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# Saponin and Sapogenol. VII.[1] Sapogenol Constituents of Five *Primulaceous* Plants

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The sapogenol constituents of five Japanese *Primulaceous* plants: i) *Primula sieboldi* E. Morren, roots (Japanese name: sakurasō), ii) *P. japonica* A. Gray, roots and fruits (kurinsō), iii) *Lysimachia mauritiana* Lam., Fruits (hamabossu), iv) *L. clethroides* Duby, roots (okatoranoō), v) *L. japonica* Thunb., roots (konasubi), have been investigated.

It has been elucidated that the major sapogenols obtained by acid hydrolysis of saponin of each plant material are primulagenin A (VIII) and/or dihydropriverogenin A (XIII), while periodate oxidation followed by alkaline treatment of saponin furnished the inconsistent sapogenol compositions and the genuine sapogenols have been shown to possess a 13$\beta$,28-oxide moiety as seen in protoprimulagenin A (XV), a newly isolated sapogenol from i), ii), iv), and v), and priverogenin B (XII). The structure of protoprimulagenin A (XV) has been established on the basis of chemical and physicochemical evidences.

Saponin and sapogenol constituents of European *Primula* species (*P. elatior* L. Schreber, *P. veris* L., and *P. vulgaris* Huds.) as well as *Cyclamen europaeum* L. have been investigated extensively by several groups, since the subterranean parts of *P. elatior* and *P. veris* have been often utilized as vegetable expectorants in Europe and saponin has been believed to be their active principle.[3,4]

In 1962 Barton and his co-workers initially obtained a sapogenol cyclamiretin[5] (now termed as cyclamiretin D (I)[6]) from the tuber of *C. europaeum*, and later Tschesche and his co-workers elucidated a genuine sapogenol named cyclamiretin A (II) from the same plant source.[6] They demonstrated that cyclamiretin D was secondarily formed from cyclamiretin A during acid hydrolysis of saponin. Cyclamiretin A was then remarked as the unprecedented example of an oleanane triterpenoid possessing a 13$\beta$,28-oxide moiety. Afterwards, Dorchai and Thomson added several minor sapogenols named cyclamigenins $A^1$ (III), $A^2$ (IV), B (V), C (VI), and D (VII) from the same kind of tuber and elucidated their structures.[7] It was in 1969 when Tschesche and his co-workers proposed the structure of a major saponin cyclamin.[8]

As for *Primula spp.* constituents, Tschesche and his co-workers examined minor sapogenols of *P. elatior* in addition to a well-known sapogenol primulagenin A (VIII) and elucidated 28-dehydro-primulagenin A (IX) and echinocystic acid (X),[3] whereas they isolated[9] pri-

1) Part VI: I. Yosioka, K. Hino, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 1499 (1972).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) R. Tschesche and F. Ziegler, *Liebig's Ann.*, **674**, 185 (1964), and the literatures cited therein.
4) R. Tschesche and G. Wulff, *Planta Medica*, **12**, 272 (1964).
5) D.H.R. Barton, A. Hameed, and J.F. McGhie, *J. Chem. Soc.*, **1962**, 5176.
6) *a*) R. Tschesche, F. Inchaurrondo, and G. Wulff, *Liebig's Ann.*, **680**, 107 (1964); *b*) R. Tschesche, H. Striegler, and H.W. Fehlhaber, *ibid.*, **691**, 165 (1966).
7) *a*) R.O. Dorchai and J.B. Thomson, *Tetrahedron Letters*, **1965**, 2223; *b*) *Idem*, *Tetrahedron*, **24**, 1377 (1968); *c*) R.O. Dorchai, H.E. Rubalcava, J.B. Thomson, and B. Zeeh, *ibid.*, **24**, 5649 (1968).
8) R. Tschesche, H.J. Mercker, and G. Wulff, *Liebig's Ann.*, **721**, 194 (1969).
9) R. Tschesche, B.T. Tjoa, and G. Wulff, *Liebig's Ann.*, **696**, 160 (1966).

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verogenin A monoacetate (XIa),[10] priverogenin B monoacetate (XIIa),[10] and dihydropriverogenin A (XIII)[10] from *P. veris*. Furthermore, they proposed the structure of a major saponin of *P. elatior*.[3] On the other hand, Hensens and Lewis investigated some reactions of primulagenin A (VIII) such as acetyl migration[11a] and acid-catalysed transformation.[11b] Very recently, Heits, *et al.* have clarified a new sapogenol anagalligenone B (XIV) isolated from *Anagallis arvensis* L. (Primulaceae).[12]

With respects to Japanese *Primulaceous* plants constituents, only limited information had been available when we extended the study on saponin and sapogenol to them. There was a report by Yanagisawa and Takashima who isolated a saponin named sakurasō-acid from the roots of *Primula sieboldi* E. Morren.[13] We have begun with a comparative study on saponin and sapogenol constituents of five Japanese *Primulaceous* plants and thereafter examined their genuine sapogenols. The present paper deals with the detailed accounts.

## Sapogenol Compositions of Five *Primulaceous* Plants[14]

Isolation of each saponin from (i) *Primula sieboldi* E. Morren, roots (Japanese name: sakurasō), (ii) *P. japonica* A. Gray, roots (kurinsō), (iii) *Lysimachia mauritiana* Lam., fruits (hamabossu), (iv) *L. clethroides*, Duby, roots (okatoranoö), and (v) *L. japonica* Thunb., roots (konasubi), was undertaken through the ordinary procedure as described in the Experimental section. The respective yields are listed in Table I. Total saponin was then hydrolysed with ethanolic hydrogen chloride at reflux and a resulting sapogenol mixture was subjected to chromatographic separation using alumina followed by direct comparisons with the authentic triterpenoids. As shown in Table I, all the plant materials have been disclosed to contain primulagenin A (VIII) and/or dihydropriverogenin A (XIII)[10] as the major sapogenol constituent, and no remarkable difference of sapogenol composition was noticed between two genera, *Primula* and *Lysimachia*, as far as the examined plant materials concerned.

Table I

| Origin | Saponin mixture[a] | Crude sapogenol mixture[b] | Primulagenin A (VIII)[c] | Dihydropriverogenin A (XIII)[c] |
|---|---|---|---|---|
| *P. sieboldi*, roots | 4—16% | 27% | 46% | — |
| *P. japonica*, roots | 6.5 | 33 | 5 | 44% |
| *L. mauritiana*, fruits | 1.4 | 32 | — | 40 |
| *L. clethroides*, roots | <2.8[d] | 23 | 16 | 2 |
| *L. japonica*, roots | 2.8 | 29 | 4 | trace |

*a*) yield from the air-dried material
*b*) yield from the crude saponin
*c*) yield from the total acid hydrolysate of crude saponin
*d*) discolored due to the concomitant coloring substance (see Experimental)

## Genuine Sapogenols[15]

It has been revealed[6a] that mild acid hydrolysis of saponin originated from *C. europaeum* or *P. veris* affords an oleanane triterpenoid possessing a 13$\beta$,28-oxide structure such as either cyclamiretin A (II),[6] cyclamigenin B (V),[7a,b] or priverogenin B monoacetate (XIIa)[9,10]

10) *a*) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971); *b*) R. Tschesche, B.T. Tjoa, and G. Wulff, *Tetrahedron Letters*, **1968**, 183; *c*) M. Kodama and S. Ito, *Chem. & Ind.*, **1967**, 1647.
11) *a*) O.D. Hensens and K.G. Lewis, *Tetrahedron Letters*, **1968**, 3213; *b*) *Idem*, *Australian J. Chem.*, **24**, 2117 (1971).
12) S. Heits, D. Billet, and D. Raulais, *Bull. Soc. Chim. France*, **1971**, 2320.
13) H. Yanagisawa and N. Takashima, *Yakugaku Zasshi*, **46**, 844 (1926).
14) I. Kitagawa, A. Matsuda, T. Nishimura, S. Hirai, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1435 (1967) (Preliminary report).
15) I. Kitagawa, A. Matsuda, and I. Yosioka, *Tetrahedron Letters*, **1968**, 5377 (Partial preliminary report).

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and the partial structure is readily transformed to a corresponding triterpenoid possessing an ordinary $\Delta^{12}$-17-$CH_2OH$ moiety under intensified acid conditions. Since the genuine sapogenols of above mentioned Japanese *Primulaceous* plant materials have also been assumed to have a 13$\beta$,28-oxide moiety which on acid hydrolysis is converted to a $\Delta^{12}$-17-$CH_2OH$ moiety as in primulagenin A (VIII) or dihydropriverogenin A (XIII), periodate treatment of saponin after Dugan and de Mayo[16] has been examined. Thus, a saponin from each plant material was subjected to sodium periodate oxidation followed by alkaline treatment and the procedure was repeated two or three times if necessary. Chromatographic separation of the final product gave the results as shown in Table II and three sapogenols (protoprimulagenin A (XV), aegicerin (XVI), and priverogenin B (XII)) were isolated additionally and are discussed later. It is noticeable that most of the sapogenol compositions thus obtained are distinctly inconsistent with those obtained by acid hydrolysis of saponin except in case from *P. japonica*.

Table II

| | | *via* acid hydrolysis[a] (yield %)[b] | | *via* periodate oxidation (yield %)[b] | |
|---|---|---|---|---|---|
| *P. sieboldi* | roots | primulagenin A(VIII) | 46 | protoprimulagenin A(XV) | 41 |
| | | | | aegicerin (XVI) | 7 |
| | | | | primulagenin A | 4 |
| *P japonica* | roots | dihydropriverogenin A (XIII) | 44 | dihydropriverogenin A | 21 |
| | | primulagenin A | 5 | protoprimulagenin A | 3 |
| | fruits | dihydropriverogenin A | 24 | dihydropriverogenin A | 23 |
| *L. mauritiana* | fruits | dihydropriverogenin A | 40 | priverogenin B(XII) | 28 |
| *L. clethroides* | roots | primulagenin A | 16 | protoprimulagenin A | 11 |
| | | dihydropriverogenin A | 2 | aegicerin | 4 |
| *L. japonica* | roots | primulagenin A | 4 | protoprimulagenin A | 9 |
| | | dihydropriverogenin A | trace | | |

*a*) refer to Table I *b*) yield from the total sapogenol mixture

The major sapogenol of *P. japonica* roots obtained by both acid hydrolysis and periodate oxidation of saponin is the same, *i.e.* dihydropriverogenin A (XIII), having a $\Delta^{12}$-17-$CH_2OH$ moiety. Since it has been suspected that a 13$\beta$,28-oxide moiety of saponin might have suffered the oxide-ring opening presumably by acidic components in the plant during the course of drying plant material or extraction of saponin with methanol at reflux, the fresh roots of *P. japonica* were extracted with methanol containing 0.5% pyridine as was devised by Kubota and Hinoh[17] for isolation of genuine sapogenols of *Bupleurum falcatum* L. Periodate oxidation of the saponin thus obtained again resulted in the similar results. Therefore, it has been concluded that dihydropriverogenin A (XIII) is the major genuine sapogenol of *P. japonica* roots. Contrary to acid hydrolysis of saponin, protoprimulagenin A (XV) was obtained as a minor genuine sapogenol from the roots of *P. japonica* while it is a major one from *P. sieboldi* roots (*vide infra*).

As described above, acid hydrolysis of saponin from both *P. japonica* roots and *L. mauritiana* fruits furnished dihydropriverogenin A (XIII) as a major sapogenol. However, periodate oxidation of both saponins has demonstrated that major genuine sapogenols of both materials are unidentical (Table II). Since two different parts of the plants were analysed respectively, fruits of *P. japonica* and roots of *L. mauritiana* were also examined. The major sapogenol of the former obtained both by acid hydrolysis and periodate oxidation

16) J.J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).
17) T. Kubota and H. Hinoh, *Tetrahedron*, **24**, 675 (1968).

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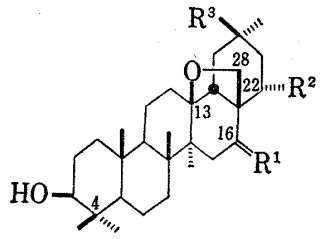
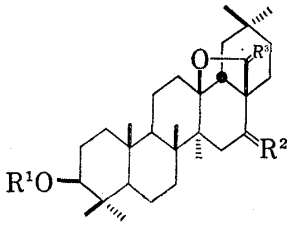
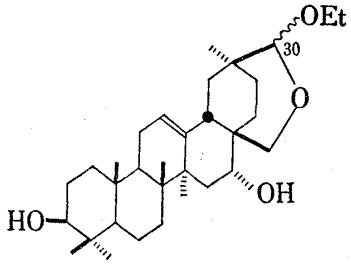
of saponin was dihydropriverogenin A (XIII) same as from roots. On the other hand, the sapogenol composition of *L. mauritiana* roots is distinctly different from other plant sources examined here. It was clarified[18] to consist of dihydropriverogenin A (XIII) and camelliagenin C (XVII)[10a,19] and four other triterpenoid sapogenols which are the subjects of further investigation.



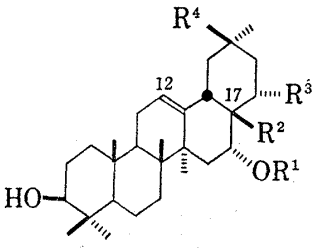
| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| II: | α–OH, β–H | H | CHO | cyclamiretin A |
| IV: | =O | H | H | cyclamigenin A[2] |
| V: | =O | H | CHO | cyclamigenin B |
| Va: | β–OAc at C-3 in place of OH in V | | | |
| VII: | =O | H | $CH(OMe)_2$ | cyclamigenin D |
| XII: | α–OH, β–H | OH | $CH_3$ | priverogenin B |
| XIIa: | α–OH, β–H | OAc | $CH_3$ | |
| XIV: | =O | H | $CH_3$ | anagalligenone B |
| | ($\alpha$–$CH_2OH$ at C-4 in place of $CH_3$) | | | |



| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| XV: | H | α–OH, β–H | $H_2$ | protoprimulagenin A |
| XVa: | Ac | α–OH, β–H | $H_2$ | |
| XVI: | H | =O | $H_2$ | aegicerin |
| XVIa: | Ac | =O | $H_2$ | |
| XVIII: | Ac | =O | =O | |



III: 30β: cyclamigenin A[1]
VI: 30α: cyclamigenin C



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | |
|---|---|---|---|---|---|
| I: | H | $CH_2OH$ | H | CHO | cyclamiretin D |
| VIII: | H | $CH_2OH$ | H | $CH_3$ | primulagenin A |
| IX: | H | CHO | H | $CH_3$ | 28-dehydro-primulagenin A |
| X: | H | COOH | H | $CH_3$ | echinocystic acid |
| XI: | H | CHO | OH | $CH_3$ | priverogenin A |
| XIa: | Ac | CHO | OH | $CH_3$ | |
| XIII: | H | $CH_2OH$ | OH | $CH_3$ | dihydropropriverogenin A |
| XVII: | H | $CH_2OH$ | OH | $CH_3$ | camelliagenin C |
| | ($\alpha$–$CH_2OH$ at C-4 in place of $CH_3$) | | | | |

Chart 1

Periodate oxidation of saponins isolated from roots of *P. sieboldi*, *L. clethroides*,[20] and *L. japonica*[20] afforded a new genuine sapogenol now named protoprimulagenin A (XV), $C_{30}H_{50}O_3$, mp 272—273°, $[\alpha]_D$ +13° ($CHCl_3$), as a major which was obtained from *P. japonica* in minority as shown in Table II.

Protoprimulagenin A (XV) was readily transformed to primulagenin A (VIII) by acid treatment, whereas it gave a monoacetate (XVa), mp 266—267°, with acetic anhydride and pyridine. The infrared (IR) spectrum of XVa indicates existence of an unacetylated hydroxyl

18) Preliminary report: I. Yosioka, A. Matsuda, U.G. Khan, and I. Kitagawa, The 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July 1970, Abstract Papers, II-181.
19) *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.
20) Saponins from these plant sources were extracted with methanol containing 0.5% pyridine as from *P. japonica* roots described above.

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function (3620 and 3450 (br.) $cm^{-1}$) and an acetyl function (1720 $cm^{-1}$). The proton magnetic resonance (PMR) spectrum of XVa provides evidences supporting the formulation of protoprimulagenin A as XV (Table III). Thus, it demonstrates presence of seven C-methyl groups and one acetyl function. A one-proton triplet-like signal at 5.55 $\tau$ is characteristically assigned to C-3$\alpha$-H geminal to C-3$\beta$-OAc. An AB type quartet signal at 6.88 and 6.56 $\tau$ ($J$=8 Hz) is assignable to a C-28 methylene function constituting a 13$\beta$,28-oxide moiety as in cyclamiretin A (II) and the assignment is supported by absence of a vinyl proton usually observed in a $\Delta^{12}$-oleanene triterpenoid. Coupling mode of a broad doublet at 6.08 $\tau$ ($J$=6 Hz) assigned to C-16$\alpha$-H is ascribed to distorted conformation of ring D as is expected by Dreiding model inspection. These observations are in good accord with the findings in saikogenins presented by Shibata and his co-workers[21] and Kubota and Hinoh.[17] Consequently, the structure (XV) has been established for protoprimulagenin A.

Oxidation of the monoacetate (XVa) with chromic anhydride and pyridine gave a monoketone-monoacetate (XVIa), mp 274—276°, which was submitted to ruthenium tetroxide oxidation. A resulting product, mp 276—277°, was proved to be a $\gamma$-lactone derivative (XVIII) by its IR absorption bands (KBr) at 1773, 1730, 1713, and 1243 $cm^{-1}$, thus additionally substantiating existence of a 13$\beta$,28-oxide moiety in protoprimulagenin A (XV).

Table III. The PMR Data of XVa nd XVIa taken in $CDCl_3$
(Chemical Shifts are given in $\tau$ Values and J Values in Hz)[a]

| | XVa (100 MHz) | XVIa (60 MHz) |
|---|---|---|
| >C-C$\underline{H}_3$ | 9.13, 9.10 (6H each, s), 9.03<br>8.85, 8.80 (3H each, s) | 9.15 (9H s), 9.08, 9.06, 8.97,<br>8.76 (3H each, s) |
| -OCOC$\underline{H}_3$ | 7.99 (3H, s) | 7.96 (3H s) |
| >C$_{(15)}\underline{H}_2$ | — | 7.48, 7.10 (1H each, ABq, $J$=10) |
| -C$_{(28)}\underline{H}_2$- | 6.88, 6.56 (1H each, ABq, $J$=8) | 6.55, 6.15 (1H each, ABq, $J$=8) |
| >C$_{(16)}\underline{H}$-OH | 6.08 (1H, br. d, $J$=6) | — |
| >C$_{(3)}\underline{H}$-OAc | 5.55 (1H, $t$-like) | 5.52 (1H, $t$-like) |

a) Abbreviations: ABq=AB type quartet, br. d=broad doublet, s=singlet, t-like=triplet like

Protoprimulagenin A is considered to be a genuine form of primulagenin A (VIII) in the roots of *P. sieboldi*, *P. japonica*, *L. clethroides*, and *L. japonica*.

Another minor ketonic sapogenol was obtained from saponins of the roots of *P. sieboldi* and *L. clethroides* through periodate oxidation. The sapogenol, $C_{30}H_{48}O_3$, mp 257.5—258°, $[\alpha]_D$ −25° ($CHCl_3$), possesses hydroxyl and carbonyl functions as revealed by its IR absorption bands (3620, 3470 (br), and 1700 $cm^{-1}$ in $CHCl_3$). On acetylation with acetic anhydride and pyridine, it gave a monoacetate (XVIa), mp 274—276°, whose PMR data (Table III) are comparable with those of protoprimulagenin A monoacetate (XVa) except the following significance. The former lacks a signal due to a proton geminal to C-16$\alpha$-OH, while it shows existence of an active methylene function by an AB quartet at 7.48 and 7.10 $\tau$ ($J$=10 Hz). Therefore, the structure of ketonic sapogenol has been assumed to be XVI and the assumption was verified by direct comparison of its monoacetate with the above described monoketone-monoacetate (XVIa) prepared from protoprimulagenin A. Lower field appearance of an AB quartet due to a C-28 methylene function in XVIa (6.55 and 6.15 $\tau$) as compared with that of XVa is ascribable to an anisotropic effect of a C-16 carbonyl function in the former, as has been observed in the PMR data of cyclamigenin B monoacetate (Va).[7b] Although direct comparison is unavailable, the established structure (XVI) is identical with that of aegicerin

21) N. Aimi, H. Fujimoto, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 641 (1968).

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(lit.[22]: mp 254—256°, $[\alpha]_D$ −23.6° ($CHCl_3$)) which was previously isolated from *Aegiceras majus* Gaertn. (Myrsinaceae) and established as XVI by Rao.[22]

It should be pointed out that the ketonic sapogenol (aegicerin) in the present study has been obtained along with protoprimulagenin A (XV) on treatment of saponin with periodate and no compound presumably derivable from the ketonic sapogenol on acid treatment has been obtained through acid hydrolysis of saponin. Hence, genuineness of the ketonic sapogenol should be a subject of further examination.

From the fruits of *L. mauritiana* was obtained another sapogenol, mp 270.5—271.5°, which was readily convertible to dihydropriverogenin A (XIII) on acid treatment and was assumed to be a 13$\beta$,28-oxide isomer of XIII. In fact, direct comparison of the sapogenol with authentic priverogenin B (XII) proved correctness of the assumption.

## Experimental[23]

### *Primula sieboldi*, Roots

**Isolation of Saponin**——1) Three extractions of the air-dried roots (215 g of cut, cultivated specimens, provided in Feb. 1967 through the courtesy of Mr. T. Arahari, the Takagamo Shrine, Kamojin, Gose-city, Nara prefecture) with MeOH at reflux followed by concentration afforded crystalline saponin of 14.0 g, 4.5 g, and 1.1 g respectively. The combined mother layer was evaporated to dryness and the residue was partitioned into *n*-BuOH–water mixture. Evaporation of the *n*-BuOH soluble portion yielded additional amount of saponin mixture (powder, 15.2 g). The total yield of saponin was 34.6 g (16.1% from dried roots). The water soluble portion gave a saccharide mixture of 42.5 g (19.8%).

2) In another experiment, from the roots (250 g) collected in Feb.—Apr. 1966, was obtained the saponin of 10.2 g (4.1%). The true reason of variation of the saponin yield is obscure, but might be ascribed to difference of the harvest period.

**Acid Hydrolysis of Saponin**——A mixture of saponin (3 g) in aq. 2N HCl (150 ml) and EtOH (150 ml) was refluxed for 4 hr and diluted with water. The resulting precipitates were collected by filtration, washed with water and dried to give a crude hydrolysate (890 mg, 27% from saponin). The hydrolysate (880 mg) was mixed with alumina (5 g) with the aid of MeOH and dried. The mixture was then put on a column of alumina (70 g)[24] and chromatographed eluting with benzene–$CHCl_3$ mixtures of increasing order of polarity. From the eluates of benzene–$CHCl_3$ (1:1) mixture was obtained a sapogenol (413 mg, 46% from the hydrolysate) of mp 234—235° (recryst. from MeOH–water), which was identified with authentic primulagenin A (VIII)[25] by mixed mp, IR, and TLC.

**Periodate Oxidation and Alkaline Treatment of Saponin**——To an ice-cooled suspension of saponin (3 g) in MeOH (100 ml)–water (100 ml) mixture, was added dropwise a solution of $NaIO_4$ (4.5 g) in water (50 ml) in a period of 30 min, and the total mixture was stirred at room temperature for further 8 hr in the dark and left standing overnight. After adding *n*-BuOH and water to the reaction mixture, MeOH was removed under reduced pressure. The mixture was then extracted with *n*-BuOH and the *n*-BuOH solution was treated with aq. KI solution to decompose excess $NaIO_4$ and liberated $I_2$ was consumed by aq. $Na_2S_2O_3$ solution. The *n*-BuOH solution was evaporated *in vacuo* to give a periodate oxidation product, which was treated under $N_2$ atmosphere with 3% KOH–EtOH (KOH 3 g, water 10 ml, EtOH 90 ml) at reflux for 1.5 hr, diluted with water and adjusted to pH 5—6 with aq. 10% $H_2SO_4$ and then to pH 3 with aq. 1% $H_2SO_4$, and extracted with *n*-BuOH. The *n*-BuOH solution was washed with water until neutral and evaporated *in vacuo*. The product thus obtained was treated again with aq. $NaIO_4$ and KOH as above, and the final product was extracted with hot benzene to give 460 mg of the soluble portion and 880 mg of the insoluble portion. The insoluble portion was treated again with $NaIO_4$ and KOH and extracted with benzene (additional soluble portion 120 mg, insoluble portion 560 mg). The combined benzene soluble portion (580 mg of a sapogenol mixture, 19.3% from saponin) was chromatographed on alumina (6+60 g) eluting with benzene–$CHCl_3$ mixtures.

22) K.V. Rao, *Tetrahedron*, **20**, 973 (1964).

23) The following instruments were used for the physical data. Melting points: Yanagimoto Micro-melting-point Apparatus (a hot-stage type) and Ishii High-meltingpoint Apparatus (a capillary type); Specific rotations: Rex Photoelectric Polarimeter NEP-2; IR spectra: Hitachi IR Spectrometers EPI-S2 and EPI-2; PMR spectra: Hitachi H-60 and Varian HA-100 NMR Spectrometers. On chromatography, alumina (Merck, neutral, activity grade I) was used for column and water was sprayed for detection on preparative TLC.

24) Alumina column chromatography thereafter was undertaken in a similar manner and the amount of alumina is given in a parenthesis as 5+70 g.

25) Kindly provided by Prof. R. Tschesche of Bonn University, Germany.

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The eluates obtained with benzene–$CHCl_3$ (6:1—1:1) mixtures afforded a sapogenol mixture, which was crystallized from $CHCl_3$–*n*-hexane to give a sapogenol. Preparative TLC ($SiO_2$, $CHCl_3$: MeOH=50:1) of the mother layer gave additional amount of the same sapogenol and another sapogenol. The combined sapogenol (240 mg, 41.4% from the total sapogenol mixture) was recrystallized from acetone–*n*-hexane to yield protoprimulagenin A (XV), mp 272—273°, $[\alpha]_D$ +13° (*c*, 1.0 in $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3640, 3560. *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.27; H, 11.02.

The second sapogenol obtained above (40 mg, 6.9% from the sapogenol mixture) by preparative TLC was recrystallized from MeOH to afford aegicerin (XVI), mp 257.5—258°, $[\alpha]_D$ −25° (*c*, 1.0 in $CHCl_3$) (in *lit.*[22]): mp 254—256°, $[\alpha]_D$ −23.6° (*c*, 0.87 in $CHCl_3$)). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 3470 (br), 1700. *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 79.00; H, 10.65. PMR (100 MHz, $CDCl_3$): 9.23, 9.17, 9.11, 9.06, 9.03, 8.98, 8.77 (3H each, all s, $-\underset{|}{\overset{|}{C}}-C\underline{H}_3\times 7$), 7.45, 7.19 (1H each, ABq, $J$=12 Hz, $-C_{(15)}\underline{H}_2-$), 6.60, 6.18 (1H each, ABq, $J$=8 Hz, $-C_{(28)}\underline{H}_2-$), 6.85 (1H, *t*-like, $-C_{(3)}\underline{H}$-OH).

The combined fractions obtained by $CHCl_3$ and $CHCl_3$–MeOH (10:1) elution were subjected to preparative TLC ($SiO_2$, $CHCl_3$: MeOH=25:1) to give primulagenin A (VIII, 22 mg, 3.8% from the above sapogenol mixture), which was crystallized from $CHCl_3$–*n*-hexane and identified with authentic sample by mixed mp, IR, and TLC. Primulagenin A thus obtained seems to be a secondary product formed during the above mentioned treatment of saponin.

**Acid Treatment of Protoprimulagenin A (XV)**——A mixture of XV (21 mg) in aq. 2N HCl (3 ml) and EtOH (3 ml) was refluxed for 1 hr, diluted with water and extracted with ether. Treatment of the ether extract in a usual manner gave a product which was revealed by TLC to comprise one major and four minor components. Preparative TLC ($SiO_2$, $CHCl_3$: MeOH=25:1) of the product furnished a major product (13.5 mg, 64%), mp 235—237° (from $CHCl_3$–*n*-hexane), being identified with primulagenin A (VIII) by mixed mp, IR, and TLC.

**3-O-Acetyl-protoprimulagenin A (XVa)**——Acetylation of protoprimulagenin A (XV, 120 mg) with $Ac_2O$ (2 ml) and pyridine (5 ml) at room temperature for 2 days followed by ordinary treatment afforded a product (127 mg), which was crystallized from MeOH to give 3-O-acetyl-protoprimulagenin A (XVa), mp 266—267°, $[\alpha]_D$ +15° (*c*, 1.0 in $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 3450 (br), 1720; $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1732, 1245. *Anal.* Calcd. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 77.01; H, 10.49. PMR: as given in Table III.

**Oxidation of 3-O-Acetyl-protoprimulagenin A (XVa)**——A solution of XVa (80 mg) in pyridine (2 ml) was treated with $CrO_3$–pyridine complex ($CrO_3$ 150 mg, pyridine 3 ml) at room temperature and the total mixture was left standing at room temperature overnight, poured into water and extracted with ether. After treatment in a usual manner, the ether extract gave an oxidation product (73 mg) which was crystallized from MeOH to afford a monoketone-monoacetate (XVIa), mp 274—276°, $[\alpha]_D$ −20° (*c*, 1.0 in $CHCl_3$). IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 1735, 1708, 1245; $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1715, 1700; $\nu_{max}^{KBr}$ $cm^{-1}$: 1730, 1700, 1243. *Anal.* Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 76.74; H, 9.92. PMR: as given in Table III.

**$RuO_4$ Oxidation of Monoketone-monoacetate (XVIa)**——i) $RuO_4$ Solution: To an ice-cooled suspension of $RuO_2$ (200 mg) in $CCl_4$ (25 ml) was added dropwise aq. $NaIO_4$ solution ($NaIO_4$ 1.6 g, water 25 ml) in a period of 1 hr. The $CCl_4$ layer was taken and filtered to remove black precipitates and shaken with aq. $NaIO_4$ solution ($NaIO_4$ 0.5 g, water 25 ml) and kept in a refrigerator.

ii) $RuO_4$ Oxidation: A mixture of monoketone-monoacetate (XVIa, 36 mg) in above prepared $RuO_4$ solution (25 ml) was left standing at room temperature overnight. The reaction mixture was then treated with isopropanol to decompose excess $RuO_4$, filtered, and evaporated to furnish a crude product (40 mg). Preparative TLC ($SiO_2$, $CHCl_3$: benzene=2:1), of the product gave a $\gamma$-lactone derivative (XVIII, 19 mg, 53%), mp 276—277° (cryst. from MeOH), $[\alpha]_D$ −107° (*c*, 1.0 in $CHCl_3$). IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 1783, 1730, 1713, 1243; $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1770, 1720 (sh), 1710; $\nu_{max}^{KBr}$ $cm^{-1}$: 1773, 1730, 1713, 1243. *Anal.* Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 75.15; H, 9.54.

**Acetylation of Aegicerin (XVI)**——Acetylation of aegicerin (XVI, 5 mg) with $Ac_2O$ (0.4 ml) and pyridine (1 ml) at room temperature for 2 days followed by usual treatment furnished an acetate (4.2 mg), which, mp 274—276° after crystallization from MeOH, was identified with the above described monoketone-monoacetate (XVIa) by mixed mp, IR, and TLC.

***Primula japonica*, Roots**

**Isolation of Saponin**——Air-dried roots (550 g of cut, collected at Kōya-san, Wakayama prefecture, and 380 g of cut, collected at Dorogawa, Nara prefecture, both in June 1966) were extracted with MeOH at reflux three times to give 101 g of an extract which was partitioned into *n*-BuOH–water mixture. Since saponin was fairly soluble in water, the aqueous layer was concentrated *in vacuo* and extracted with *n*-BuOH repeatedly. The combined *n*-BuOH layer was evaporated *in vacuo* and the residue was treated with ether to give a saponin mixture (60.6 g, 6.5% from dried roots).

**Acid Hydrolysis of Saponin**——A mixture of saponin (1 g) in aq. 2N HCl (30 ml) and EtOH (30 ml) was refluxed for 4 hr and poured into water to precipitate a hydrolysate (330 mg, 33% from saponin mixture). The total hydrolysate was subjected to alumina (1.5+30 g) column chromatography eluting with benzene–$CHCl_3$ mixtures.

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The combined fractions obtained by benzene–$CHCl_3$ (1:1) elution were purified further by preparative TLC ($SiO_2$, $CHCl_3$: MeOH=15:1) to furnish primulagenin A (VIII, 15 mg, 5% from the hydrolysate), mp 230—234° (from MeOH), being identical with authentic sample by mixed mp, IR, and TLC.

The eluates obtained with $CHCl_3$ afforded dihydropriverogenin A (XIII, 144 mg, 44% from the hydrolysate), mp 268—270° (from MeOH–water), being identified with authentic sample[25] by mixed mp, IR, and TLC.

**Periodate Oxidation and Alkaline Treatment of Saponin**——The saponin mixture (1 g) was treated with $NaIO_4$ and KOH repeatedly twice as described for *Primula sieboldi* roots saponin. The total product (517 mg) was extracted with ether under heating to give an ether soluble portion (200 mg of a sapogenol mixture, 20% from saponin), which was chromatographed on alumina (2+20 g) eluting with benzene–$CHCl_3$ and $CHCl_3$–MeOH mixtures successively.

The fractions obtained by benzene–$CHCl_3$ (6:1—1:1) mixture elution were further purified by preparative TLC ($SiO_2$, $CHCl_3$: MeOH=100:1) to furnish protoprimulagenin A (XV, 6.7 mg, 3.4% from the sapogenol mixture), mp 226—227° (from $CHCl_3$–*n*-hexane), being identified with the above obtained sample by IR and TLC.

The eluates obtained with $CHCl_3$ and $CHCl_3$–MeOH (97: 3) afforded dihydropriverogenin A (XIII, 42.5 mg, 21.3% from the sapogenol mixture), mp 257—258° (from MeOH), being identical with authentic sample by mixed mp, IR, and TLC.

**Isolation of Saponin from Fresh Roots**——Fresh roots of *Primula japonica* (1.5 kg of cut, collected at Kōya-san in May 1968) were extracted three times with MeOH containing 0.5% pyridine at reflux for several hours. The combined MeOH extracts were partitioned into *n*-BuOH–water mixture and the water layer was concentrated *in vacuo* and further extracted with *n*-BuOH several times. The combined *n*-BuOH soluble portion was evaporated *in vacuo* and the residue was treated with ether to furnish a saponin mixture (36 g, 2.4% from fresh roots. The yield corresponds to 16% in terms of dried roots).

**Periodate Oxidation and Alkaline Treatment of the Above Saponin**——The above described saponin (1 g) was treated with $NaIO_4$ and KOH similarly as undertaken for saponin obtained from air-dried roots after the ordinary MeOH extraction. The total sapogenol mixture thus obtained (160 mg) was subjected to alumina column chromatography and preparative TLC to afford protoprimulagenin A (XV) and dihydropriverogenin A (XIII) similarly as obtained above. Priverogenin B (XII)[25] was not detected in the total sapogenol mixture although the mixture was examined minutely by TLC. It was found that $SiO_2$ impregnated $AgNO_3$ worked nicely to distinguish dihydropriverogenin A (XIII) from priverogenin B (XII) although $SiO_2$ only was unsatisfactory.

***Primula japonica*, Fruits**

**Isolation of Saponin**——Crushed fresh fruits (85 g) were extracted four times with MeOH (300 ml) at reflux for 10 hr respectively to afford totally 5 g of extracts, which after ordinary procedure (*n*-BuOH–water partition, ether treatment) gave a saponin mixture (360 mg, 0.4% from fresh fruits).

**Acid Hydrolysis of Saponin**——A mixture of saponin (80 mg) in aq. 2N HCl (6 ml) and EtOH (6 ml) was refluxed for 3 hr and treated in a usual manner to give a hydrolysate (22 mg, 28% from saponin). Preparative TLC ($SiO_2$, $CHCl_3$: MeOH=15:1) furnished a major sapogenol (5.2 mg, 6.5% from the hydrolysate), mp 252—253° (from MeOH), which was identified with dihydropriverogenin A (XIII) by IR and TLC.

**Periodate Oxidation and Alkaline Treatment of Saponin**——The saponin (250 mg) was subjected to $NaIO_4$ and KOH treatment as above and the product was extracted with ether to give a sapogenol mixture (29 mg, 11.6% from saponin) from which dihydropriverogenin A (XIII, 6.6 mg, 23%) was isolated by preparative TLC ($SiO_2$, $CHCl_3$: MeOH=20:1) and identified by IR and TLC.

***Lysimachia mauritiana*, Fruits**

**Isolation of Saponin**——Air-dried fruits (powdered 500 g, collected in July 1966 at Kada, Wakayama prefecture) was extracted twice with MeOH at reflux and a saponin mixture (7.0 g, 1.4% from dried fruits) was obtained after ordinary procedure (*n*-BuOH–water partition, ether treatment).

**Acid Hydrolysis of Saponin**——A mixture of saponin (1 g) in aq. 2N HCl (30 ml) and EtOH (30 ml) was refluxed for 4 hr and poured into water to give a hydrolysate (320 mg, 32% from saponin), which was chromatographed on alumina (2+30 g) eluting with benzene–$CHCl_3$ mixtures and $CHCl_3$. From the $CHCl_3$ eluates was obtained dihydropriverogenin A (XIII, 125 mg, 40% from the total hydrolysate), mp 259—261° (from MeOH), being identified with authentic sample by mixed mp, IR, and TLC.

**Periodate Oxidation and Alkaline Treatment of Saponin**——The saponin (1 g) was treated with $NaIO_4$ and KOH twice as before and the total product (640 mg) was extracted with ether under heating to furnish a sapogenol mixture (127 mg, 12.7% from saponin), which was subjected to alumina (2+20 g) column chromatography eluting with benzene–$CHCl_3$ and $CHCl_3$–MeOH mixtures successively.

The eluates obtained with benzene–$CHCl_3$ (1:1) afforded a sapogenol (35 mg, 27.6% from the sapogenol mixture), which was crystallized from MeOH–water and then from acetone–$CCl_4$ to give crystals of mp 270.5—271.5°, being identical with authentic priverogenin B (XII)[25] by mixed mp, IR, and TLC.

***Lysimachia clethroides*, Roots**

**Isolation of Saponin**——Air-dried roots (cut 92.5 g, collected at Mt. Kongō, Osaka prefecture and To-no-mine, Hyōgo prefecture in June—July 1966) were extracted three times with MeOH at reflux for

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several hours. The combined MeOH extracts were partitioned into *n*-BuOH–water mixture and *n*-BuOH soluble portion was treated with ether to give crude saponin (2.6 g, 2.8% from dried roots) as precipitates. Although crude saponin thus obtained was still contaminated with significant amount of coloring substances as revealed by TLC, further purification was not undertaken but the crude saponin was subjected to acid hydrolysis.

**Acid Hydrolysis of Crude Saponin**——A mixture of above obtained crude saponin (1.5 g) in aq. 2N HCl (35 ml) and EtOH (35 ml) was refluxed for 4 hr, poured into water, and extracted with ether. Working up in a usual manner of the ether extract gave reddish brown resinous product (340 mg, 23% from crude saponin), which was purified by alumina (2+30 g) column chromatography eluting with benzene–$CHCl_3$ and $CHCl_3$–MeOH mixtures successively.

Elution with benzene–$CHCl_3$ (1:1) afforded primulagenin A (VIII, 56 mg, 16% from the hydrolysate), which was recrystallized from $CHCl_3$ and then from MeOH–water, mp 236°, and identified with authentic sample by mixed mp, IR, and TLC.

The fractions obtained by $CHCl_3$–MeOH (100:1) elution afforded dihydropriverogenin A (XIII, 7.4 mg, 2% from the hydrolysate), mp 258—261° (from MeOH–water) being identical with authentic sample by mixed mp, IR, and TLC.

**Isolation of Saponin from Fresh Roots**——Air-dried roots (cut, 280 g, collected at Futatabi-san, Hyōgo prefecture in Aug. 1968) were extracted three times with MeOH containing 0.5% pyridine at reflux for several hours. Ordinary working up (*n*-BuOH–water partition, ether treatment) of the extracts afforded a crude saponin mixture (9.2 g, 3.3% from dried roots).

**Periodate Oxidation and Alkaline Treatment of Saponin**——The above obtained crude saponin mixture (3 g) was treated twice with $NaIO_4$ and KOH as before and the crude product (852 mg) was extracted with ether under heating to give a sapogenol mixture (205 mg, 6.8% from crude saponin), which was subjected to alumina (1.5+20 g) column chromatography eluting with benzene–$CH_2Cl_2$ mixtures. Although TLC of the total sapogenol mixture disclosed minor existence of priverogenin B (XII), primulagenin A (VIII) and dihydropriverogenin A (XIII), in addition to major protoprimulagenin A (XV) and aegicerin (XVI), only latter two sapogenols were isolated as shown below due to shortage of the material.

The eluates obtained with $CH_2Cl_2$ were further purified by preparative TLC ($SiO_2$, $CH_2Cl_2$: MeOH= 20:1) to furnish protoprimulagenin A (XV, 21 mg, 10.5% from the sapogenol mixture), mp 250° (from acetone–*n*-hexane), being identical with authentic sample by mixed mp, IR, and TLC.

Following eluates of $CH_2Cl_2$ were again purified by preparative TLC ($SiO_2$, $CH_2Cl_2$: MeOH=20:1) to furnish aegicerin (XVI, 7.7 mg, 3.8% from the sapogenol mixture), mp 227—229° (from acetone–*n*-hexane), being identical with the above described specimen.

***Lysimachia japonica*, Roots**

**Isolation of Saponin**——Air-dried roots (cut 15.6 g, collected at several places in June—July 1966) were extracted three times with MeOH at reflux for several hours. The MeOH extract (1.7 g) was then partitioned into *n*-BuOH–water and treated as usual to give a saponin mixture (440 mg, 2.8% from dried roots).

**Acid Hydrolysis of Saponin**——A mixture of saponin (420 mg) in aq. 2N HCl (15 ml) and EtOH (15 ml) was refluxed for 4 hr, poured into water and extracted with ether. Usual working up of the ether extract gave a resinous hydrolysate (124 mg, 29% from saponin mixture), which was subjected to alumina (1+ 10 g) column chromatography eluting with benzene–$CHCl_3$ mixtures.

The eluates of benzene–$CHCl_3$ (1:1) furnished primulagenin A (VIII, 5.1 mg, 4% from the total hydrolysate), mp 223—224° (from MeOH–water), being identical with authentic sample by IR and TLC.

From the eluate of $CHCl_3$ was obtained a trace amount of a sapogenol probably identical (TLC only) with dihydropriverogenin A (XIII). However, the direct comparison was not undertaken due to shortage of the material.

**Isolation of Saponin from Fresh Roots**——Air-dried roots (cut 6 g, collected at Suzuka-city, Mie prifecture in Aug. 1968) was extracted three times with MeOH containing 0.5% pyridine and the total extracts were treated as usual to give a saponin mixture (160 mg, 2.7% from dried roots).

**Periodate Oxidation and Alkaline Treatment of Saponin**——The above described saponin (160 mg) was subjected to $NaIO_4$ and KOH treatment twice as before and the crude product (73 mg) was extracted with ether to give a sapogenol mixture (27 mg, 16.9% from saponin), which was purified by preparative TLC ($SiO_2$, $CH_2Cl_2$: MeOH=20:1). Protoprimulagenin A (XV, 2.5 mg, 9.2% from the sapogenol mixture) was obtained as a major product and identified with authentic sample by IR and TLC.

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