whose coupling constants ($J_1=J_2=10$, and $J_3=7$ c.p.s.) reveal the configuration of the C-9 oxygen functions as being β.[2] The C-6 hydrogen signals of the derivatives (II～V) appear as slightly multiplying doublets with the coupling constants 6 c.p.s. which show the C-6 oxygen functions to be α-oriented.[3] The ketone (V) was reduced with sodium borohydride to give the epimeric alcohol (IX). In the NMR spectra, the C-15 proton signal (0.94) of the epimeric alcohol (IX) is shifted to lower field as compared with those (0.81 and 0.80) of the original alcohol (III) and cyperene (X), while the C-13 proton signal (0.89) of the alcohol (III) shows downward shift as compared with those (0.73 and 0.75) of its epimer (IX) and cyperene (X). These observations indicate that the C-3 hydroxyl of the epimeric alcohol (IX) is closely located to the C-10α methyl and, therefore, α-situated, and likewise, the C-3 hydroxyl of the original alcohol (III) is closely oriented to the C-11α methyl and, therefore, β-situated.

The absolute stereochemistry of sugetriol is consequently elucidated as shown in formula I.

It is of interest to note that the Japanese nutgrass oil contains a series of substances having the isopatchoulane skeleton and with different degrees of oxidation, *i.e.*, cyperene (X),[4] cyperotundone (XI),[1] sugeonol (XII),[3] and sugetriol (I).

Pharmaceutical Institute,
Tohoku University
School of Medicine,
Kita-4-bancho, Sendai

Hiroshi Hikino (ヒキノヒロシ)
Keitaro Aota (青田恵太郎)
Tsunematsu Takemoto (竹本常松)

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3) H. Hikino, K. Aota, T. Takemoto: This Bulletin, in press.
4) *Idem.*: Unpublished data.

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Comparative Study on the Sapogenin Constituents of Five Primulaceous Plants

Some kinds of roots belonging to *Primula* genus (Primulaceae) have long been familiar as valuable expectorants. Especially in Europe, the roots and rhizomes of *Primula elatior* L. Schreber and *P. veris* L. em. Hudson (*P. officinalis* L. Hill) have often been used as well as the other vegetable expectorants such as Senega roots.[1] Among several kinds of the root constituents, the saponin has been believed responsible for the physiological activity[2] although the conclusive work in this line is still lacking, and this appears why saponins and sapogenins originated from these European *Primulaceous* plants (including also *P. vulgaris* Huds.) have been investigated extensively by Tschesche and coworkers and other groups.[1,3]

In connection with the effort to find rich saponin source expecting its biological activity at the same time, we have first attempted a comparative study on saponin and sapogenin constituents of *Primulaceous* plants which are easily available in this country.

1) R. Tschesche, F. Ziegler: Liebig's Ann., **674**, 185 (1964), and literatures cited therein.
2) R. Tschesche, G. Wulff: Planta Medica, **12**, 272 (1964).
3) R. Tschesche, B. T. Tjoa, G. Wulff: Liebig's Ann., **696**, 160 (1966), and literatures cited therein.

In this communication, we wish to describe the preliminary study on five plant materials, namely, *Primula sieboldi* E. MORREN (Japanese name : sakura-sō) roots, *P. japonica* A. GRAY (kurin-sō) roots, *Lysimachia mauritiana* LAM. (hamabossu) fruits, *L. clethroides* DUBY (okatoranoö) roots, and *L. japonica* THUNB. (konasubi) roots. Among them a saponin named sakuraso-acid has already been isolated from *P. sieboldi* roots by Yanagisawa and Takashima,[4] however, no decisive chemical evidence has been provided thereafter.

The methanol extracts of the above mentioned plant materials were fractionated by *n*-butanol-water mixture as usual, and *n*-butanol soluble parts were then treated with ether repeatedly furnishing crude saponins with yields as shown in Table I. The crude saponins thus obtained were next hydrolyzed by refluxing for 4 hours in 1*N* ethanolic hydrogen chloride and the separations of sapogenin mixtures were accomplished by column chromatography using neutral alumina.

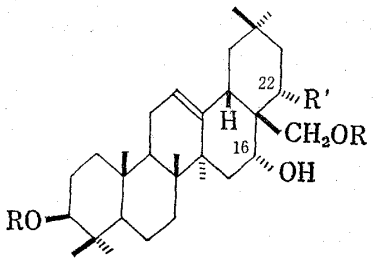
TABLE I.

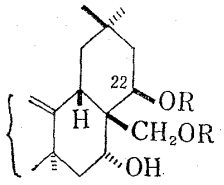
Origin	*Primula sieboldi* roots	*P. japonica* roots	*Lysimachia mauritiana* fruits	*L. japonica* roots	*L. clethroides* roots
Harvested period	February~April	June~July	July	June~July	June~July
Yield (%)[a] of crude saponin	4.1~16.1	6.5	1.4	2.8	<2.8[b]
Yield (%)[c] of sapogenin (I)	46	5	nil	4	16
Yield (%)[c] of sapogenin (II)	nil	44	40	trace	2

a) Based on the air-dried plant materials.
b) Contaminated with significant amount of coloring substance.
c) Based on the total sapogenin mixtures.

As described in Table I, primulagenin A[1] (I) was found to be the major sapogenin of *P. sieboldi*, *L. clethroides*, and *L. japonica*, whereas dihydropriverogenin A[3] (II) was the major of *P. japonica* and *L. mauritiana*. The respective identities of the sapogenins isolated here with authentic specimens*[1] were achieved by mixed melting point determinations and comparisons of infrared spectra (IR) and thin-layer chromatographic behavior (TLC). In regard to the sapogenin composition, no significant remark can be found between two genera as far as the plants tested.

On the way of the present investigation, we unexpectedly found the identity of dihydropriverogenin A and camelliagenin A[5] (mixed m.p., IR, and TLC). Tschesche and





I : R=R'=H, primulagenin A
II : R=H, R'=OH dihydropriverogenin A
=camelliagenin A
IV : R=Ac, R'=OAc

III : R=H
V : R=Ac

Chart 1.

*1 Kindly provided by Prof. Tschesche.
4) H. Yanagisawa, N. Takashima : Yakugaku Zasshi, **46**, 844 (1926).
5) S. Ito, M. Kodama, M. Konoike : Tetrahedron Letters, **1967**, 591; H. Itokawa, N. Sawada, T. Murakami : *Ibid.*, **1967**, 597.

co-workers initially proposed[3] the structure of dihydropriverogenin A possessing 22β-hydroxyl function (axial) (Ⅲ), while very recently camelliagenin A was assigned with 22α-hydoxyl (equatorial).[5] We examined the nuclear magnetic resonance spectra of dihydropriverogenin A triacetate (Ⅳ) and 22-epimeric triacetate (Ⅴ) in relation to the acetyl derivatives of theasapogenols[6] and found that dihydropriverogenin A (=camelliagenin A) should be expressed by Ⅱ, which will be discussed in detail elsewhere from our laboratory.[7]

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Faculty of Pharmaceutical Sciences,
Osaka University,
Toyonaka, Osaka

Isao Kitagawa (北川 勲)
Akiko Matsuda (松田明子)
Tadashi Nishimura (西村 正)
Shin-ichi Hirai (平井真一)
Itiro Yosioka (吉岡一郎)

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6) I. Yosioka, T. Nishimura, A. Matsuda, I. Kitagawa: Tetrahedron Letters, **1966**, 5973, 5979; I. Yosioka, A. Matsuda, T. Nishimura, I. Kitagawa: Chem. and Ind., **1966**, 2202.
7) I. Yosioka, T. Nishimura, N. Watani, I. Kitagawa: Tetrahedron Letters, to be published.

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A New Colorimetric Method of Sodium Cyclamate by Solvent Extraction with Tris(1,10-Phenanthroline)iron^{+2} Chelate*1,2

Cyclamate has been commercially available as a synthetic sweetening agent since 1950. Common method for determination of cyclamate involves titrimetry, gravimetry and gas chromatography. However, relatively few colorimetric determinations have been applied to food and drugs because the procedures are tedious and usually do not give a very accurate result, as pointed out in the review described recently by M. L. Richardson.[1]

It is the purpose of this investigation to make a new colorimetric procedure in determination of cyclamate which includes a separation treatment from saccharin sodium using a molecular sieve column.

It occurs to the authors that a small amount of cyclamate in an aqueous solution is selectively extracted into nitrobenzene if a considerable amount of tris(1,10-phenanthroline)iron^{+2} sulfate is added, and that there is a linear relationship between the red color intensity of the organic phase and the amount of cyclamate present. Fig. 1 shows the visible absorption spectra in the organic phase. It can be observed that the tris(1,

*1 Part XXIX in the series entitled "The Spectrophotometric Determination of Anions by Solvent Extraction with Metal Chelate Cations." Part XXVIII. K. Hiiro, T. Tanaka, Y. Yamamoto: Bunseki Kagaku, to be published.
*2 This work is reported in part at the 20th Annual Meeting of the Chemical Society of Japan, Abstracts of Papers Ⅱ, p. 337 (Tokyo, April, 1967).
1) M. L. Richardson: Talanta, **14**, 385 (1967).