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Saponin and Sapogenol. VIII.[1] Photochemical Cleavage of Glycoside Linkage in Saponin. (1). Photolysis of Some Saponins and Their Structural Features

Isao Kitagawa, Masayuki Yoshikawa, Yasuhiro Imakura, and Itiro Yosioka

Faculty of Pharmaceutical Sciences, Osaka University[2]

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In search of a new hydrolysis method of saponin, it has been found that ultraviolet irradiation is a convenient procedure for hydrolysis of some oleanane triterpenoid saponins resulting in liberation of their genuine sapogenols.

The subsequent investigation on structure requirement for ready photolysis of glycoside linkage in saponin has revealed that an uronic acid moiety directly connected to the sapogenol is an essential constituent in the carbohydrate portion.

It has been generally known that the ordinary acid hydrolysis of saponin often gives rise to some undesired secondary alteration of the sapogenol during the procedure. In order to split the glycoside linkage without such unfavorable change of the sapogenol, some devices have been made chemically (periodate oxidation,[3] heterogeneous acid hydrolysis,[4] and chemical modification of saponin prior to hydrolysis[5]), enzymatically,[6] and microbiologically.[7] For these years, we have demonstrated that the soil bacterial hydrolysis method is a useful and widely applicable procedure in the structure elucidation of various types of genuine sapogenols and aglycones.[8]

In the present paper, we wish to report that ultraviolet irradiation is a facile procedure for the cleavage of glycoside linkage in some types of oleanane triterpenoid saponins resulting in the liberation of their genuine sapogenols, and it has become clear as described below that a uronic acid moiety linking directly to the sapogenol portion is an essential constituent for ready photolysis.

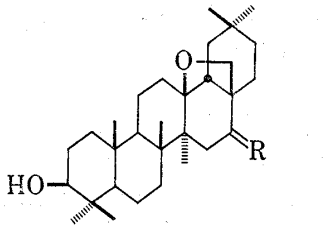
Photolysis of Some Kinds of Saponins[9] and Glycosides

During the study on behavior of several kinds of triterpenoid glycosides upon electron impact, it has been recognized that the glycosides seldom give the molecular ions and the

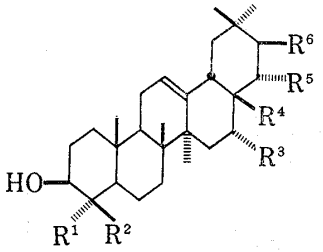
1) Part VII: I. Kitagawa, A. Matsuda, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2226 (1972).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) *a*) F. Smith and A.M. Unran, *Chem. & Ind.*, **1959**, 881; *b*) G.W. Hay, B.A. Lewis, and F. Smith, *Methods in Carbohydrate Chemistry*, **5**, 357 (1965); *c*) J.J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).
4) R. Tschesche, F. Inchaurrondo, and G. Wulff, *Ann.*, **680**, 107 (1964).
5) *a*) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966); *b*) R. Tschesche, G. Lüdke, and G. Wulff, *Chem. Ber.*, **102**, 1253 (1969).
6) *a*) R. Tschesche and G. Wulff, *Tetrahedron*, **19**, 621 (1963); *b*) T. Kawasaki, I. Nishioka, T. Yamauchi, K. Miyahara, and M. Enbutsu, *Chem. Pharm. Bull.* (Tokyo), **13**, 435 (1965); *c*) P. Tunmann, W. Gerner, and G. Stapel, *Ann.*, **694**, 162 (1966).
7) *a*) C.H. Hassal and B.S.W. Smith, *Chem. & Ind.*, **1957**, 1570; *b*) Ch. Tamm and A. Gubler, *Helv. Chim. Acta*, **42**, 239 (1959); *c*) W.A. Lourens and M. B. O'Donovan, *S. African J. Agr. Sci.*, **4**, 293 (1961) [*C.A.*, **56**, 10196 (1962)].
8) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; *b*) I. Yosioka, T. Sugawara, K. Yoshikawa, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2450 (1972), and the preceding papers of the series cited therein.
9) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. & Ind.*, **1973**, 276 (preliminary communication).

fragment ions are derived abundantly from the carbohydrate portion and scarcely from the aglycone part. These observations have led us to assume that a glycoside linkage in saponin might have been cleaved photochemically. After several preliminary examinations, ultraviolet irradiation of sakurasō-saponin[1] (isolated from the root of *Primula sieboldi* E. Morren) was undertaken using a high pressure mercury lamp and the genuine sapogenol protoprimulagenin A (I)[1] was liberated in a good yield. Protoprimulagenin A was initially isolated through periodate oxidation of sakurasō-saponin along with minor quantity of primulagenin A (II) and aegicerin (III),[1] the latter two being suspected as the secondary products during the procedure. However, in the present photolysis, protoprimulagenin A was a sole sapogenol and no indication of the presence of II or III was observed in the total photoreaction product.

To search for the most pertinent reaction condition, sakurasō-saponin was subjected to photolysis under a variety of conditions. It has been clarified that the photochemical hydrolysis is effected smoothly in a quartz tube and would not proceed in a Pyrex tube or in an alkaline medium, and the reaction rate was markedly reduced by using a 30W low pressure mercury lamp, in a stream of oxygen, or in a Vycor tube.



I : R = $-\beta$H, $-\alpha$OH
III : R = O



	R^1	R^2	R^3	R^4	R^5	R^6
II :	CH_3	CH_3	OH	CH_2OH	H	H
IV :	CH_3	CH_3	OH	CH_2OH	OH	H
V :	CH_3	CH_3	OH	CH_2OH	OH	OH
VI :	CH_3	CH_2OH	OH	CH_2OH	OH	OH
VII :	CH_2OH	CH_3	OH	CH_2OH	OH	OH
VIII :	CH_2OH	CH_3	OH	CH_2OH	OH	H
IX :	CHO	CH_3	OH	CH_2OH	OH	OH
X :	CH_3	CH_2OH	H	CH_3	H	αOH

Chart 1

Since the successful photochemical hydrolysis of sakurasō-saponin has appeared promising its general applicability, some other saponins and glycosides at hand have been submitted to photolysis and it has been found that some yield their genuine sapogenols whereas some do not as shown below.

The following triterpenoid saponins provided the favorable results although the yields of some sapogenols were unsatisfactory, which are listed in Table I: kurinsō-saponin[1] (from the root of *Primula japonica* A. Gray), desacyl-jegosaponin[10] (prepared by alkaline hydrolysis of jegosaponin[11] isolated from the pericarps of *Styrax japonica* Sieb. et Zucc.), a desacyl derivative[10] of horse-chestnuts saponin[12] (from the seeds of *Aesculus turbinata* Blume), a desacyl derivative[10] of tea seeds saponin[13] (from the seeds of *Thea sinensis* L.), and soya-

10) Since these saponins have been known to be the mixtures of acylated derivatives,[11,12a,13a] their desacyl preparations were subjected to photolysis to simplify composition of the photoreaction products.

11) *a*) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353; *b*) I. Yosioka, S. Saijoh, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 564 (1972).

12) *a*) G. Wulff and R. Tschesche, *Tetrahedron*, **25**, 415 (1969); *b*) I. Yosioka, A. Matsuda, K. Imai, T. Nishimura, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1200 (1971).

13) *a*) R. Tschesche, A. Weber, and G. Wulff, *Ann.*, **721**, 209 (1969); *b*) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970); *c*) *Idem, ibid.*, **18**, 1621 (1970); *d*) *Idem, ibid.*, **19**, 1186 (1971).

saponin I[14] (from the seeds of *Glycine max* MERRILL). The sapogenol compositions thus obtained are approximately in parallel with those previously elucidated by combination of acid hydrolysis, periodate oxidation, and soil bacterial hydrolysis.

TABLE I. Photolysis Products of Various Saponins

Saponins	Products[a]	
Sakurasō-saponin	protoprimulagenin A (I)	61.0%
Kurinsō-saponin	dihydropriverogenin A (IV)	17.0
	protoprimulagenin A (I)	2.0
Desacyl-jegosaponin	barringtogenol C (V)	61.0
Desacyl derivative of	barringtogenol C (V)	2.2
Horse-chestnuts saponin	protoaescigenin (VI)	9.6
Desacyl derivative of	dihydropriverogenin A (IV)	3.0
Tea seeds saponin[b]	barringtogenol C (V)	3.4
	theasapogenol A (VII)	3.3
	camelliagenin C (VIII)	3.0
Soyasaponin I	soyasapogenol B (X)	30.0

a) The yields were calculated on the basis of estimated molecular weights of the consumed major saponins or desacyl saponins.
b) Theasapogenol E (IX) was not obtained. It is assumed for one reason that the theasapogenol E moiety in the parent saponin would be converted to the theasapogenol A (VII) moiety on the preparation of desacyl saponin,[13d] and another reason is ascribed to its photo-instability.

However, the saponins and glycosides given below gave the unfavorable results: senegin[3c,8a,15] (oleanene-type saponin from the root of *Polygala senega* L., Mi-saponin[16] (oleanene-type saponin from the seed kernels of *Madhuca longifolia* L.), ginsenoside Rb_2[17] (dammarane-type saponin from the *Ginseng* root), a steroidal glycoside mixture[18] (from the root of *Metanarthecium luteo-viride* MAXIM.), paeoniflorin[19] (a momoterpene glucoside from the root of *Paeonia albiflora* PALLAS), antirrhinoside[20] (an iridoid glucoside from *Linaria japonica* L.). Among them, Mi-saponin and ginsenoside Rb_2 afforded a few unidentified products other than sapogenols respectively as detected by thin-layer chromatography (TLC), which will be the subjects of future investigation.

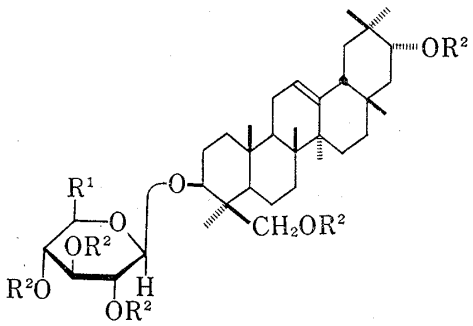
Structural Features for Ready Photolysis[21]

Examination of the chemical structures of above described saponins and glycosides[22] has disclosed that one of the significant structural differences between two photoreactive and unreactive groups is a fact that a glucuronic acid (or its methyl ester) moiety in the carbohydrate ingredients of the formers directly attaches to an aglycone while no glucuronic acid moiety in the latters. The other significance lies of course in photostability of the sapogenol

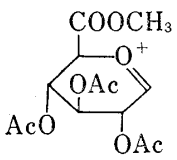
14) *a*) G. Cainelli, J.J. Britt, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **41**, 2053 (1958); *b*) H.M. Smith, J.M. Smith, and F.S. Spring, *Tetrahedron*, **4**, 111 (1958); *c*) For isolation, see the Experimental section.
15) Y. Tsukitani and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **21**, 1564 (1973), and the literatures cited therein.
16) I. Kitagawa, A. Inada, I. Yosioka, R. Somanathan, and M.U.S. Sultanbawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 630 (1972).
17) M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972).
18) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1971**, 1117, and the literatures cited therein.
19) N. Aimi, M. Inaba, M. Watanabe, and S. Shibata, *Tetrahedron*, **25**, 1825 (1969).
20) I. Kitagawa, T. Tani, K. Akita, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **21**, 1978 (1973) (preliminary communication: *idem*, *Tetrahedron Letters*, **1972**, 419).
21) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, **1973**, 3997 (preliminary communication).
22) The unknown chemical structures of some of the saponins and glycosides listed here are now under investigation in our laboratory.

or aglycone part. To establish the structural requirement for ready photolysis, glycyrrhizin, a diglucuronide of glycyrrhetic acid, was then subjected to photolysis. In this case, however, due to photo-instability of the aglycone part, a complex mixture resulted, although a small amount of glycyrrhetic acid was detected by TLC in the mixture.

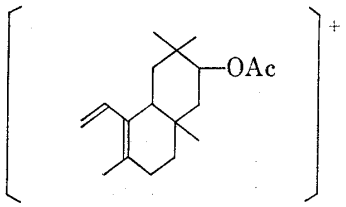
Next, in order to verify the real participation of glucuronic acid moiety in the photochemical cleavage, a prosapogenol (XIa) obtained by partial acid hydrolysis of soybean saponin has been submitted to photolysis.



XIa : R^1=$COOCH_3$, R^2=H
XIb : R^1=$COOCH_3$, R^2=Ac
XIIa : R^1=CH_2OH, R^2=H
XIIb : R^1=CH_2OAc, R^2=Ac
XIIc : R^1=CH_2OMe, R^2=Me



m/e 317 (i)



m/e 276 (ii)

Chart 2

Mild acid hydrolysis of soybean saponin followed by silica gel column chromatography furnished a prosapogenol (XIa), mp 249—250°, which exhibits a broad hydroxyl absorption band at 3480 cm^{-1} along with an ester carbonyl band at 1744 cm^{-1} in its infrared (IR) spectrum. On further acid treatment, the prosapogenol gave soyasapogenol B (X)[14] and glucuronic acid, while acetylation of the prosapogenol with acetic anhydride and pyridine gave a pentaacetate (XIb), mp 266—269°, whose proton magnetic resonance (PMR) spectrum shows a one-proton triplet-like signal at δ 3.19 due to C-3 α H, a three-proton singlet at δ 3.74 due to a methoxycarbonyl and a two-proton singlet at δ 4.16 ascribable to an acetoxymethylene function. In the mass spectrum of XIb, are observed a base peak at *m/e* 317 (i, derivable from the methyl glucuronate moiety) and a prominent fragment ion peak at *m/e* 276 (ii, derivable from the soyasapogenol B part through a reverse Diels-Alder type fragmentation[23]) in addition to a weak molecular ion peak at *m/e* 858. Therefore, the location of methyl glucuronate moiety in the prosapogenol has been determined at C-3 β OH of soyasapogenol B (X).

Treatment of XIa with $NaBH_4$ yielded a product (XIIa), mp 254—256°, which shows absence of an ester carbonyl absorption band in its IR spectrum and was hydrolyzed with acid to soyasapogenol B and glucose. On acetylation with acetic anhydride and pyridine, XIIa was converted to a hexaacetate (XIIb), mp 167—169°, which shows absence of a methoxycarbonyl function and presence of two acetoxymethylene functions in its PMR spectrum. Exhaustive methylation of XIIa furnished a hexamethyl ether (XIIc), mp 189—191°, whose PMR spectrum exhibits a one-proton doublet (δ 4.24, J=7 Hz) ascribable to $C_{1'}$-αH thus substantiating β-orientation of the glucoside linkage. On methanolysis, XIIc afforded methyl 2,3,4,6-tetra-O-methyl-glucopyranoside.

23) 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.

The accumulated evidence has led to formulations of the prosapogenol and its reduction product as 3-O-β-D-glucuronopyranosyl-soyasapogenol B methyl ester (XIa) and 3-O-β-D-glucopyranosyl-soyasapogenol B (XIIa) respectively.

Irradiation of XIa and XIIa under the same reaction conditions revealed completely different behavior of both compounds. From XIa, soyasapogenol B (X) was liberated in an excellent yield while XIIa was recovered unchanged. Consequently, it has now become apparent that a glucuronic acid moiety attached directly to a sapogenol portion is indispensable for ready photolysis.

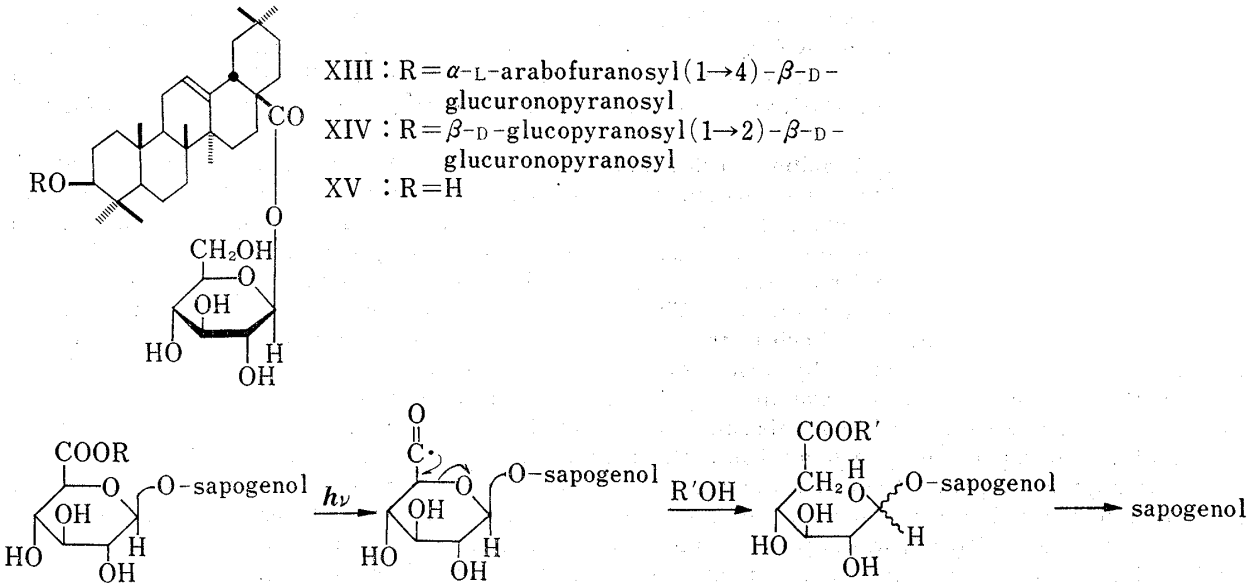


Chart 3

To achieve an additional support for the above finding, chikusetsusaponin IV (XIII)[24] and V (XIV)[25] were subjected to photolysis and a prosapogenol (designated as compound O (XV)[8b] was liberated from both in a good yield as expected. The ester glucoside linkage in both saponins was found less photoreactive, however, prolonged irradiation resulted in the formation of further degradation product.

As for the reaction pathway, a scheme depicted in Chart 3 is attractive as one of the plausible paths, which will be a subject of further investigation. From the mechanistic viewpoint, it should be mentiond here that other uronide linkage such as a galacturonide moiety could be also photoreactive.

The present photochemical cleavage of uronide linkage appears to be a first example of the photolysis of glycoside which possesses no ordinary photoreactive function in the aglycone part, although some related studies have been reported on the photolysis of aryl glycosides[26] and cellulose.[27] Since the uronide moiety has been known to occur often in the carbohydrate ingredients of saponins,[28] the present finding seems to offer a new approach on the structure determination of some kinds of saponins and an effective degradation method of the other kinds of uronide derivatives.

24) N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969).
25) N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).
26) *a*) T. Kariyone, M. Takahashi, K. Takaishi, and W. Kamisako, *Yakugaku Zasshi*, **78**, 939 (1958); *b*) U. Zehavi and A. Patchornik, *J. Org. Chem.*, **37**, 2285 (1972).
27) *a*) R.L. Desai, *Pulp. Pap. Mag. Canad.*, **69**, T322 (1968); *b*) C. Kujirai, *Sen-i Gakkaishi*, **22**, 84 (1966).
28) *a*) K. Hiller, M. Keipert, and B. Linzer, *Pharmazie*, **21**, 713 (1966); *b*) N. Basu and R.P. Rastogi, *Phytochemistry*, **6**, 1249 (1967); *c*) H.-D. Woitke, J.-P. Kayser, and K. Hiller, *Pharmazie*, **25**, 213 (1970).

Experimental[29]

Photolysis of Sakurasō-saponin——1) A solution of sakurasō-saponin (700 mg) in MeOH (700 ml) was irradiated internally with a 500 W high pressure mercury lamp (Eikōsha PIH-500) for one hour. The temperature of reaction mixture was 29—32° during the period. After neutralizing with aq. 10% K_2CO_3,[30] MeOH was removed under reduced pressure while diluting with water and the aqueous mixture thus obtained was extracted with $CHCl_3$ and *n*-BuOH successively. The both extracts obtained by evaporation of the solvent (200 mg and 310 mg respectively) were subjected to preparative TLC (developing with $CHCl_3$: MeOH= 30:1) to give protoprimulagenin A (I, 146 mg, 46%) which was further recrystallized from $CHCl_3$ to give colorless needles of mp 271—273°, being identical with the authentic sample by mixed mp, IR (KBr), and TLC.

2) In another experiment, sakurasō-saponin (200 mg) was irradiated in MeOH (400 ml) with stirring for one hour and neutralized with aq. K_2CO_3 as above and the reaction mixture was evaporated under reduced pressure while adding water. The resulting precipitate was collected by filtration, washed and dried to yield a crude product (110 mg), which was crystallized from MeOH to give protoprimulagenin A (57 mg, 61%) as colorless needles.

Examination of Photolytic Conditions using Sakurasō-saponin——1) Internal Irradiation: a) A solution of sakurasō-saponin (50 mg) in MeOH (500 ml) was irradiated internally with a 500 W high pressure mercury lamp for 30 min (reaction temp.: 21—23°). TLC of the total reaction mixture showed some saponin still being left unattacked. The product obtained as above was purified by preparative TLC to give 7 mg of protoprimulagenin A(I). b) Under the same conditions as above with a stream of nitrogen, 6 mg of I was obtained starting from 50 mg of saponin. c) With a stream of oxygen, one mg of I was obtained.

2) External irradiation: a) Each methanolic solution (5 ml) of saponin (5 mg) in a quartz, Vycor, or Pyrex tube was Irradiated externally (distance=2 cm) with a 500 W high pressure mercury lamp for 30 min, and the reaction mixture was examined by TLC. Protoprimulagenin A(I) was smoothly liberated in a quartz tube, however, in a Vycor tube, the product was found to be a mixture of saponin and a trace amount of I, and saponin was unchanged in a Pyrex tube. b) A solution of saponin (5 mg) in MeOH (5 ml) was adjusted to pH 9—10 or 11 with aq. 2 N NaOH or 10% K_2CO_3 and irradiated as above in a quartz tube for 30 min and I was not detected in the total reaction mixture in each case. c) A solution of saponin (5 mg) in MeOH (5 ml) was adjusted to pH 2—3 with aq. H_2SO_4 and irradiated in a quartz tube for 30 min. TLC examination of the reaction mixture showed liberation of I as above a).

Photolysis of Kurinso-saponin——1) A methanolic solution (600 ml) of kurinsō-saponin (600 mg) was irradiated for one hour as above, treated with aq. 10% K_2CO_3 (to pH= *ca.* 10) under heating for a while[31] and evaporated under reduced pressure to remove MeOH. Resulting white precipitate (120 mg) was collected by filtration and the aqueous filtrate was extracted with *n*-BuOH. The *n*-BuOH extraction recovered saponin (200 mg),[32] while purification of the white precipitate by preparative TLC($CHCl_3$: MeOH=30: 1) afforded dihydropriverogenin A (IV, 48 mg, colorless needles of mp 279—283° from $CHCl_3$–MeOH mixture) and protoprimulagenin A (I, 6 mg, colorless needles of mp 270—272° from $CHCl_3$), being identical with their authentic samples by mixed mp, IR(KBr), and TLC.

Photolysis of Desacyl-jegosaponin——1) Desacyl-jegosaponin: A solution of jegosaponin (6 g) in an alkaline medium (KOH 6 g, water 60 ml, and EtOH 200 ml) was refluxed for 4 hr, diluted with water, and evaporated under reduced pressure while keeping the mixture neutral with dil. HCl. An aqueous solution thus obtained was extracted repeatedly with *n*-BuOH saturated with water. The *n*-BuOH solution was then washed with water and evaporated to dryness *in vacuo* to afford desacyl-jegosaponin (5.89 g), which was crystallized from aqueous EtOH to give colorless needles of mp 248—251°.

2) Photolysis: A solution of desacyl-jegosaponin (700 mg) in MeOH (700 ml) was irradiated for 40 min as above (reaction temp.: 20—30°), treated with aq. 10% K_2CO_3 (pH=*ca.* 8), evaporated under reduced pressure to remove MeOH, and diluted with water. The aqueous suspension was then extracted with $CHCl_3$–MeOH (20: 1) mixture and *n*-BuOH saturated with water successively and the $CHCl_3$–MeOH

29) The following instruments were used for the physical data. Melting points: Yanagimoto Micro-melting-point Apparatus (a hot-stage type) and recorded uncorrected; Specific rotations: Rex Photoelectric Polarimeter NEP-2 (1=1 dm); IR spectra: Hitachi IR Spectrometer EPI-S2 or EPI-G3; PMR spectra (in $CDCl_3$, tetramethylsilane as an internal standard): Hitachi R-20A and R-22 NMR Spectrometers; Mass spectra: Hitachi RMU-6D Mass Spectrometer (direct inlet). For chromatography, silica gel (Merck, 70—230 mesh for column) and Silica gel D-5 (Camag, for TLC) were used and on preparative TLC, water was sprayed for detection.

30) The reaction mixtures after irradiation always showed acidic, however the reason is still obscure.

31) Since dihydropriverogenin A seems to constitute saponin partly as its acetyl derivative, alkaline hydrolysis under heating could simplify the sapogenol composition.

32) To avoid secondary photochemical change of the sapogenols, all the reactions were terminated in shorter period and starting saponins were recovered.

in vacuo, and subjected to paper partition chromatography (PPC): Toyo Filter Paper No. 50, developing layer was washed with water and evaporated *in vacuo* to give white powder (132 mg) which was crystallized from $CHCl_3$–MeOH mixture to afford barring togenol C(V) being identical with the authentic sample by mixed mp, IR(KBr), and TLC. From the *n*-BuOH extract was recovered 201 mg of desacyl-jegosaponin.

Photolysis of Desacyl Derivative of Horse-chestnuts Saponin——Horse-chestnuts saponin (6 g) was treated with an alkaline mixture (KOH 7.5 g, water 30 ml, and EtOH 200 ml) as above to give a crude desacyl derivative (5.4 g). A solution of the crude desacyl saponin (450 mg) in EtOH (450 ml) was irradiated for 40 min as above and the product was extracted with $CHCl_3$–MeOH (20: 1) mixture and *n*-BuOH saturated with water. Yellowish brown powder (70 mg) obtained from $CHCl_3$–MeOH extract was subjected to preparative TLC ($CHCl_3$: MeOH: water=20: 3: 0.5, lower layer) to afford protoaescigenin (VI, 22 mg) and barringtogenol C(V, 5 mg), both of which were recrystallized from $CHCl_3$–MeOH mixture and identified with the authentic samples by mixed mp, IR(KBr), and TLC. The *n*-BuOH extraction recovered a mixture (128 mg), which was revealed by TLC to contain the starting desacyl saponin as the major.

Photolysis of Desacyl Derivative of Tea Seeds Saponin——Commercial "teaponin" (3 g, Izome Yuka Kogyo Co., Shizuoka) was treated with an alkaline mixture (KOH 3.75 g, water 15 ml and EtOH 100 ml) as above to give a crude desacyl derivative (2.01 g). A solution of the crude desacyl saponin (800 mg) in MeOH (800 ml) was irradiated and extracted as above. The $CHCl_3$–MeOH extract (147 mg, sapogenol mixture) was subjected to preparative TLC ($CHCl_3$: MeOH: water=20: 4: 0.5) to afford theasapogenol A (VII, 13 mg), barringtogenol C (V, 12 mg), camelliagenin C (VIII, 9 mg), and dihydropriverogenin A (IV, 10 mg), which were recrystallized from $CHCl_3$–MeOH mixture and identified with the authentic samples respectively by mixed mp, IR(KBr), and TLC. The *n*-BuOH extraction recovered a mixture (180 mg) which contains the starting desacyl saponin as the major as revealed by TLC.

Isolation of Soybean Saponin——Powdered soybean (7 kg) was defatted with hot *n*-hexane and extracted with MeOH at reflux. The MeOH extract was then partitioned in *n*-BuOH–water (1: 1) mixture as usual and *n*-BuOH soluble portion was evaporated *in vacuo* to give a residue which was dissolved in a small amount of MeOH and defatted again with *n*-hexane. The MeOH soluble portion was then treated with a large amount of ether to precipitate crude saponin which was collected by filtration, dissolved again in MeOH and passed through a column (active charcoal :150 g, Tokusei-shirasagi, Takeda Chem. Ind.; Celite 535: 150 g, Wako Pure Chem. Ind.) eluting with MeOH. A saponin mixture thus obtained (60 g) was treated with *n*-BuOH and the insoluble portion collected by filtration was then treated with aq. 5% NaOH to give a suspension, from which the insoluble portion (15 g) was collected by a centrifuge. The precipitate was then crystallized from weakly acidic aq. MeOH to give a crystalline saponin mixture (10 g). The saponin (8 g) was then mixed with silica gel (40 g) with aid of MeOH, dried, put on a column of silica gel (560 g) and chromatographed developing with a lower layer of $CHCl_3$–MeOH–water (7: 3: 1) mixture to afford soyasaponin I (4.5 g, major), soyasaponin II (0.5 g), soyasaponin III (0.12 g), and their mixture (I+II: 1.1 g; other mixture: 1 g).

Photolysis of Soyasaponin I——A solution of soyasaponin I (700 mg) in MeOH (700 ml) was irradiated for one hour at room temperature (reaction temp.: 32—33°), neutralized, and concentrated *in vacuo* as for sakurasō-saponin. The resulting precipitate (300 mg) was collected by filtration and purified by preparative TLC ($CHCl_3$: MeOH=30: 1) to give soyasapogenol B (X, 90 mg), which was recrystallized from $CHCl_3$–MeOH mixture to give colorless needles of mp 261—263°, being identical with the authentic sample by mixed mp, IR (KBr), and TLC.

Isolation of Prosapogenol (XIa) from Soyasaponin I——A mixture of soyasaponin I (2.2 g) in aq. 10% H_2SO_4–MeOH (1: 2) (30 ml) was refluxed for 14 hr and poured into cold water. The precipitate (1.5 g) collected by filtration was mixed with silica gel (5 g) with aid of MeOH, dried, put on a column of silica gel (70 g), and chromatographed eluting with $CHCl_3$ and $CHCl_3$–MeOH mixture successively. Elution with $CHCl_3$–MeOH (400: 1) gave soyasapogenol B (X, 180 mg) and that with $CHCl_3$–MeOH (40: 1–30: 1) furnished a prosapogenol (XIa, 200 mg), which was recrystallized from MeOH to give colorless needles of mp 249—250°, $[\alpha]_D^{12}$ +34.1° (c=0.3, $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{60}O_9 \cdot 1/2H_2O$: C, 67.55; H, 9.35. Found: C, 67.43; H, 9.17. IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1744 ($COOCH_3$).

Pentaacetyl-prosapogenol (XIb)——XIa (20 mg) was treated with Ac_2O (0.1 ml) and pyridine (0.2 ml) at room temperature overnight followed by usual work-up. White powder (25 mg) thus obtained was crystallized from MeOH to give colorless prisms of mp 266—269°, $[\alpha]_D^{12}$ +40.1° (c=0.5, MeOH). *Anal.* Calcd. for $C_{47}H_{70}O_{14}$: C, 65.71; H, 8.21. Found: C, 65.26; H, 8.14. IR ν_{max}^{Nujol} cm^{-1}: 1760, 1728 (OAc, $COOCH_3$). PMR (60 MHz) δ: 0.81, 0.90, 1.00, 1.06, 1.13 (3H, each, s), 0.97 (6H, s) (seven methyls), 2.02, 2.04 (totally 15H, s, five acetoxyls), 3.19 (1H, t-like, J=7 Hz, $C_{(3)}\underline{H}$), 3.74 (3H, s, $-COOC\underline{H}_3$), 4.16 (2H, s, $C_{(24)}\underline{H}_2OAc$), 4.59 (1H, d, J=*ca.* 8 Hz, $C_{(1')}\underline{H}$), 4.63 (1H, $C_{(21)}\underline{H}$).[33] Mass Spectrum (m/e %): 858 (M^+, 2), 525 (10), 317 (i, 100), 276 (ii, 36), 249 (45), 216 (ii-AcOH, 98).

Acid Hydrolysis of XIa——A mixture of XIa (5 mg) in aq. 10% H_2SO_4–MeOH (1: 1) (2 ml) was refluxed for 20 hr, poured into cold water, and filtered to collect a precipitated product, which was crystallized from $CHCl_3$–MeOH mixture to give soyasapogenol B (X) being identical with the authentic sample by mixed mp,

33) The coupling pattern is unclear due to overlapping with the signal of $C_{(1')}H$.

IR(KBr), and TLC. The aqueous filtrate was neutralized with aq. saturated $Ba(OH)_2$, filtered, concentrated for 24 hr with i) iso-PrOH–*n*-BuOH–water (7: 1: 2) (Rf=0.10) and ii) *n*-BuOH–pyridine–water–benzene (5: 3: 3: 1, upper layer) (Rf=0.10), and glucuronic acid was detected.[34]

$NaBH_4$ Reduction of XIa——To a solution of XIa (50 mg) in EtOH (2 ml), was added a solution of $NaBH_4$ (10 mg) in EtOH (1 ml) and the total mixture was stirred at room temperature for 20 min, treated with acetone (5 ml), diluted with EtOH (5 ml), and passed through columns of Amberlite IRA-400 (OH^-) (5 g) and Dowex $50^W \times 8$ (H^+) (5 g) successively to give a reduction product (46 mg), which was crystallized from MeOH–$CHCl_3$–acetone mixture to give colorless needles of XIIa, mp 254—256°, $[\alpha]_D^{12}$ +38.7° (c=0.5, MeOH). *Anal.* Calcd. for $C_{36}H_{60}O_8$: C, 69.64; H, 9.74. Found: C, 69.17; H, 9.68. IR ν_{max}^{KBr} cm^{-1}: 3330 (OH), 1640 (C=C).

Acid Hydrolysis of XIIa——A mixture of XIIa (5 mg) in 1 N H_2SO_4 (in dioxane –water (1: 3) mixture) (1 ml) was refluxed for 6 hr, poured into water, and the precipitate was collected by filtration and subjected to preparative TLC ($CHCl_3$: MeOH=30: 1) to give soyasapogenol B (X, 3 mg), being identical with the authentic sample by mixed mp, IR(KBr), and TLC. The aqueous filtrate was passed through a column of Amberlite IR-45(OH^-) (5 g), concentrated *in vacuo*, and subjected to PPC: Toyo Filter Paper No. 50, developing with iso-PrOH: *n*-BuOH: water=7: 1: 2 for 24 hr, detection by aniline hydrogen phthalate, to identify glucose (Rf=0.45).

Acetylation of XIIa giving Hexaacetate (XIIb)——Acetylation of XIIa (40 mg) with Ac_2O (0.3 ml)–pyridine (1 ml) in the usual way at room temperature afforded a product, which was crystallized from aqueous MeOH to give a hexaacetate (XIIb) as colorless plates of mp 167—169°, $[\alpha]_D^{11}$ +42° (c=0.4, MeOH). *Anal.* Calcd. for $C_{46}H_{70}O_{13}$: C, 66.03; H, 8.31. Found: C, 65.63; H, 8.47. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1765 (OAc). PMR (60 MHz) δ: 0.83, 0.92, 1.07, 1.15 (3H, each, s), 0.99 (9H, s) (totally seven methyls), 2.01, 2.06 (totally 18H, six acetoxyls), 3.22 (1H, t-like, J=7 Hz, $C_{(3)}\underline{H}$), 4.20 (4H, br. s, $W_{h/2}$=3.5 Hz, $C_{(24)}\underline{H_2}OAc$, $C_{(6')}\underline{H_2}OAc$).

Methylation of XIIa giving Hexamethyl Ether (XIIc)——To a stirred solution of XIIa (30 mg) in DMSO (2 ml) under N_2 atmosphere, was added a solution (5 ml) of DMSO carbanion (prepared by heating a mixture of 1 g of NaH which was washed with petr. ether beforehand and 20 ml of DMSO under N_2 atmosphere at 50—60° for one hour) and the total mixture was stirred at room temperature for 4 hr, treated with CH_3I (5 ml) and left standing overnight. After diluting with water, the mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was purified by preparative TLC ($CHCl_3$: MeOH=200: 1) to give a hexamethyl ether (XIIc), which was recrystallized from benzene–MeOH to colorless needles of mp 189—191°, $[\alpha]_D^{21}$ +24.5° (c=0.3, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{72}O_8$: C, 71.55; H, 10.30. Found: C, 71.21; H, 10.08. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1100 (C–O–C). PMR (90 MHz) δ: 0.84, 0.88, 0.95, 1.08, 1.15 (3H, each, s), 0.98 (6H, s) (totally seven methyls), 3.10 (1H, m, $C_{(3)}\underline{H}$), 3.23, 3.27, 3.37, 3.50 (3H, each, s), 3.62 (6H, s) (totally six methoxyls), 4.24 (1H, d, J=7 Hz, $C_{(1')}\underline{H}$), 5.20 (1H, m, $=C_{(12)}\underline{H}$–).

Methanolysis of XIIc——A mixture of XIIc (7 mg) in 2 N HCl–MeOH (2 ml) was refluxed for one hour, poured into water, and filtered to give an aqueous filtrate, which was extracted with ether. The ether extract was subjected to gas liquid chromatography (column: 3% SE-30 on chromosorb W, 3 mm × 1 m, column temp.: 190°; detector temp.: 200°; carrier gas: N_2 3 kg/cm^2), and methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (retention time: 2 min 40 sec) was identified.

Photolysis of XIa and XIIa——1) A solution of XIa (50 mg) in EtOH (500 ml) was irradiated for 30 min as before (reaction temp.: 17—27°), neutralized with aq. 10% K_2CO_3, and evaporated *in vacuo* while adding water. The white precipitate (47 mg) collected by filtration was then subjected to preparative TLC ($CHCl_3$: MeOH= 30: 1) to afford recovered prosapogenol (XIa, 15 mg) and soyasapogenol B (X, 20 mg) which was recrystallized from $CHCl_3$–MeOH mixture to give colorless needles of mp 258—260° (identified by mixed mp, IR(KBr), and TLC).

2) A solution of XIIa (5 mg) in EtOH (5 ml) was irradiated in a quartz tube with a 500 W high pressure mercury lamp (distance=2 cm) for 30 min, passed through a column of Amberlite IR-45 (OH^-) (5 g), and evaporated *in vacuo* to recover the starting material (5 mg). However, it was found by TLC that further irradiation for 3 hr resulted in formation of an unidentified product which was non-identical with soyasapogenol B.

Photolysis of Chikusetsusaponin IV (XIII) and V (XIV)——1) Preliminary Examination: A solution of chikusetsusaponin IV (XIII, 5 mg) in MeOH (5 ml) was irradiated for 20 min (reaction temp.: 24—38°) and worked up as described above to give a product which was identified with compound (O)(XV) by TLC. Irradiation of chikusetsusaponin V (XIV, 5 mg) also furnished compound (O) as a sole product as detected by TLC.

2) A solution of a 1: 1 mixture of chikusetsusaponin IV and V (350 mg) in MeOH (700 mg) was irradiated for 25 min as before (reaction temp.: 16—25°), neutralized with aq. 10% K_2CO_3, and evaporated to remove MeOH while adding water. The resulting aqueous suspension was extracted with ether and *n*-

34) Although distinguishable by coloration with aniline hydrogen phthalate, PPC was yet unsatisfactory to differentiate glucuronic acid from galacturonic acid even by development with a recommended solvent system ii (N. Albon and D. Gross, *Analyst*, **75**, 454 (1950)).

BuOH. The ether extract obtained by evaporation of the solvent was treated with benzene and the insoluble product (50 mg) was purified by preparative TLC ($CHCl_3$: MeOH=5:1) to give compound (O) (XV, 42 mg), which was recrystallized from MeOH to give colorless needles of mp 233—234.5° being identical with the authentic sample by mixed mp, IR (KBr), and TLC. The *n*-BuOH extraction recovered a mixture of starting saponins (280 mg).

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