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Saponin and Sapogenol. XVI.[1] Structure of Desacyl-boninsaponin A obtained from the Bark of *Schima mertensiana* Koidz.

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A piscicidal saponin mixture (named boninsaponin) isolated from the bark of *Schima mertensiana* Koidz. (syn. *S. boninensis* Nakai, Theaceae) was treated with alkali and the major desacylated product of boninsaponin was isolated and designated as desacyl-boninsaponin A.

On the basis of chemical (including the photolytic cleavage of the glucuronopyranoside linkage) and physicochemical evidence, the structure of desacyl-boninsaponin A has been determined as A_1-barrigenol (3)-[β-D-glucopyranosyl ($1_{glu}\rightarrow 2_{glr}$)] [$\alpha$-L-rhamnopyranosyl-($1_{rham}\rightarrow 2_{gal}$)-$\beta$-D-galactopyranosyl ($1_{gal}\rightarrow 4_{glr}$)]-$\beta$-D-glucuronopyranoside (**9a**), in which the structure of oligosaccharide portion is identical with that of desacyl-jegosaponin obtained from the pericarps of *Styrax japonica* Sieb. et Zucc. (Styracaceae).

The bark of *Schima mertensiana* Koidz. (syn. *S. boninensis* Nakai, Theaceae) (Japanese name: himetsubaki), which grows in Bonin Islands, has been considered to contain some piscicidal substances since it has been known as useful to catch fish in the stream. We have procured the bark[3] and isolated a saponin mixture (designated as boninsaponin) which shows piscicidal activity. As a continuative study on saponin,[1] we have been working on the structure elucidation of boninsaponin. This paper provides the full account on the structure elucidation of desacyl-boninsaponin A which is obtained as the major desacylated derivative of boninsaponin.

Boninsaponin, which was isolated from the methanol extractive of the bark through the ordinary procedure, comprises some saponins (at least six) as shown by thin-layer chromatography (TLC) (Fig. 1). As reported previously,[4] boninsaponin afforded, on acid hydrolysis followed by alkaline treatment, five olean-12-ene type sapogenols: primulagenin A (**1**), dihydropriverogenin A (**2**), A_1-barrigenol (**3a**), barringtogenol C (**4**), and R_1-barrigenol (**5**), among which A_1-barrigenol was the major constituent (35.8% of the total sapogenol mixture). Since i) the acid hydrolysis of boninsaponin yielded a mixture of acylated derivatives of triterpenoids and the successive alkaline treatment was indispensable for liberating free sapogenols and ii) the soil bacterial hydrolysis[5] of boninsaponin gave a mixture of acylated sapogenols[6] similarly as in the soil bacterial hydrolysis of jegosaponin,[7] boninsaponin was considered to be a mixture of acylated saponins and was subjected to alkaline treatment. Desacyl-boninsaponin thus obtained showed seven spots on TLC (Fig. 1) and the major component was isolated by silica gel column chromatography and designated as desacyl-boninsaponin A.

The infrared (IR) spectrum of desacyl-boninsaponin A (**9a**) shows the absorption bands ascribable to hydroxyl (3390 (br) cm^{-1}) and carboxyl (1734 cm^{-1}). On acid hydrolysis,

1) Part XV: I. Kitagawa, T. Sugawara, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 275 (1976).
2) Location: *133-1, Yamada-kami, Suita, Osaka, 565, Japan.*
3) Kindly supplied by Dr. H. Ishii of Chiba University to whom the authors' deepest thanks are due.
4) I. Kitagawa, A. Inada, M. Utsunomiya, and I. Yosioka, *Phytochemistry*, **14**, 314 (1975).
5) *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.
6) A preliminary work using the soil bacterial strain YSB-26.
7) I. Yosioka, S. Saijoh, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 564 (1974).

desacyl-boninsaponin A yielded A_1-barrigenol (**3a**), glucose, galactose, rhamnose, and a uronic acid.[8] On the other hand, ultraviolet irradiation of desacyl-boninsaponin A in methanol readily liberated A_1-barrigenol (**3a**) thus suggesting that, in the carbohydrate portion of the saponin, the uronic acid moiety is the one directly linked to the aglycone.[9]

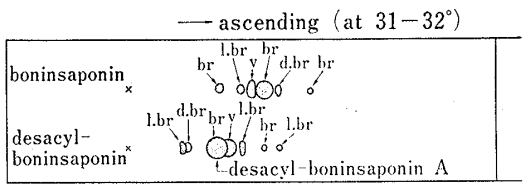
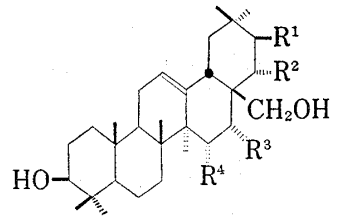
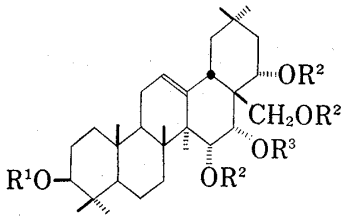


Fig. 1. TLC Patterns of Boninsaponin and Desacyl-boninsaponin

solvent: $CHCl_3$–MeOH–water (65 : 35 : 10, lower layer)
abbreviations: br=brown, l.br=light brown, d.br=dark brown, v=violet

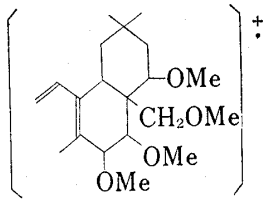
On mild acid hydrolysis followed by silica gel column chromatography and preparative TLC, desacyl-boninsaponin A furnished A_1-barrigenol (**3a**) and three prosapogenols designated as DB-1 (**6b**), DB-2 (**7b**), and DB-3 (**8b**), all of which possess A_1-barrigenol as the common aglycone.



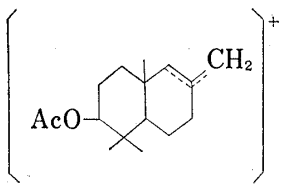


	R^1	R^2	R^3	R^4	
1	H	H	OH	H	primulagenin A
2	H	OH	OH	H	dihydropriverogenin A
4	OH	OH	OH	H	barringtogenol C
5	OH	OH	OH	OH	R_1-barrigenol

3a	$R^1=R^2=R^3=H$ A_1-barrigenol
3b	$R^1=H$, $R^2=R^3=Me$
3c	$R^1=Ac$, $R^2=R^3=Me$
3d	$R^1=R^2=Ac$, $R^3=H$



i *m/e* 338



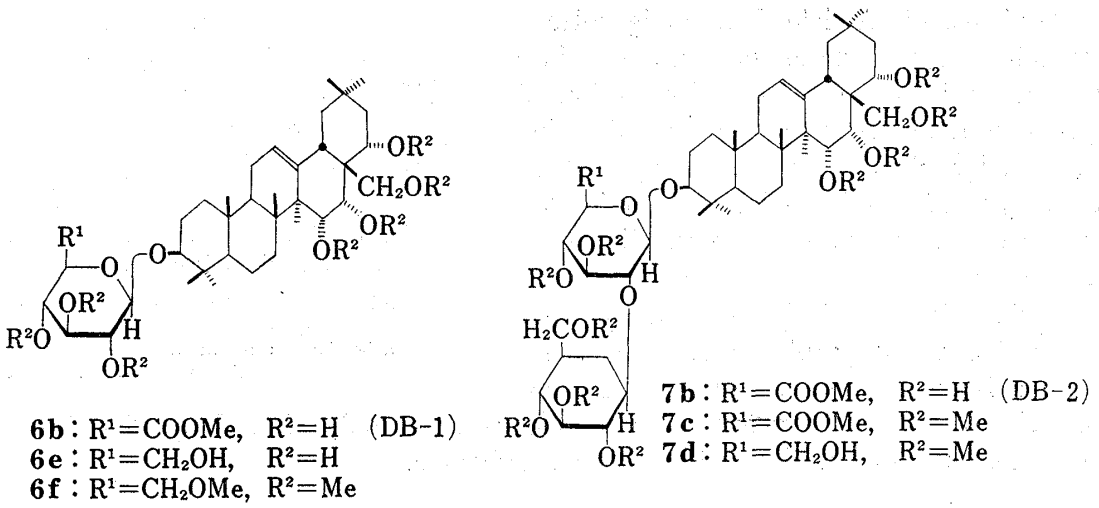
ii *m/e* 249

Chart 1

DB-1 (**6b**) is a uronide-methyl ester of A_1-barrigenol as shown by acid hydrolysis and the IR spectrum ($COOCH_3$: 1745 cm^{-1}). The methoxycarbonyl group was formed by esterification of the uronic acid function during methanolic acid hydrolysis of desacyl-boninsaponin A as in the case of desacyl-jegosaponin.[10] The same group also presents in the other prosapogenols DB-2 (**7b**) and DB-3 (**8b**). Reduction of DB-1 with sodium borohydride gave a product (**6e**), which possesses no methoxycarbonyl function (IR) and was converted to a per-O-methyl derivative (**6f**) by complete methylation with methyl iodide-sodium hydride-dimethyl sulfoxide.[11] The IR spectrum (CCl_4) of **6f** no longer shows free hydroxyl absorption band. On methanolysis with 10% methanolic hydrogen chloride, **6f** furnished methyl 2,3,4,6-tetra-O-methyl-glucopyranoside and a methylated aglycone (**3b**, *vide infra* for the structure elucidation), thus disclosing that DB-1 is a glucuronopyranoside of A_1-barrigenol.

DB-2 (**7b**) is a glucoside of DB-1. The IR spectrum of DB-2 shows the absorption bands due to hydroxyl (3386 (br) cm^{-1}) and methoxycarbonyl (1745 cm^{-1}). Complete methylation

8) Confirmed to be glucuronic acid on the following basis.
9) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1339 (1974).
10) I. Kitagawa, Y. Imakura, T. Hayashi, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1520 (1975).
11) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).



6b: R^1=COOMe, R^2=H (DB-1)
6e: R^1=CH_2OH, R^2=H
6f: R^1=CH_2OMe, R^2=Me

7b: R^1=COOMe, R^2=H (DB-2)
7c: R^1=COOMe, R^2=Me
7d: R^1=CH_2OH, R^2=Me

Chart 2

of DB-2 as for **6e** yielded an undeca-O-methyl derivative (**7c**) (IR: no hydroxyl), which shows two anomeric proton signals at δ 4.41 and δ 4.62 (1H each, both d, J=7 Hz) in its proton magnetic resonance (PMR) spectrum.

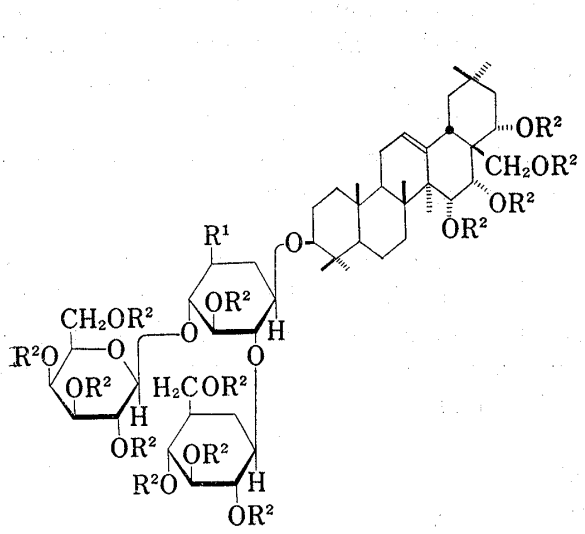
DB-3 (**8b**) is a galactoside of DB-2. The IR spectrum of DB-3 also shows the hydroxyl and methoxycarbonyl absorption bands (3380 (br), 1742 cm^{-1}) as in the IR spectra of DB-1 and DB-2. The tetradeca-O-methyl derivative (**8c**) prepared by complete methylation of DB-3 possesses no free hydroxyl (IR) and shows three anomeric proton signals at δ 4.58, 4.85, and 4.95 (1H each, all d, J=7 Hz) in its PMR spectrum.

Respective treatment of the undeca-O-methyl derivative (**7c**) and the tetradeca-O-methyl derivative (**8c**) with lithium aluminium hydride ($LiAlH_4$) afforded the corresponding reduction products (**7d** and **8d**) which show the weak hydroxyl absorption bands in their IR spectra (3586, 3481 and 3597, 3496 cm [1]). On methanolysis with 10% methanolic hydrogen chloride, **7d** liberated methyl 2,3,4,6-tetra-O-methyl-glucopyranoside and methyl 3,4-di-O-methyl-glucopyranoside while **8d** furnished methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, and methyl 3-O-methyl-glucopyranoside as the methylated carbohydrate ingredients, respectively.

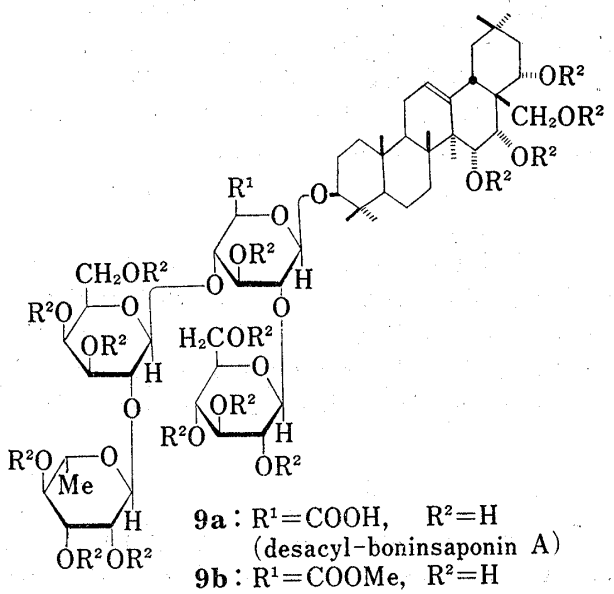
The methylated aglycone (**3b**) commonly obtained by methanolysis of **6f**, **7d**, and **8d** was characterized as the monoacetate (**3c**) which possesses one acetoxyl (1744, 1247 cm^{-1} in IR and δ 2.04 (3H, s) in PMR spectra) and four methoxyls (PMR) but no free hydroxyl (IR).[12] The location of acetoxyl function in **3c** was determined to be at C-3 since a characteristic proton signal assignable to 3α-H geminal to 3β-OAc is observed at δ 4.51 (1H, t-like) in its PMR spectrum[13] and the position and coupling pattern are comparable to those of 3α-H signal in the PMR spectrum of A_1-barrigenol tetraacetate (**3d**): δ 4.47 (1H, t-like). In addition, the fragment ion peaks observed at *m/e* 338 (**i**) and 249 (**ii**) in the mass spectrum of **3c**, which are derived through the reverse Diels-Alder type fragmentation of ring C in **3c**, also support the formulation **3c**.[13] It follows therefore that the methylated aglycone is formulated as **3b** and the carbohydrate moieties in the above mentioned prosapogenols (DB-1, DB-2 and DB-3) connect at 3β-OH of A_1-barrigenol.

Based on the accumulated evidence described above, the structures of DB-1, DB-2, and DB-3 are respectively formulated as **6b**, **7b**, and **8b**, in which all the monosaccharide components of D series are connected with β-orientation as based on the anomeric proton coupling patterns in the PMR spectra of **7c** and **8c** (monosaccharide moieties being considered to take Cl form).

12) Authentic **3c** was prepared by methanolysis of **9d** followed by acetylation (*vide infra*).
13) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).



8b: R^1=COOMe, R^2=H (DB-3)
8c: R^1=COOMe, R^2=Me
8d: R^1=CH_2OH, R^2=Me



9a: R^1=COOH, R^2=H (desacyl-boninsaponin A)
9b: R^1=COOMe, R^2=H
9c: R^1=COOMe, R^2=Me
9d: R^1=CH_2OH, R^2=Me

Chart 3

Finally, the structure of desacyl-boninsaponin A (**9a**) has been determined on the basis of the following evidence. Thus, complete methylation of desacyl-bonisaponin A as above afforded the hexadeca-O-methyl derivative (**9c**), which exhibits the methoxycarbonyl absorption band at 1761 cm^{-1} but no hydroxyl absorption band in its IR spectrum (CCl_4). The PMR spectrum of **9c** taken in a mixture of deuterochloroform and hexadeuterobenzene shows four anomeric proton signals at δ 4.46, 4.79, 5.09 (1H each, all d, J=7 Hz) and δ 5.31 (2H, br.s, overlapped with the signal due to a vinyl proton at C-12). Reduction of **9c** with $LiAlH_4$ furnished a pentadeca-O-methyl derivative (**9d**) which possesses no methoxycarbonyl function but a hydroxyl (IR: 3593 (w), 3493 (w, br) cm^{-1}). Methanolysis of **9d** with 10% methanolic hydrogen chloride yielded 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, and methyl 3-O-methyl-glucopyranoside as the methylated carbohydrate components and **3b** as the methylated aglycone which was identified as its monoacetate (**3c**) as described above.

Consequently, the structure **9a** except the anomeric configuration in the terminal rhamnose moiety has become rational for desacyl-boninsaponin A and the application of the Klyne's rule[14] for **9b**[15] and **8b** has finally led us to express the full structure of desacyl-boninsaponin A as A_1-barrigenol(3)-[β-D-glucopyranosyl($1_{glu}\rightarrow 2_{glr}$)][$\alpha$-L-rhamnopyranosyl($1_{rham}\rightarrow 2_{gal}$)-$\beta$-D-galactopyranosyl ($1_{gal}\rightarrow 4_{glr}$)]-$\beta$-D-glucuronopyranoside (**9a**): $[M]_D$ of **9b**−$[M]_D$ of **8b**=−126°; $[M]_D$ of methyl α-L-rhamnopyranoside=−109°; $[M]_D$ of methyl β-L-rhamnopyranoside=+169°.[16]

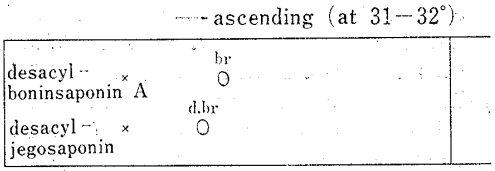


Fig. 2. TLC of Desacyl-boninsaponin A and Desacyl-jegosaponin[10]
solvent: $CHCl_3$-MeOH-water(65:40:10)

14) W. Klyne, *Biochem. J.*, **47**, xli (1950).
15) Prepared from **9a** by diazomethane methylation.
16) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1972).
17) *a*) S.G. Errington, D.E. White, and M.W. Fuller, *Tetrahedron Letters*, **1967**, 1289; *b*) S. Ito, T. Ogino, T. Sugiyama, and M. Kodama, *ibid.*, **1967**, 2289.

A_1-Barrigenol (**3a**)[17] has hitherto been isolated as the sapogenol from the several species of plants: *Barringtonia asiatica* Kurz. (Lecythidaceae),[18a] *Schima kankaoensis* Hay.,[18b] *S. liukiuensis* Nakai[18c] (Theaceae), *Pittosporum undulatum* Vent. (Pittosporaceae),[18d] *Camellia sasanqua* Thunb. (Theaceae),[18e] and *Ternstroemia japonica* Thunb. (Theaceae),[18e] but no report on the structure elucidation of the parent saponin has been provided. The present study is the first example of the structure elucidation of saponin possessing A_1-barrigenol as the aglycone.

It is interestingly pointed out that the structure of oligosaccharide portion in desacyl-boninsaponin A (**9a**) is identical with that in desacyl-jegosaponin[10] which was obtained from the pericarps of *Styrax japonica* Sieb. et Zucc. (Styracaceae) and whose aglycone is barringtogenol C (**4**),[13] a hydroxyl position isomer of A_1-barrigenol (**3a**). Both desacylated saponins can be distinguished from each other on TLC as shown in Fig. 2.

Experimental[19]

Isolation of Boninsaponin——Air-dried bark (cut, 10.1 kg) of *Schima mertensiana* Koidz. collected in Bonin Islands[3] was extracted with MeOH three times under reflux. Evaporation of the combined extracts gave a dark brown residue (1.3 kg) which was partitioned into a *n*-BuOH–water mixture as usual. The *n*-BuOH soluble portion (1.0 kg) was dissolved in a small amount of MeOH and poured into a much larger amount of ether with stirring to give brown precipitate. The precipitate was collected, dissolved again in MeOH, and poured into a larger amount of ether to yield precipitate. Three more similar precipitations gave a saponin fraction as brown powder (522 g). A solution of saponin fraction (50 g) in MeOH was passed through an active charcoal column (100 g of charcoal, Tokusei-shirasagi, Takeda Chem. Ind. and 100 g of Celite 535, Wako Pure Chem. Ind.) and successive elution was made with MeOH to give a saponin mixture (boninsaponin, 28 g). The sapogenols obtained by acid hydrolysis (aq. 15% H_2SO_4–EtOH=1: 1, refluxing for 7 hr) followed by alkaline treatment (aq. 5% KOH–EtOH=1: 1, refluxing for 3 hr) of boninsaponin were reported previously.[4] The piscicidal activity (LC_{50}) of boninsaponin using *Oryzias latipes* (killie-fish, himedaka)[20] was 1.5 ppm,[21] and the hemolytic index[22] of boninsaponin was 19000.

Desacyl-boninsaponin A——A solution of boninsaponin (40 g) in aq. 50% EtOH–KOH mixture (500 ml—15 g) was heated under reflux for 4.5 hr, treated with 10% HCl to make pH 6.0, and concentrated under reduced pressure to remove EtOH. The aqueous mixture was then extracted with aq. *n*-BuOH and the *n*-BuOH layer was taken, washed with water, and evaporated to dryness under reduced pressure to give a desacylated saponin mixture (desacyl-boninsaponin, 30 g). Desacyl-boninsaponin (28 g) was adsorbed on silica gel (30 g) with the aid of MeOH, dried *in vacuo*, and put on a column of silica gel (1.2 kg) made with $CHCl_3$–MeOH–water (65: 35: 10, lower layer). Successive development of the column with the same solvent mixture gave desacyl-boninsaponin A (6.4 g)[23] and the mixture containing the other desacylated saponins (combined weight 11.9 g). Desacyl-boninsaponin A thus obtained was crystallized from the same solvent mixture as used for elution of the column, dissolved in MeOH, and treated with Dowex 50w × 8 (H^+) until the solution

18) *a*) T. Nozoe, *Nippon Kagaku Kaishi*, **56**, 689, 704 (1935); T. Nozoe and T. Kinugasa, *ibid.*, **56**, 864 (1935); *b*) T. Nozoe and T. Kinugasa, *ibid.*, **56**, 883 (1935); *c*) T. Takahashi, M. Miyazaki, M. Yasue, H. Imakura, and O. Honda, *Nippon Mokuzai Gakkaishi*, **9**, 59 (1963); *d*) A.R.H. Cole, D.T. Downing, J.C. Watkins, and D.E. White, *Chem. & Ind.*, **1955**, 254; *e*) I. Yosioka, R. Takeda, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 1237 (1972).

19) 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 the internal standard): Hitachi 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) 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 on a hot plate for 5 min. 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).

20) K. Sakata, K. Kawazu, and T. Mitsui, *Agr. Biol. Chem.*, 35, 1084 (1971).

21) Kindly undertaken by Dr. K. Kawazu of Okayama University to whom the authors' deepest thanks are due.

22) M. Fujita and K. Nishimoto, *Yakugaku Zasshi*, **72**, 1645 (1952).

23) Desacyl-boninsaponin A obtained at this stage was contaminated with its carboxylate form, so that the acidic ion-exchange treatment was needed to procure the pure carboxylic acid form (*vide infra*).

showed weakly acidic (0.5 g of ion-exchange resin was used for 300 mg of desacyl-boninsaponin A). The analytical sample of desacyl-boninsaponin A (**9a**) was obtained by recrystallization from aq. MeOH as colorless needles of mp 235—237°, $[\alpha]_D^{16}$ −17.1° (c=0.5, MeOH). *Anal.* Calcd. for $C_{54}H_{88}O_{25}\cdot H_2O$: C, 56.14; H, 7.85. Found: C, 56.14; H, 8.15. IR ν_{max}^{KBr} cm^{-1}: 3390 (br, OH), 1734 (COOH).

Complete Acid Hydrolysis of Desacyl-boninsaponin A (9a)——A solution of **9a** (50 mg) in aq. 15% H_2SO_4–EtOH (1: 1, 12 ml) was heated under reflux for 5 hr, poured into cold water, and extracted with EtOAc three times. The combined extract was washed with water, dried with anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure to give A_1-barrigenol (**3a**, 16 mg) which was recrystallized from acetone as colorless needles and identified with the authentic sample[18e] by mixed mp, IR (KBr), and TLC. The aqueous layer was neutralized with aq. saturated $Ba(OH)_2$ and Dowex 44 (OH^-) successively and concentrated to give a syrupy residue, which was then subjected to PPC developing with iso-PrOH–*n*-BuOH–water (7: 1: 2) twice. The spots were detected by the aniline hydrogen phthalate reagent and identified with glucuronic acid, glucose, galactose, and rhamnose, respectively.

Photolysis of Desacyl-boninsaponin A (9a)——A methanolic solution (60 ml) of desacyl-boninsaponin A (**9a**, 50 mg) in a quartz tube was irradiated externally (distance: 2 cm) with a 500 W high pressure mercury lamp (Eikōsha, PIH-500) for 90 min with ice-water cooling (reaction temp.: 25—28°), treated with 10% K_2CO_3, and evaporated under reduced pressure to remove MeOH. After dilution with water, the total reaction mixture was extracted with EtOAc several times, and the combined extract was evaporated under reduced pressure and subjected to preparative TLC ($CHCl_3$–MeOH=10: 1, detection with water) to give a product (9.5 mg), which was crystallized from acetone as colorless needles and identified with A_1-barrigenol (**3a**) by mixed mp, IR (KBr), and TLC.

Mild Acid Hydrolysis of Desacyl-boninsaponin A (9a) giving DB-1 (6b), DB-2 (7b), and DB-3 (8b)——A solution of **9a** (6 g) in aq. 3% H_2SO_4–MeOH (1: 1, 600 ml) was refluxed for 4.5 hr and MeOH was removed by evaporation under reduced pressure. The resulted aqueous mixture was neutralized with 10% Na_2CO_3 and extracted several times with *n*-BuOH saturated with water, and the organic layer was taken, washed with water, and evaporated to dryness under reduced pressure to give a residue (4.3 g). The partial hydrolysate thus obtained was adsorbed on silica gel (10 g) with the aid of MeOH, dried *in vacuo*, and put on a silica gel column (1.2 kg) made with $CHCl_3$–MeOH–water (65: 30: 10, lower layer). Successive elution with the same solvent mixture gave A_1-barrigenol (**3a**, 600 mg), a mixture mainly comprising DB-1 (87 mg), a mixture mainly comprising DB-2 (240 mg), DB-3 (**8b**, 350 mg), and mixtures (combined weight, 1.8 g). Preparative TLC purification ($CHCl_3$–MeOH=10: 1 for DB-1 and $CHCl_3$–MeOH–water=100: 25: 1 for DB-2, detection with water for both) gave pure DB-1 (**6b**, 50 mg) and DB-2 (**7b**, 185 mg), respectively.

DB-1 (**6b**), mp 215—217° (colorless needles from acetone–*n*-hexane), $[\alpha]_D^{31}$ −12.2° (c=0.25, acetone). *Anal.* Calcd. for $C_{37}H_{60}O_{11}\cdot 2H_2O$: C, 61.99; H, 9.00. Found: C, 62.22; H, 9.26. IR ν_{max}^{KBr} cm^{-1}: 3405 (br, OH), 1745 ($COOCH_3$).

DB-2 (**7b**), mp 263—265° (colorless needles from MeOH–$CHCl_3$), $[\alpha]_D^{27}$ −6.1° (c=1.0, MeOH). *Anal.* Calcd. for $C_{43}H_{70}O_{16}\cdot 2H_2O$: C, 58.75; H, 8.49. Found: C, 58.66; H, 8.53. IR ν_{max}^{KBr} cm^{-1}: 3386 (br, OH), 1745 ($COOCH_3$).

DB-3 (**8b**), mp 254—256° (colorless needles from aq. EtOH), $[\alpha]_D^{14}$ −6.5° (c=1.0, MeOH). *Anal.* Calcd. for $C_{49}H_{80}O_{21}\cdot 2H_2O$: C, 56.52; H, 8.13. Found: C, 56.49; H, 8.55. IR ν_{max}^{KBr} cm^{-1}: 3380 (br, OH), 1742 ($COOCH_3$).

A few mg of DB-1 (**6b**), DB-2 (**7b**), or DB-3 (**8b**) was treated with aq. 15% H_2SO_4–95% EtOH (1: 1, 2 ml) under reflux for 4.5 hr and the aglycone was taken up with EtOAc and identified with A_1-barrigenol (**3a**) by TLC ($CHCl_3$–MeOH=10: 1).

$NaBH_4$ Reduction of DB-1 (6b) (giving 6e) followed by Methylation (giving 6f) and Methanolysis of 6f——To a solution of **6b** (17 mg) in MeOH was added $NaBH_4$ (5 mg) and the total mixture was kept stirring for 30 min at room temperature and treated with acetone (2 ml) to decompose excess $NaBH_4$ with further stirring for about 10 min. The reaction mixture was diluted with MeOH (4 ml), passed through the columns of Amberlite IRA-400 (OH^-, 0.5 g) and Dowex 50w×8 (H^+) successively, and evaporated to dryness to give a reduction product **6e** (14 mg), IR ν_{max}^{KBr} cm^{-1}: 3407 (br, OH), no ester carbonyl. Purity of the product was secured by TLC and it was directly used for the following methylation. To a solution of **6e** (10 mg) in dimethyl sulfoxide (DMSO) (2 ml) was added DMSO carbanion (1 ml)[1] dropwise and the total mixture was kept stirring at room temperature under N_2 atmosphere. After 30 min, 0.5 ml of newly distilled CH_3I was added and the reaction mixture was kept stirring for 2 hr in the dark, poured into ice-water, and extracted with EtOAc. The EtOAc extract was washed with water and evaporated to dryness to give an oily product, which was subjected to preparative TLC (benzene–acetone=8: 1, detection by spraying water) to afford the per-O-methyl derivative (**6f**, 5.5 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH. A solution of **6f** (5 mg) in anhydrous 10% HCl–MeOH (2 ml) was refluxed for 1.5 hr, neutralized with Ag_2CO_3, filtered, and subjected to preparative TLC (benzene–acetone=5: 1, detection with I_2 vapor) to give a methylated aglycone (**3b**, 1 mg) and a methylated monosaccharide. The methylated aglycone (**3b**) was acetylated with Ac_2O (0.2 ml) and pyridine (0.4 ml) as usual and identified with **3c** (*vide infra*) by IR and TLC. The methylated monosaccharide was identified with methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (I) by GLC and TLC (benzene–acetone=4: 1). GLC: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 180°; carrier gas: N_2 30 ml/

min; t_R (min): 4′57″, 6′30″.

Methylation of DB-2 (7b) giving Undeca-O-methyl Derivative (7c)——A solution of **7b** (100 mg) in DMSO (4 ml) was treated with DMSO carbanion (2 ml) for 30 min as described above, added with CH_3I (1.2 ml) and kept stirring for further 2 hr in the dark. The reaction mixture was treated as above and the EtOAc extractive (92 mg) was purified by preparative TLC (benzene–acetone=5: 1, detection with water) to give **7c** (35 mg, amorphous), $[\alpha]_D^{21}$ −3.2° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{90}O_{16}$: C, 64.74; H, 9.23. Found: 64.92; H, 9.25. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1759 ($COOCH_3$). PMR ($CDCl_3$) δ: 3.28, 3.31 (3H each, both s), 3.35 (6H, s), 3.42 (3H, s), 3.50 (6H, s), 3.55, 3.58, 3.60 (3H each, all s) ($OCH_3\times10$), 3.77 (3H, s, $COOCH_3$), 4.41, 4.62 (1H each, both d, J=7 Hz, anomeric H×2), 5.26 (1H, m, 12-H).

$LiAlH_4$ Reduction of 7c (giving 7d) followed by Methanolysis——To a solution of **7c** (30 mg) in dry ether (4 ml) was added $LiAlH_4$ (15 mg) and the total mixture was heated under reflux for 2 hr. The reaction mixture was treated with aqueous ether and the precipitate was removed by filtration and washed with ether. The combined filtrate and washings were washed with water, dried with anhydrous $MgSO_4$, and evaporated to dryness to give a residue (28 mg). Preparative TLC (benzene–acetone=3: 1, detection with water) of the residue gave a reduction product **7d** (26 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3586 (w), 3481 (w, br) (OH), no ester carbonyl. A solution of **7d** (25 mg) in anhydrous 10% HCl–MeOH (2 ml) was refluxed for 1.5 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 a methylated aglycone (**3b**, 8.5 mg), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (I), and methyl 3,4-di-O-methyl-glucopyranoside (II). The methylated aglycone (**3b**) was acetylated with Ac_2O (0.5 ml) and pyridine (1 ml) as usual, crystallized from acetone–MeOH (giving colorless needles), and identified with **3c** (*vide infra*) by mixed mp, IR, and TLC. The identification of methylated monosaccharides were undertaken by GLC and TLC. GLC: column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 200°; carrier gas: N_2 40 ml/min; t_R (min): I, 4′48″, 6′01″; II, 16′27″, 18′35″. TLC: benzene–acetone=4: 1 for I; benzene–acetone=2: 1 for II.

Methylation of DB-3 (8b) giving Tetradeca-O-methyl Derivative (8c)——A solution of **8b** (160 mg) in DMSO (6.4 ml) was treated with DMSO carbanion (3.2 ml) for 30 min as described above, added with CH_3I (2 ml), and kept stirring for further 2 hr in the dark. The reaction mixture was then poured into ice-water and extracted with EtOAc several times. After working-up of the EtOAc extract in a usual manner, the product was purified by preparative TLC (benzene–acetone=4: 1, detection with water) to give **8c** (amorphous, 54 mg), $[\alpha]_D^{22}$ −3.7° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{62}H_{106}O_{21}$: C, 62.71; H, 9.00. Found: C, 62.56; H, 8.81. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1762 ($COOCH_3$). PMR ($CDCl_3+C_6D_6$=1: 1) δ: 3.19, 3.21, 3.27 (3H each, all s), 3.30 (6H, s), 3.33, 3.37, 3.40, 3.44, 3.47, 3.49 (3H each, all s), 3.54 (6H, s) ($OCH_3\times13$), 3.57 (3H, s, $COOCH_3$), 4.58, 4.85, 4.95 (1H each, all d, J=7 Hz) (anomeric H×3), 5.32 (1H, m, 12-H).

$LiAlH_4$ Reduction of 8c (giving 8d) followed by Methanolysis——To a solution of **8c** (35 mg) in dry ether (5 ml) was added $LiAlH_4$ (18 mg) and the total mixture was heated under reflux for 2 hr, treated as for **7c** to give a product (**8d**, 32 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3597 (w), 3496 (w, br) (OH), no ester carbonyl. A solution of **8d** (28 mg) in anhydrous 10% HCl–MeOH (4 ml) was refluxed for 2 hr, treated as for **7d**, and then subjected to preparative TLC (benzene–acetone=3: 1, detection with I_2) to give the methylated aglycone (**3b**, 9 mg), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (I), methyl 2,3,4,6-tetra-O-methyl-galactopyranoside (III), and methyl 3-O-methyl-glucopyranoside (IV). The methylated aglycone was acetylated with Ac_2O (0.7 ml) and pyridine (1.5 ml), crystallized from acetone–MeOH (giving colorless needles), and identified with **3c** (*vide infra*) by mixed mp, IR, and TLC. The methylated monosaccharides were identified by GLC and TLC. GLC: i) column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 200°; carrier gas: N_2 40 ml/min; t_R (min): I, 3′33″, 4′25″; III, 4′56″, 7′33″. ii) column: 3% SE-30 on chromosorb W (80—100 mesh), 3 mm×2 m; column temp.: 200°; carrier gas: N_2 30 ml/min; t_R (min): IV, 3′15″. To obtain an additional prove for methyl 3-O-methyl-glucopyranoside (IV), it was acetylated with Ac_2O and pyridine and identified with an authentic sample of methyl 3-O-methyl-2,4,6-tri-O-acetyl-D-glucopyranoside (V) by GLC: column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 105°; carrier gas: N_2 20 ml/min; t_R (min): V, 3′54″. TLC: benzene–acetone=4: 1 for I and III; $CHCl_3$–MeOH=4: 1 or EtOAc for IV.

Methylation of Desacyl-boninsaponin A (9a) giving Hexadeca-O-methyl Derivative (9c)——A solution of **9a** (350 mg) in DMSO (14 ml) was treated with DMSO carbanion (6.5 ml), kept stirring under N_2 atmosphere for 30 min at room temperature, added with CH_3I (4 ml), and kept stirring for further 2 hr in the dark. The reaction mixture was then poured into ice-water and extracted with EtOAc. The residue obtained from the EtOAc extract after usual working-up was purified by preparative TLC (benzene–acetone=2: 1, detection with water) to give **9c** (165 mg, amorphous), $[\alpha]_D^{22}$ −3.7° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{70}H_{120}O_{25}$: C, 61.74; H, 8.88. Found: C, 61.38; H, 8.58. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1761 ($COOCH_3$). PMR (C_6D_6) δ: 0.93, 0.98, 1.01 (3H each, all s), 1.10 (6H, s), 1.13 (3H, s) (*tert.* $CH_3\times6$), 1.43 (3H, d, J=6 Hz, rhamnose CH_3), 1.80 (3H, s, 14α–CH_3); ($C_6D_6+CDCl_3$=1: 1) δ: 4.46, 4.79, 5.09 (1H each, all d, J=7 Hz, anomeric H×3), 5.31 (2H, br. s, 12-H and anomeric H), 3.10—3.75 (48H, $OCH_3\times16$); ($CDCl_3$) δ: 4.52 (2H, t-like), 4.87 (1H, d, J=7 Hz), 5.17 (1H, s-like) (anomeric H×4), 5.28 (1H, m, 12-H).

$LiAlH_4$ Reduction of 9c (giving 9d) followed by Methanolysis——To a solution of **9c** (120 mg) in dry ether

(25 ml) was added $LiAlH_4$ (80 mg) and the total mixture was refluxed for 2 hr and treated as above to give a product (**9d**, 107 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3593 (w), 3493 (w, br) (OH), no ester carbonyl. A solution of **9d** (100 mg) in anhydrous 10% HCl–MeOH (5 ml) was refluxed for 1 hr, neutralized with Ag_2CO_3 and filtered. The product obtained by concentration of the filtrate under reduced pressure was subjected to preparative TLC (benzene–acetone=2: 1, detection with I_2) to give the methylated aglycone (**3b**, 31 mg) and methylated monosaccharides. The methylated aglycone (30 mg) was acetylated with Ac_2O (2 ml) and pyridine (4 ml) to give a monoacetate (32 mg), which was recrystallized from acetone–MeOH to give an analytical sample of **3c** as colorless needles of mp 209—210°, $[\alpha]_D^{22}$ +32.3° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{60}O_6$: C, 73.43; H, 10.27. Found: C, 73.69; H, 10.32. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1744, 1247 (OAc). PMR ($CDCl_3$) δ: 0.87 (6H, s), 0.90, 0.92 (3H each, both s), 0.98 (6H, s), 1.42 (3H, s) (*tert.* $CH_3\times7$), 2.04 (3H, s, OAc), 3.28, 3.32, 3.36, 3.44 (3H each, all s, $OCH_3\times4$), 4.51 (1H, t-like, 3α-H), 5.32 (1H, m, 12-H); (C_6D_6) δ: 0.88 (6H, s), 0.94 (3H, s), 1.07 (6H, s), 1.11 (3H, s) (*tert.* $CH_3\times6$), 1.78 (3H, s, 14α-CH_3), 1.90 (3H, s, OAc), 3.14, 3.16, 3.36, 3.59 (3H each, all s, $OCH_3\times4$), 4.52 (1H, t-like, 3α-H), 5.42 (1H, m, 12-H). Mass Spectrum *m/e* (%): 588 (M^+, 10), 556 (M^+–MeOH, 14), 524 (M^+–2MeOH, 24), 511 (M^+–CH_2OMe–MeOH, 44), 479 (M^+–CH_2OMe–2MeOH, 55), 447 (M^+–CH_2-OMe–3MeOH, 11), 419 (M^+–AcOH–CH_2OMe–2MeOH, 7), 338 (**i**, 19), 306 (**i**–MeOH, 7), 293 (**i**–CH_2OMe, 15), 274 (**i**–2MeOH, 8), 261 (**i**–CH_2OMe–MeOH, 100), 242 (**i**–3MeOH, 4), 229 (**i**–CH_2OMe–2MeOH, 51), 197 (**i**–CH_2-OMe–3MeOH, 9), 249 (**ii**, 10), 189 (**ii**–AcOH, 16). *cf.* A_1-Barrigenol tetraacetate (**3d**): PMR ($CDCl_3$) δ: 0.84, 0.88, 0.93, 0.96, 0.99, 1.01 (3H each, all s, *tert.* $CH_3\times6$), 1.52 (3H, s, 14α-CH_3), 2.06 (9H, s), 2.09 (3H, s) (OAc$\times$ 4), 3.70, 4.00 (2H, ABq, J=11.7 Hz, 28–H_2), 4.22 (1H, d, J=4 Hz, 16β–H), 4.47 (1H, t-like, 3α–H), 5.10 (1H, d, J=4 Hz, 15β–H), 5.23 (1H, d.d, J=5 & 7 Hz, 22β–H), 5.41 (1H, m, 12–H).

The methylated monosaccharides were identified with methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (I), methyl 3-O-methyl-glucopyranoside (IV), methyl 3,4,6-tri-O-methyl-galactopyranoside (VI), and methyl 2,3,4-tri-O-methyl-rhamnopyranoside (VII) by GLC and TLC. Methyl 3-O-methyl-glucopyranoside (IV) was further identified as its triacetate (V) as above by GLC and TLC. GLC: i) column: 3% SE-30 on chromosorb W (80—100 mesh), 3 mm$\times$2 m; column temp.: 200°; carrier gas: N_2 30 ml/min; t_R (min): IV, 3′15″. ii) column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm$\times$2 m; column temp.: 105°; carrier gas: N_2 20 ml/min; t_R (min): V, 3′54″. iii) column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm$\times$2 m; column temp.: 200°; carrier gas: N_2 30 ml/min; t_R (min): VI, 9′38″, 13′35″. iv) column: 15% NPGS on chromosorb WAW (80—100 mesh), 3 mm$\times$2 m; column temp.: 180°; carrier gas: N_2 30 ml/min; t_R (min): I, 4′57″, 6′18″; VII, 2′46″, 3′36″. TLC: benzene–acetone=4: 1 for I, V, and VII; $CHCl_3$–MeOH=4: 1 or EtOAc for IV; benzene–acetone=2: 1 for VI.

Diazomethane Methylation of Desacyl-boninsaponin A (9a) giving 9b——A solution of desacyl-boninsaponin A (**9a**, 100 mg) in MeOH (250 ml) was treated with ethereal CH_2N_2 at room temperature overnight and evaporated to dryness to give **9b**. The analytical sample was prepared by recrystallization from MeOH as colorless needles of mp 275—278°, $[\alpha]_D^{23}$ −15.5° (c=1.0, MeOH). *Anal.* Calcd. for $C_{55}H_{90}O_{25}\cdot6H_2O$: C, 52.45; H, 8.16. Found: C, 52.41; H, 8.15. IR ν_{max}^{KBr} cm^{-1}: 3410 (br, OH), 1748 ($COOCH_3$).

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