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Saponin and Sapogenol. V.[1] Sapogenol Constituents of Seeds of *Camellia sasanqua* Thunb. and Leaves of *Ternstroemia japonica* Thunb.

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The sapogenol constituents of two *Theaceous* plant materials: seeds of *Camellia sasanqua* Thunb. and leaves of *Ternstroemia japonica* Thunb., have been examined in connection with the previous investigation on tea seeds sapogenols.

In addition to eight known oleanane triterpenoids: dihydropriverogenin A (II), camelliagenin B (III), A_1-barrigenol (IV), camelliagenin C (V), barringtogenol C (VI), camelliagenin D (VII), theasapogenol E (VIII), and theasapogenol A (IX), a new sapogenol designated as 22*a*-hydroxy-erythrodiol (I) has been isolated from the former seeds while oleanolic acid, primulagenin A (XIV), dihydropriverogenin A, and A_1-barrigenol have been obtained from the latter leaves. Isolation of primulagenin A from the *Theaceous* plant material seems to be unprecedented.

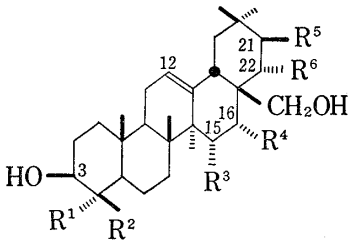
In relation to the investigation on tea seeds sapogenols,[3–5] we have further examined the sapogenol constituents of two related *Theaceous* plant materials: seeds of *Camellia sasanqua* Thunb. (Japanese name: sazanka) and leaves of *Ternstroemia japonica* Thunb. (Japanese name: mokkoku), anticipating chemotaxonomical significance if any. The present paper deals with the details of our efforts on these two materials.

Sapogenol Constituents of *Camellia sasanqua* Seeds[6]

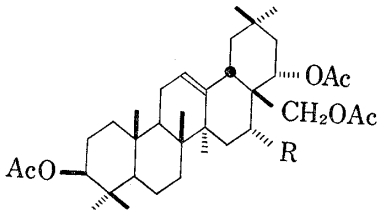
When we started the examination on the seeds sapogenols of *Camellia sasanqua* Thunb., practically no chemical study had been made on the sapogenols, but only the preliminary account on the saponin (named Sasanqua saponin)[7] and fat ingredient[8] had been reported. Eventually, we have been able to eluicidate nine sapogenols as described below. However, it has been noticed later on that Ito and Ogino have also investigated simultaneously the sapogenol composition and reached the similar conclusion as ours although they have obtained only six sapogenols including unidentified one.[9] Besides, it should be mentioned here that Yamada, *et al.* have worked on the constituents of *Camellia sasanqua* independently and isolated sasanquin (=3-methoxy-4-(β-primeverosidoxy)-1-allylbenzene) from the young leaves[10] and recently they have also reported the purification and biological activities of seeds saponin of the same plant.[11]

1) Part IV: I. Yosioka, A. Matsuda, K. Imai, T. Nishimura and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1200 (1971).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) I. Yosioka, T. Nishimura, A. Matsuda and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).
4) I. Yosioka, T. Nishimura, A. Matsuda and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1621 (1970).
5) I. Yosioka, T. Nishimura, A. Matsuda and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971).
6) Preliminary reports on the subjects: *a*) I. Yosioka, A. Matsuda and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **15**, 547 (1967); *b*) I. Yosioka, A. Matsuda, R. Takeda and I. Kitagawa, *Chem. & Ind.*, **1968**, 745.
7) S. Aoyama, *Yakugaku Zasshi*, **50**, 454 (1930).
8) S.R. Chakrabarty and M.M. Chakrabarty, *Indian Soap J.*, **20**, 16 (1954) (*Chem. Abstr.*, **49**, 16469 (1955)).
9) S. Ito and T. Ogino, *Tetrahedron Letters*, **1967**, 1127.
10) T. Yamada, H. Aoki, T. Tamura and Y. Sakamoto, *Agr. Biol. Chem.* (Tokyo), **31**, 85 (1967).
11) T. Yamada, H. Aoki and M. Namiki, *Nippon Nogeikagaku Kaishi*, **44**, 580 (1970).

Acid hydrolysis followed by alkaline treatment of the crude saponin mixture obtained by the ordinary fractionation of the methanol extract of defatted seeds afforded a sapogenol mixture in a 22% yield (based on the crude saponin). Repeated column and thin-layer chromatographic (TLC) separation of the sapogenol mixture furnished nine sapogenol ingredients: 22α-hydroxy-erythrodiol (I),[6b] dihydropriverogenin A (II),[5] camelliagenin B (III),[12] A_1-barrigenol (IV),[13] camelliagenin C (V),[12] barringtogenol C (VI),[3] camelliagenin D (VII),[5,9] theasapogenol E (VIII),[5] and theasapogenol A (IX),[4] in the respective yields of 0.1, 10, 1, 1, 0.6, 1, 0.15, 2, and 30% (based on the total sapogenol mixture). All of them are oleanane triterpenoids and have been identified with the authentic specimens by direct comparisons except 22α-hydroxy-erythrodiol (I) which is a new sapogenol and whose structure has been established as mentioned later.



I—IX, XIV



X : R=H
XI : R=OH

	R^1	R^2	R^3	R^4	R^5	R^6
22α-hydroxy-erythrodiol (I):	CH_3	CH_3	H	H	H	OH
dihydropriverogenin A (II):	CH_3	CH_3	H	OH	H	OH
camelliagenin B (III):	CHO	CH_3	H	OH	H	OH
A_1-barrigenol (IV):	CH_3	CH_3	OH	OH	H	OH
camelliagenin C (V):	CH_2OH	CH_3	H	OH	H	OH
barringtogenol C (VI):	CH_3	CH_3	H	OH	OH	OH
camelliagenin D (VII):	CH_3	CHO	H	OH	OH	OH
theasapogenol E (VIII):	CHO	CH_3	H	OH	OH	OH
theasapogenol A (IX):	CH_2OH	CH_3	H	OH	OH	OH
primulagenin A (XIV):	CH_3	CH_3	H	OH	H	H

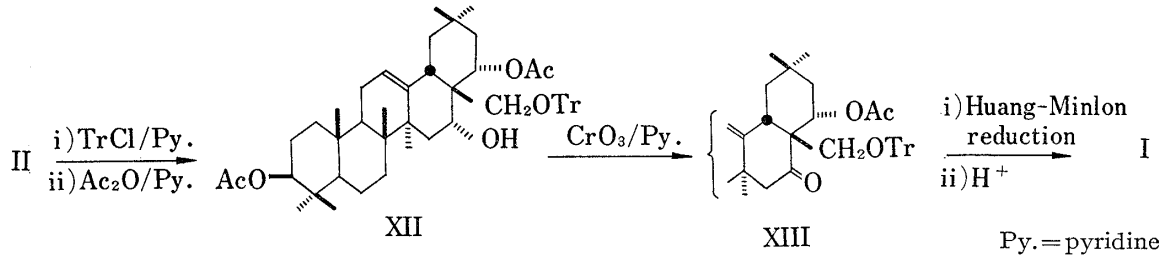


Chart 1

It is interestingly pointed out from the chemotaxonomical view-point that both of theasapogenol A (IX), the major sapogenol of *Thea sinensis* L. seeds, and dihydropriverogenin A (II), the major one of *Camellia japonica* L. seeds, have been isolated as the conspicuous constituents of seeds sapogenols of *Camellia sasanqua* L. In addition, the sapogenol composition in the present seeds is the richest in variety among three related plant materials (*T. sinensis*, *C. japonica*, and *C. sasanqua*). It contains A_1-barrigenol (IV) which has been

12) *a*) H. Itokawa, N. Sawada and T. Murakami, *Chem. Pharm. Bull.* (Tokyo), **17**, 474 (1969); *b*) S. Ito, M. Kodama and M. Konoike, *Tetrahedron Letters*, **1967**, 591.
13) *a*) S.G. Errington, D.E. White and M.W. Fuller, *Tetrahedron Letters*, **1967**, 1289; *b*) S. Ito, T. Ogino, H. Sugiyama and M. Kodama, *Tetrahedron Letters*, **1967**, 2289.

isolated from the other plant sources such as *Barringtonia asiatica* KURZ. (Myrtaceae),[14a] *Schima kankaoensis* HAY. (Theaceae),[14b] and *Pittosporum undulatum* VENT. (Pittosporaceae).[13] Furthermore, as for genuineness of the sapogenols it should be noted that the relative yield of theasapogenol E (VIII) and theasapogenol A (IX) is a subject of the further examination since the former aldehydic substance is readily convertible to the latter during acid or alkaline treatment and camelliagenin D (VII), the only compound isolated here which carries the axial oxygenated function at C-4, is most likely an artefact derivable from theasapogenol E (VIII) during the isolation procedure as demonstrated in our previous paper.[5]

A new sapogenol (I), now designated as 22α-hydroxy-erythrodiol, has been elucidated as based on the following evidence. The substance (I), $C_{30}H_{50}O_3 \cdot \frac{1}{2} H_2O$; mp 279—282°; $[\alpha]_D$+37° (pyridine), exhibits the absorption bands at 3388 cm^{-1} (OH) and 1624 cm^{-1} (C=C) in its infrared (IR) spectrum. Ordinary acetylation of I furnished a triacetate (X) whose IR spectrum shows the absence of free hydroxyl function but shows the acetoxyl absorption band at 1736 cm^{-1}. The proton magnetic resonance (PMR) spectrum of X indicates the presence of seven tertiary methyls, one primary acetoxyl function, two secondary acetoxyl functions, and one vinylic proton in the triacetate (X) (Table I). Assuming the olean-12-ene framework for the substance since it occurs together with the aforementioned eight oleanane triterpenoids (II—IX), the PMR signals are rationally explained as follows. A characteristic one-proton triplet-like signal at 5.52 τ is assigned to $C_{(3)}$-α-H, whereas a two-proton broad singlet at 6.07 τ is assigned to -$C\underline{H}_2$OAc.[5,15] Judging from the chemical shift of $C_{(3)}$-α-H and -$C\underline{H}_2$OAc, the D or E ring has become likely for the location of the primary acetoxyl function. Here again, taking into consideration of the hydroxylation pattern of co-occurring triterpenoids (II—IX), the primary acetoxyl and another equatorial secondary acetoxyl functions are assumed to be at $C_{(17)}$ and $C_{(22)}$ respectively, and therefore it follows that the structure of triacetate is formulated as X and the substance (I) corresponds to a 16-desoxy derivative of dihydropriverogenin A (II). In fact, the comparison of PMR data between X and XI[5] as given in Table I discloses the considerable resemblance of both compounds except the absence of a signal due to a proton geminal to an α-hydroxyl function at $C_{(16)}$ in the former, thus agreeing with the above assumption.

TABLE I. The PMR Data of X and XI taken in $CDCl_3$ (Chemical Shifts are given in τ Values and J Values in Hz)[a]

	X (at 60 MHz)	XI (at 100 MHz)[5]
>C–C$\underline{H}_3$	9.13 (6H, s), 9.04 (9H, s) 8.99, 8.79 (3H each, s)	9.12 (6H, s), 9.09, 9.07, 9.03 8.97, 8.57[b] (3H each, s)
-OCOC$\underline{H}_3$	8.00 (3H, s), 7.95 (6H, s)	7.96 (9H, s)
>$C_{(17)}$-C$\underline{H}_2$OAc	6.07 (2H, br. s)	6.34 6.23 (2H, ABq, J=11)
>$C_{(16)}\underline{H}$–OH	—	5.76 (1H, m)
>$C_{(3)}\underline{H}$–OAc	5.52 (1H, t-like)	5.50 (1H, t-like)
>$C_{(22)}\underline{H}$–OAc[c]	4.84 (1H, q, J=6 & 11)	4.69 (1H, q, J=6 & 12)
=$C_{(12)}\underline{H}$–[c]	4.74 (1H, m)	4.68 (1H, m)

a) abbreviations: ABq=AB type quartet, br. s=broad singlet, m=multiplet, q=quartet, s=singlet, t-like=triplet like

b) This lowest methyl signal is assinged to $C_{(14)}$-C$\underline{H}_3$ since it locates in the allylic position and suffers the anisotropic effect of $C_{(16)}$-α-OH additionally.[5] The corresponding methyl signals of X is observed at 8.79 τ.

c) The chemical shifts can not be so accurate due to the overlapping of each other.

14) *a*) T. Nozoe and T. Kinugasa, *J. Chem. Soc. Japan*, **56**, 864 (1935), and the literatures cited therein; *b*) T. Nozoe and T. Kinugasa, *J. Chem. Soc. Japan*, **56**, 883 (1935).

15) *a*) A. Gaudemer, J. Polonsky and E. Wenkert, *Bull. Soc. Chim. France*, **1964**, 407; *b*) M. Shamma, R. E. Glick and R.C. Mumma, *J. Org. Chem.*, **27**, 4512 (1962); *c*) J.C. Mani, *Ann. Chim.*, **10**, 533 (1965).

Finally, correctness of the assumption has been substantiated by the unequivocal transformation starting from dihydropriverogenin A (II) to the substance (I). Tritylation of dihydropriverogenin A (II) followed by acetylation in the usual way afforded a monotrityldiacetate (XII), which in turn was treated with CrO_3–pyridine complex to afford a monoketone (XIII). The ketone was then submitted to the Huang-Minlon reduction followed by acid treatment to furnish a triol, which was found identical in all respects with the substance (I) obtained from the sapogenol mixture as mentioned above.

Sapogenol Constituents of *Ternstroemia japonica* Leaves[16]

As for the chemical constituents of *Ternstroemia japonica* Thunb., Kondo and his coworkers[17] have isolated A_1-barrigenol (IV) as the sapogenol of the wood, however, no work has been done on the leaves composition.

On treatment with acid followed by repeated chromatographic separation, the crude saponin mixture, obtained in a 3.8% yield from the fresh leaves, afforded four oleanane triterpenoids: oleanolic acid (4.2%, based on the total hydrolysate), primulagenin A (XIV, 2.5%), dihydropriverogenin A (II, 0.7%), and A_1-barrigenol (IV, 3.7%), each identified by direct comparison with the authentic compound. Present isolation of primulagenin A seems to be the first instance from the *Theaceous* plant, although it is the common triterpenoid among the *Primulaceous* plants.[18]

Experimental[19]

Isolation of Saponin from Seeds of *Camellia sasanqua* Thunb.——The total MeOH extract of defatted seeds powder (1.0 kg, obtained by courtesy of Prof. I. Nishioka of Kyūshū Univ.) was partitioned into the *n*-BuOH–water mixture as usual. The *n*-BuOH soluble portion was dissolved in a small amount of MeOH and poured into a large quantity of ether to precipitate a crude saponin mixture, which was washed with ether and dried. Yield, 85 g (8.5% from the defatted seeds).

Hydrolysis of Crude Saponin Mixture——A mixture of the crude saponin mixture (25 g), MeOH (300 ml), and 10% aq. H_2SO_4 (300 ml) was refluxed for 5 hr. The precipitates obtained by addition of water while removing MeOH by distillation were collected by filtration and treated with 5% KOH–MeOH (300 ml) at reflux for one hour. After neutralization of the reaction mixture with dil. H_2SO_4, MeOH was removed by distillation while adding water gradually. The resulting crude sapogenol mixture weighed 5.6 g (22% from the crude saponin mixture).

The similar treatment of crude saponin (154 g) using MeOH (1 liter), 10% aq. H_2SO_4 (1 liter), and 5% KOH–MeOH (600 ml) furnished 46.5 g of the crude sapogenol mixture (30% from the crude saponin mixture).

Isolation of Sapogenols (I—IX)——A homogeneous blend of the crude sapogenol mixture (5 g) and Al_2O_3 (neutral, 30 g) was put on a column of Al_2O_3 (neutral, 250 g) made with the aid of $CHCl_3$ and the column was eluted with the $CHCl_3$–MeOH mixture by increasing gradually the composition of MeOH as 3, 5, 10, 20, 30, and 100%.

Earlier eluates obtained by 3% MeOH–$CHCl_3$ afforded a pure sapogenol (*ca.* 500 mg, 10% from the total sapogenol mixture), mp 278—280° after crystallization from aq. MeOH, was identified with dihydropriverogenin A (II) by mixed mp, IR (KBr), and TLC.

The following eluates obtained by 3% MeOH–$CHCl_3$ afforded a sapogenol mixture which was purified by preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (20:1) and detecting by I_2 vapor) to give a sapogenol (50 mg, 1%). The pure sapogenol of mp 219—222° obtained by crystallization from aq. MeOH was found identical with camelliagenin B (III) by mixed mp, IR (KBr), and TLC.

The final fractions obtained by eluting with 3% MeOH–$CHCl_3$ afforded a sapogenol, which was further

16) Presented at the 88th Annual Meeting of the Pharmaceutical Society of Japan (Tokyo, April, 1968), Abstract Papers, p. 257.

17) N. Watanabe, I. Saeki, M. Sumimoto, T. Kondo and S. Kurotori, *J. Japan Wood Res. Soc.* (Mokuzai Gakkaishi), **12**, 236 (1966).

18) *a*) R. Tschesche and F. Ziegler, *Ann.*, **674**, 185 (1964), and the literatures cited therein; *b*) I. Kitagawa, A. Matsuda, T. Nishimura, S. Hirai and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1435 (1967); *c*) I. Kitagawa, A. Matsuda and I. Yosioka, *Tetrahedron Letters*, **1968**, 5377.

19) The following instruments were used for the physical data. Specific rotation: the Rex Photoelectric Polarimeter NEP-2, IR Spectra: the Hitachi IR Spectrometer EPI-2 and EPI-S2, PMR Spectra: the Hitachi H-60 NMR Spectrometer.

purified by preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (20:1) and detected by I_2 vapor) to give the pure sapogenol (50 mg, 1%) of mp 275—278° (crystallized from acetone), being identical with A_1-barrigenol (IV) by mixed mp, IR (KBr), and TLC.

The earlier eluates obtained by 10% MeOH–$CHCl_3$ were further purified by preparative TLC (SiO_2 using $CHCl_3$–MeOH (9:1) and I_2 vapor as above) to give a sapogenol (50 mg, 1%), mp 277—279° after crystallization from aq. MeOH, which was identified with barringtogenol C (VI) by mixed mp, IR (KBr), and TLC.

The following eluates obtained by 10% MeOH–$CHCl_3$ were also purified by preparative TLC (SiO_2, $CHCl_3$–MeOH (9:1) and I_2 vapor as above) to give 100 mg (2%) of a sapogenol. The sapogenol, mp 262—263° after crystallization from aq. MeOH, was found identical with theasapogenol E (VIII) by mixed mp, IR (KBr), and TLC.

The final eluates obtained by 10% MeOH–$CHCl_3$ and the eluates by 20% MeOH–$CHCl_3$ afforded a sapogenol (1.7 g, 30%), which was crystallized from aq. MeOH to give the pure sapogenol of mp 295—300° being identical with theasapogenol A (IX) by mixed mp, IR (KBr), and TLC.

To isolate the other minor sapogenols, the crude sapogenol mixture (46.5 g) was chromatographed on Al_2O_3 (neutral, 2.4 kg) eluting with the $CHCl_3$–MeOH mixture. The sapogenol mixture (362 mg) obtained by elution with the $CHCl_3$–MeOH (15:1) mixture was chromatographed again on SiO_2 (100 times by weight) with the aid of $CHCl_3$–MeOH (100:1) mixture. The sapogenol (88 mg) thus obtained was further purified by preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (18:1) and detected by I_2 vapor) to give a pure substance (60 mg, 0.1%). Crystallization from MeOH furnished the analytical sample of 22α-hydroxy-erythrodiol (I), mp 279—282° (colorless rods); $[\alpha]_D$ +37° (c=1.0, pyridine); IR ν_{max}^{KBr} cm^{-1}: 3388, 1624. *Anal.* Calcd. for $C_{30}H_{50}O_3 \cdot \frac{1}{2}H_2O$: C, 77.03; H, 10.99. Found: C, 77.24; H, 10.93.

A part of the following eluates (1 g) obtained by the $CHCl_3$–MeOH (9:1) mixture was chromatographed again on SiO_2 (100 times by weight) using the $CHCl_3$–MeOH (100:3) mixture as an eluant. The product (291 mg, 0.6% from the sapogenol mixture) was then purified by crystallization from AcOEt to give crystals of mp 256—257.5°, which was identified with camelliagenin C (V) by mixed mp, IR (KBr), and TLC.

A fraction (288 mg) obtained by eluting with the $CHCl_3$–MeOH (5:1) mixture was further chromatographed on SiO_2 (100 times by weight) using the $CHCl_3$–MeOH (100:1) mixture and submitted to preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (8:1) and detected by spraying water). The sapogenol (73 mg, 0.15%) thus obtained, mp 249—253° after crystallization from MeOH was identified with camelliagenin D (VII) by mixed, mp IR (KBr), and TLC.

22α-Hydroxy-erythrodiol Triacetate (X)——Acetylation of I (42 mg) with Ac_2O (1 ml) and pyridine (2.5 ml) by keeping at room temperature for 4 days followed by usual treatment afforded a triacetate (X, amorphous, 42 mg). All the efforts to crystallize the triacetate were unsuccessful, however, since TLC using three different combinations of the solvent systems assured purity of the triacetate, it was used for the IR and NMR spectral measurement. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1736, 1243, no hydroxyl. PMR data are as given in Table I.

3,22-Di-O-acetyl-28-O-trityl-dihydropriverogenin A (XII)——To a solution of dihydropriverogenin A (II, 655 mg) in pyridine (20 ml), was added trityl chloride (1.5 g) and the total mixture was refluxed for 4 hr in an oil bath (temp.: 135—145°). The reaction mixture was then poured into water and extracted with ether. The crude product obtained after removing the solvent was submitted to column chromatography using Al_2O_3 (50 times by weight). Isolation of the pure 28-O-trityl derivative was unsuccessful by this procedure, however a mixture of monotrityl and ditrityl derivatives was obtained in total amount of 892 mg. A fraction (145 mg) which contained mainly 28-O-trityl derivative was acetylated with Ac_2O (2 ml) and pyridine (5 ml) by keeping at room temperature for 5 days and treating in a usual manner. Crystallization of the product (123 mg) from *n*-hexane yielded 3,22-di-O-acetyl-28-O-trityl-dihydropriverogenin A (XII), mp 187—191°; $[\alpha]_D$ +74° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{68}O_6$: C, 79.46; H, 8.56. Found: C, 79.56; H, 8.77. IR ν_{max}^{KBr} cm^{-1}: 3550, 1728, 1600, 1490, 1465, 1445, 1245, 780, 767, 750, 703. PMR ($CDCl_3$, 60 MHz) τ: 9.68 (3H, s),[20] 9.13, 9.03 (6H each, s), 8.77, 8.61 (3H each, s) (totally seven methyls), 8.11, 7.95 (3H each, s, $-OCOC\underline{H}_3 \times 2$), 7.34, 7.16 (ABq, J=8 Hz, $-C_{(28)}\underline{H}_2-OTr$), 5.99 (1H, m, $>C_{(16)}-\beta-\underline{H}$), 5.51 (1H, t-like, $>C_{(3)}-\alpha-\underline{H}$), 4.64 (1H, m, $=C_{(12)}\underline{H}-$), 4.21 (1H, q, J=7 & 11 Hz, $>C_{(22)}-\beta$-H), 2.5—2.9 (15H, m, aromatic protons).

3,22-Di-O-acetyl-28-O-trityl-16-dehydro-dihydropriverogenin A (XIII)——A solution of XII (20 mg) in pyridine (1 ml) was treated with CrO_3-pyridine complex (CrO_3 40 mg and pyridine 1 ml) at room temperature with stirring for 4 hr. The product (16 mg) obtained by treating the reaction mixture with water followed by filtration, washing and drying, was crystallized from EtOH to give XIII, mp 276—280°; $[\alpha]_D$ +59° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{66}O_6$: C, 79.66; H, 8.32. Found: C, 79.30; H, 8.42. IR ν_{max}^{KBr} cm^{-1}: 1742 (sh), 1730, 1715 (sh), 1695, 1600, 1490, 1463, 1445, 1242, 778, 765, 750, 710 (sh), 700.

Huang-Minlon Reduction of XIII followed by Acid Treatment——A solution of XIII (110 mg) in small amount of EtOH was added with diethylene glycol (15 ml) and 80% hydrazine hydrate (6 ml) and the total mixture was refluxed for 4 hr in an oil bath (temp. 150—160°). After adding KOH (1.2 g), the bath tem-

20) Diamagnetically shifted due to the anisotropic shielding effect of trityl function.[4]

perature was raised gradually up to 230° with a down-ward condenser to remove the lower boiling fraction, and the residual mixture was refluxed further for 2 hr (oil bath temp. 230—240°). After pouring into water, the reaction mixture was neutralized with 3% HCl, and the resulting precipitates were collected by filtration and refluxed with 3% HCl–MeOH for one hour. MeOH was then removed from the total mixture while adding water, and the mixture was extracted with ether. Treatment of the ether extract in a usual way yielded a crude product (72 mg), which was purified by SiO_2 (100 times by weight) column chromatography to furnish a pure product (29 mg), mp 276—279° after crystallization from MeOH, being identical with 22*α*-hydroxyerythrodiol (I) by mixed mp, IR (KBr), and TLC.

Isolation of Saponin from Leaves of *Ternstroemia japonica* THUNB.——Fresh leaves (500 g, collected at our campus in March, 1967) was homogenized with a blender and extracted 3 times with MeOH at reflux each for 6 hr. The combined MeOH extracts were partitioned into the *n*-BuOH–water mixture and the *n*-BuOH soluble portion was dissolved again in a small amount of MeOH and poured into a large amount of ether to precipitate a crude saponin mixture, which was washed repeatedly with ether. Yield, 19.0 g (3.8%).

Hydrolysis of Saponin and Isolation of Sapogenols——A mixture of saponin (16.8 g) in 7% aq. HCl (250 ml) and EtOH (300 ml) was refluxed for 6.5 hr and diluted with water. The precipitates were collected by filtration to give a crude hydrolysate (4.45 g). A part of the total hydrolysate was treated with 5% KOH–MeOH at reflux, however no significant change was observed on TLC. Therefore, the crude hydrolysate (2.5 g) was mixed with SiO_2 (17.5 g) and put on a column of SiO_2 (118 g) made with $CHCl_3$ and the column was eluted with the $CHCl_3$–MeOH mixture of increasing polarity.

The earlier eluates obtained by 1% MeOH–$CHCl_3$ afforded a sapogenol (106 mg, 4.2% from the total hydrolysate), mp 246—252° after crystallization from MeOH, which was identified with oleanolic acid by mixed mp, IR (KBr), and TLC.

The following eluates obtained by the same solvent mixture afforded a sapogenol mixture, which on preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (20:1) and detected by spraying water) gave a pure sapogenol (61 mg, 2.5%) of mp 235—237° (crystallized from aq. MeOH). The sapogenol was identified with primulagenin A (XIV) by mixed mp, IR (KBr), and TLC.

Preparative TLC (SiO_2, developing with $CHCl_3$–MeOH (15:1) and detected by spraying water) of the eluates obtained by 3% MeOH–$CHCl_3$ afforded 17 mg (0.7%) of a pure sapogenol, mp 271—273° after crystallization from MeOH, which was identified with dihydropriverogenin A (II) by mixed mp, IR (KBr), and TLC.

The eluates obtained by 5% MeOH–$CHCl_3$, on crystallization from acetone, afforded a pure sapogenol (92 mg, 3.7%) of mp 276—278°, which was idetnified with A_1-barrigenol (IV) by mixed mp, IR (KBr), and TLC.

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