in the dark at room temperature for 20 hr, poured into ice-water and extracted with $CHCl_3$. After treating the $CHCl_3$ extract as above, the product was purified by preparative TLC (benzene–acetone=4:1, detection with water) and treated with acetone to give Vc (42 mg, amorphous), $[\alpha]^{8}_{D}-6.3°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{62}H_{106}O_{21}$: C, 62.71; H, 9.00. Found: C, 62.61; H, 8.99. IR $\nu^{CCl_4}_{max}$ cm^{-1}: no OH, 1755 (COOMe). PMR ($CDCl_3+C_6D_6=1:1$, 90 MHz) δ: 4.50 (1H, d, $J=7$ Hz), 4.86 (1H, d, $J=7$ Hz), 4.97 (1H, d, $J=7$ Hz) (anomeric $H \times 3$), 5.26 (1H, m, 12-H).

$LiAlH_4$ Reduction of Vc giving Ve——To a solution of Vc (70 mg) in dry ether (12 ml) was added $LiAlH_4$ (40 mg) and the total mixture was heated under reflux for 2 hr and treated as above. The product was purified by silica gel (6 g) column chromatography eluting with benzene–acetone (6:1) and treated with acetone to give Ve (54 mg, amorphous), $[\alpha]^{23}_{D}-5.1°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{61}H_{106}O_{20}$: C, 63.19; H, 9.22. Found: C, 62.73; H, 9.26. IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3600 (w, OH), no COOMe. PMR ($CDCl_3$, 90 MHz) δ: 4.43 (1H, d, $J=7$ Hz), 4.88 (1H, d, $J=7$ Hz), 4.96 (1H, d, $J=7$ Hz) (anomeric $H \times 3$), 5.28 (1H, m, 12-H).

Methanolysis of IVe and Ve and Acetylation of If giving 3-O-Acetyl-16,21,22,28-tetra-O-methyl-barringtogenol C (Ig)——a) A solution of IVe (25 mg) in anhydrous 9% HCl–MeOH (5 ml) was refluxed for 2 hr, neutralized with Ag_2CO_3, and filtered. The filtrate was concentrated under reduced pressure to give a product, which was subjected to preparative TLC (benzene–acetone=4:1, detection with I_2) to give the methylated aglycone (If) (10 mg), methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (**1**), and methyl 3,4-di-O-methyl-D-glucopyranoside (**2**). The methylated monosaccharides were identified by GLC and TLC. If (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (1.5 ml) at 32° for 15 hr, treated as usual, purified by preparative TLC (benzene–EtOAc=20:1, detection with I_2), and treated with acetone to give Ig (8.5 mg, amorphous), $[\alpha]^{8}_{D}+20.0°$ ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{60}O_6$: C, 73.47; H, 10.20. Found: C, 73.26; H, 10.22. IR $\nu^{CCl_4}_{max}$ cm^{-1}: no OH, 1737, 1246 (OAc). PMR ($CDCl_3$, 90 MHz) δ: 2.04 (3H, s, OAc), 3.27, 3.28, 3.52, 3.56 (3H each, s, $OCH_3 \times 4$), 4.53 (1H, t-like, 3α-H). Mass Spectrum *m/e* (%): 588 (M^+, 4), 556 (M^+–MeOH, 6), 528 (M^+–AcOH, 2), 524 (M^+–2MeOH, 14), 511 (M^+–CH_2OMe-MeOH, 100), 479 (M^+–CH_2OMe-2MeOH, 11), 419 (M^+–AcOH–CH_2OMe–2MeOH, 6), 338 (**i**, 4), 249 (**ii**, 4), 229 (**i**–CH_2OMe–2MeOH, 74), 197 (**i**-CH_2OMe–3MeOH, 53), 189 (**ii**-AcOH, 9). b) Ve (20 mg) was treated with anhydrous 9% HCl–MeOH (5 ml) under reflux for 2 hr as above to give a product which was purified by preparative TLC (benzene–acetone=3:1, detection with I_2). The methylated aglycone (If) thus obtained was acetylated and the acetate (6 mg) was identified with Ig by IR (CCl_4), TLC, and PMR. The methylated monosaccharides were identified with methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (**1**), methyl 2,3,4,6-tetra-O-methyl-D-galactopyranoside (**3**), and methyl 3-O-methyl-D-glucopyranoside (**4**), respectively, by GLC and TLC. GLC: i) Column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temp.: 190°, carrier gas: N_2 55 ml/min; t_R (min): **1**, 3.15, 4.02; **2**, 15.25, 17.35. ii) Column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm ×

2 m; column temp: 190°; carrier gas: N_2 30 ml/min; t_R (min): **1**, 4.63, 5.95; **3**, 6.70, 6.85; **2**, 22.72, 25.00. iii) Column: 3% SE-30 on chromosorb W (80—100 mesh), 3 mm × 2 m; column temp.: 170°; carrier gas: N_2 20 ml/min; t_R (min): **1**, 2.63, 2.97; **3**, 2.97, 3.10; **4**, 3.80. iv) Column: 3% SE-30 on chromosorb W (80—100 mesh), 3 mm × 2 m; column temp.: 175°; carrier gas: N_2 15 ml/min; t_R (min): **4**, 4.18. v) Column: 2% silicone OV-17 on chromosorb WAWDMCS (80—100 mesh), 3 mm × 2 m; column temp.: 170°; carrier gas: N_2 15 ml/min; t_R (min): **4**, 4.70. TLC (*Rf*): i) Benzene–acetone (4:1): **1**, 0.31, 0.45; **2**, 0.03. ii) Benzene–acetone (3:1): **1**, 0.43, 0.56; **2**, 0.06; **3**, 0.36, 0.43. iii) Benzene–acetone (1:2): **2**, 0.38; **4**, 0.18. iv) $CHCl_3$–MeOH (20:3): **4**, 0.23.

Methylation of Desacyl-jegosaponin (VIf) giving VIc——A solution of VIf (200 mg) in DMSO (11 ml) was treated with DMSO carbanion (NaH 550 mg, DMSO 12 ml) at room temperature for 1 hr with stirring under N_2 atmosphere. The reaction mixture was then added with CH_3I (10 ml), kept stirring in the dark at room temperature for 19 hr, poured into ice-water, and extracted with $CHCl_3$. The product obtained from the $CHCl_3$ extract as above was purified by silica gel (25 g) column chromatography (benzene–acetone = 6:1) and recrystallized from *n*-hexane to give colorless needles of VIc (125 mg), mp 219—221°, $[\alpha]_D^8 -26.7°$ (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{70}H_{120}O_{25}$: C, 61.75; H, 8.88. Found: C, 61.95; H, 8.86. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1762 (COOMe). PMR ($CDCl_3+C_6D_6$=1:1, 90 MHz) δ: 0.82 (6H, s), 0.84 (3H, s), 1.06 (6H, s), 1.11, 1.41 (3H each, s) ($CH_3 \times 7$), 1.37 (3H, d, J=6 Hz, rhamnose CH_3), 4.47, 4.96, 5.07 (1H each, d, J=7 Hz, anomeric H × 3), 5.29 (2H, br. s, 12-H & anomeric H), 3.09—3.66 (48H, $OCH_3 \times 16$).

$LiAlH_4$ Reduction of VIc giving VIe——To a solution of VIc (87 mg) in dry ether (20 ml) was added $LiAlH_4$ (50 mg) and the total mixture was refluxed for 2 hr and treated as above. The product was crystallized from *n*-hexane to give colorless needles of VIe (77 mg), mp 210—212°, $[\alpha]_D^{15} -15.7°$ (c=0.7, $CHCl_3$). *Anal.* Calcd. for $C_{69}H_{120}O_{24}$: C, 62.16; H, 9.07. Found: C, 62.55; H, 9.17. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3592 (w, OH), no COOMe. PMR ($CDCl_3$, 90 MHz) δ: 0.87 (6H, s), 0.91 (9H, s), 1.04, 1.31 (3H each, s) ($CH_3 \times 7$), 4.38, 4.59, 4.96 (1H each, d, J=7 Hz), 5.19 (1H, br. s) (anomeric H × 4), 5.27 (1H, m, 12-H), 3.27—3.60 (45H, $OCH_3 \times 15$).

Methanolysis of VIe——A solution of VIe (170 mg) in anhydrous 6% HCl–MeOH (20 ml) was refluxed for 2 hr, neutralized with Ag_2CO_3 and filtered. The product obtained by concentration of the filtrate under reduced pressure was chromatographed on a silica gel (15 g) column (benzene–acetone = 7:1) to give the methylated aglycone (If, 45 mg) and a mixture of methylated monosaccharides. Acetylation of If (43 mg) with Ac_2O (5 ml) and pyridine (4 ml) at 30° for 12 hr followed by usual work-up and preparative TLC (benzene–EtOAc = 20:1, detection with water) gave Ig (42 mg) being identified by IR (CCl_4), TLC, and PMR. The components of the methylated monosaccharides were separated by preparative TLC (benzene–acetone = 2:1, detection with I_2) and identified with methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (**1**), methyl 3-O-methyl-D-glucopyranoside (**4**), methyl 3,4,6-tri-O-methyl-D-galactopyranoside (**5**), and methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside (**6**) by GLC and TLC. GLC: i) Column: 2% silicone OV-17 on chromosorb WAWDMCS (80—100 mesh), 3 mm × 2 m; column temp.: 170°; carrier gas: N_2 15 ml/min; t_R (min): **1**, 2.50, 3.10; **4**, 4.70; **5**, 3.90, 4.35; **6**, 1.70. ii) Column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm × 2 m; column temp.: 190°; carrier gas: N_2 55 ml/min; t_R (min): **1**, 3.13, 4.02; **5**, 8.95, 12.90; **6**, 1.73. TLC (*Rf*): i) Benzene–acetone (2:1): **1**, 0.58, 0.69; **4**, 0.03; **5**, 0.22; **6**, 0.74. ii) Benzene–acetone (1:1): **4**, 0.06; **5**, 0.28. iii) Benzene–MeOH (20:5): **5**, 0.29, 0.32.

Methyl 2-O-Phenylcarbamoyl-3,4-di-O-isopropylidene-6-O-trityl-α- & -β-D-galactopyranosides (IXa, IXb)——A solution of methyl D-galactopyranoside (2 g) in pyridine (30 ml) was treated with trityl chloride (5 g) and Drierite (500 mg), heated under reflux for 3 hr, and poured into ice-water. The precipitates were removed by filtration and the filtrate was extracted with $CHCl_3$ and the $CHCl_3$ extract was washed with water, dired over $MgSO_4$, and evaporated to dryness to give a product which was purified by preparative TLC (benzene–acetone = 2:1, detection by UV) and methyl 6-O-trityl-(α,β)-D-galactopyranosides(VIIb, 1.8 g) were obtained as syrup (trityl group being confirmed by IR (CCl_4) and PMR). A solution of VIIb (420 mg) in 2,2-dimethoxypropane (10 ml) was added with *p*.TsOH·H_2O (*ca.* 10 mg) and left standing at 30—32° for 30 min with occasional shaking. After ice-cooling, the reaction mixture was poured into $CHCl_3$ and the $CHCl_3$ solution was washed with aq. 5% $KHCO_3$ and water successively, dried over $MgSO_4$, and evaporated to dryness to give a product which was purified by preparative TLC (benzene–MeOH = 24:1, detection by UV) to afford methyl 3,4-di-O-isopropylidene-6-O-trityl-α-D-galactopyranoside (VIIIa, 265 mg, syrup), $[\alpha]_D^{24} +48.3°$ (c=2.2, MeOH), PMR (CCl_4, 90 MHz) δ: 4.63 (1H, d, J=4 Hz, anomeric H), and β-anomer (VIIIb, 119 mg, syrup), $[\alpha]_D^{24} -14.7°$ (c=0.6, MeOH), PMR (CCl_4, 90 MHz) δ: 4.11 (1H, d, J=8 Hz, anomeric H).

To a solution of VIIIa (190 mg) in pyridine (10 ml) was added phenylisocyanate (0.1 ml) and after keeping the total mixture at 30° for 10 min, the mixture was heated under reflux for 4 hr, cooled with ice, added with MeOH (10 ml), refluxed again for 20 min, and poured into ice-water. The precipitates collected by filtration were purified by preparative TLC (benzene–acetone = 40:1, detection by UV) to give a product (218 mg), which was crystallized from *n*-hexane–ether to afford colorless needles of IXa, mp 190—191°, $[\alpha]_D^{26} +58.3°$ (c=0.9, MeOH). *Anal.* Calcd. for $C_{36}H_{37}O_7N$: C, 72.59; H, 6.26; N, 2.35. Found: C, 72.51; H, 6.27; N, 2.38. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3445 (NH), 1751, (–OCONH–), 1600, 1524 (aryl C=C, amide II). PMR (CCl_4, 90 MHz) δ: 1.29, 1.50 (3H each, s, $CH_3 \times 2$), 3.34 (3H, s, OCH_3), 4.80 (2H, br. s, $W_{h/2}$=6 Hz, 2-H, 1-H), 6.80—7.50 (aromatic protons).

The β-anomer (VIIIb, 114 mg) in pyridine (8 ml) was treated with phenylisocyanate (0.1 ml) similarly as for VIIIa and the product (120 mg) obtained by preparative TLC was crystallized from *n*-hexane–ether to give fine needles of IXb, mp 148—150°, $[\alpha]_D^{26} -5.8°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{36}H_{37}O_7N$: C, 72.59; H, 6.26; N, 2.35. Found: C, 72.20; H, 6.34; N, 2.57. IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3450 (NH), 1746 (–OCONH–), 1601, 1522 (aryl C=C, amide II). PMR (CCl_4, 90 MHz) δ: 1.32, 1.53 (3H each, s, $CH_3 \times 2$), 3.49 (3H, s, OCH_3), 4.27 (1H, d, $J=8$ Hz, 1-H), 4.77 (1H, d. d, $J=6$ & 8 Hz, 2-H), 6.50—7.50 (aromatic protons).

Methyl 3,4,6-Tri-O-methyl-α-&-β-D-galactopyranosides (Xa, Xb)——A mixture of IXa and IXb (900 mg) in anhydrous 5% HCl–MeOH (30 ml) solution was heated under reflux for 2 hr, neutralized with Ag_2CO_3, and filtered. The residue obtained after concentration of the filtrate under reduced pressure was purified by preparative TLC (benzene–acetone = 1:2, detection by UV) and the product (395 mg) was dissolved in DMF (20 ml) and added with CH_3I (13 ml) and Ag_2O (4 g), and the total mixture was kept stirring in the dark at room temperature for 24 hr, filtered, added again with CH_3I (5 ml) and Ag_2O (3 g), kept stirring for 24 hr, and filtered. The filtrate was then diluted with $CHCl_3$, washed with water, dried over $MgSO_4$, and evaporated under reduced pressure to give a product which was purified by preparative TLC (benzene–acetone = 20:3, detection by UV) and α-anomer (185 mg) and β-anomer (145 mg) were obtained as syrup. A solution of the α-anomer (185 mg) in dry ether (20 ml) was treated with $LiAlH_4$ (100 mg), refluxed for 2 hr, treated in a usual manner, and purified by preparative TLC (benzene–acetone = 1:1, detection with I_2) to give a product (82 mg), which was crystallized from acetone to afford colorless needles of Xa, mp 84—86°, $[\alpha]_D^{25} +124.7°$ ($c=1.0$, MeOH). *Anal.* Calcd. for $C_{10}H_{20}O_6$: C, 50.83; H, 8.53. Found: C, 50.91; H, 8.38. IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3580, 3490 (OH). PMR (CCl_4, 90 MHz) δ: 2.03 (1H, m, OH, exchangeable with D_2O), 3.30, 3.36, 3.44, 3.47 (3H each, s, $OCH_3 \times 4$), 4.58 (1H, d, $J=4$ Hz, 1-H). The β-anomer (145 mg) in dry ether (20 ml) was treated similarly with $LiAlH_4$ (70 mg) and the reaction product was purified by preparative TLC to give Xb (45 mg) as syrup, $[\alpha]_D^{24} -10.4°$ ($c=1.6$, MeOH). IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3600, 3460 (OH). PMR (CCl_4, 90 MHz) δ: 2.82 (1H, m, OH, exchangeable with D_2O), 3.38, 3.50, 3.51, 3.52 (3H each, s, $OCH_3 \times 4$), 4.12 (1H, d, $J=8$ Hz, 1-H).

Acknowledgement The authors would like to express their 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 monosaccharide specimens, and to the Res. Lab. of Dainippon Pharm. Co. for the elemental analyses.