Chem. Pharm. Bull. 21(4) 791—799 (1973)

UDC 547.597'457.1.02 : 581.192 : 615.322.011.5

Studies on the Constituents of Senegae Radix. II.[1] The Structure of Senegin-II, a Saponin from *Polygala senega* LINNE var. *latifolia* TORRY et GRAY

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(Received August 22, 1972)

The chemical structure of senegin-II(I), $C_{70}H_{104}O_{32}\cdot 4H_2O$, mp 247—248°, $[\alpha]_D^{20}$ −6.2° (MeOH), which was isolated from Senegae Radix (root of *Polygala senega* LINNE var. *latifolia* TORRY et GRAY) was established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-[β-D-galactopyranosyl($1_{gal}\rightarrow 4_{xyl}$)-$\beta$-D-xylopyranosyl($1_{xyl}\rightarrow 4_{rham}$)-$\alpha$-L-rhamnopyranosyl($1_{rham}\rightarrow 2_{fuc}$)-4-(3′,4′-dimethoxycinnamoyl)-β-D-fucopyranoside], on the basis of physical data of senegin-II, its derivatives and degradation products.

In the previous paper[1] we reported the isolation and characterization of four glycosides, namely senegin-I -II, -III and -IV, from the butanol soluble fraction of methanol extract of senegae radix (root of *Polygala senega* LINNE var. *latifolia* TORRY et GRAY (Polygalaceae)). As we pointed out in the previous paper, several investigations on the senega saponins have been reported, and most of them concerned with the structure elucidation of genuine aglycone of the saponin mixture ("senegin") of *Polygala senega*, while a few of them have reported the constitution of saponins.

Brieskorn and Renke[3] reported that the saponin mixture from the root of *P. senega* var. *typica* was purified and separated into eight saponins. The aglycone for all eight saponins is presenegenin and the numbers of glucose, galactose, xylose, fucose and rhamnose for each saponin were 3,1,2,1,1 (saponin A), 2,1,1,1,1 (saponin B), 2,1,1,1,1 (saponin C), 3,1,1,0,1 (saponin D), 3,0,1,1,1 (saponin E), 3,1,1,1,2 (saponin A′), 1,1,1,1,1 (saponin B′) and 3,1,0,0,1 (saponin C′), respectively. Furthermore, Takiura, *et al.*[4a,b] reported the isolation and characterization of four kinds of saponins, named senega saponin-A, -B, -C and -D from senegae radix. Presenegenin was found in these saponins together with glucose, galactose, xylose, fucose and rhamnose. The molar ratios of these sugars were 2:1:1:2:2 in S-A, S-B and S-D, while 3:1:1:2:2 in S-C. Furthermore, 4-methoxy- and 3,4-dimethoxycinnamic acid were detected in S-A and S-B (molar ratio, 1:1). 4-Methoxycinnamic acid was also detected in S-C, while the cinnamic acid derivative was not found in S-D.

Recently, Pelletier, *et al.*[5] have reported that the basic hydrolysis of the saponin of either *P. senega* or *P. tenuifolia* has given tenuifolin as the major prosapogenin whose structure has been confirmed to be 2β,27-dihydroxy-23-carboxy-oleanolic acid 3β-O-glucoside(=presenegenin 3β-O-glucoside).

The present paper deals mainly with the study on the chemical structure of senegin-II, the main saponin of this drug, which leads to the assignment of the structure I.

As we reported in the preliminary communication,[6] senegin-II (I), $C_{70}H_{104}O_{32}\cdot 4H_2O$, mp 247—248°, $[\alpha]_D^{20}$−6.2° (in methanol), is obtained as colorless needles, whose composition is

1) Part I: J, Shoji, S. Kawanishi, and Y. Tsukitani, *Yakugaku Zasshi*, **91**, 198 (1971).
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3) C.H. Brieskorn and F. Renke, *Deut. Apoth. Ztg.*, **108**, 1601 (1968).
4) *a*) Y. Akada, H. Yuki, and K. Takiura, *Yakugaku Zasshi*, **91**, 1178 (1971); *b*) *Idem., ibid*, **92**, 232 (1972).
5) S.W. Pelletier, S. Nakamura, and R. Soman, *Tetrahedron*, **27**, 4417 (1971).
6) J. Shoji, S. Kawanishi, and Y. Tsukitani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1740 (1971).

one mole each of presenegenin, glucose, fucose, rhamnose, xylose, galactose and 3,4-dimethoxycinnamic acid. The infrared (IR) spectrum of I indicates the presence of many hydroxyl groups (3500—3300 cm^{-1}), two ester groups (1750 cm^{-1} and 1730 cm^{-1}), carboxyl group (1710 cm^{-1}), double bond (1635 cm^{-1}) and benzenoid system (1610 cm^{-1} and 1515 cm^{-1}), while the ultraviolet (UV) spectrum (λ_{max}^{EtOH} mμ (log ε): 317 (4.28)) suggests the presence of 3,4-dimethoxycinnamoyl ester.

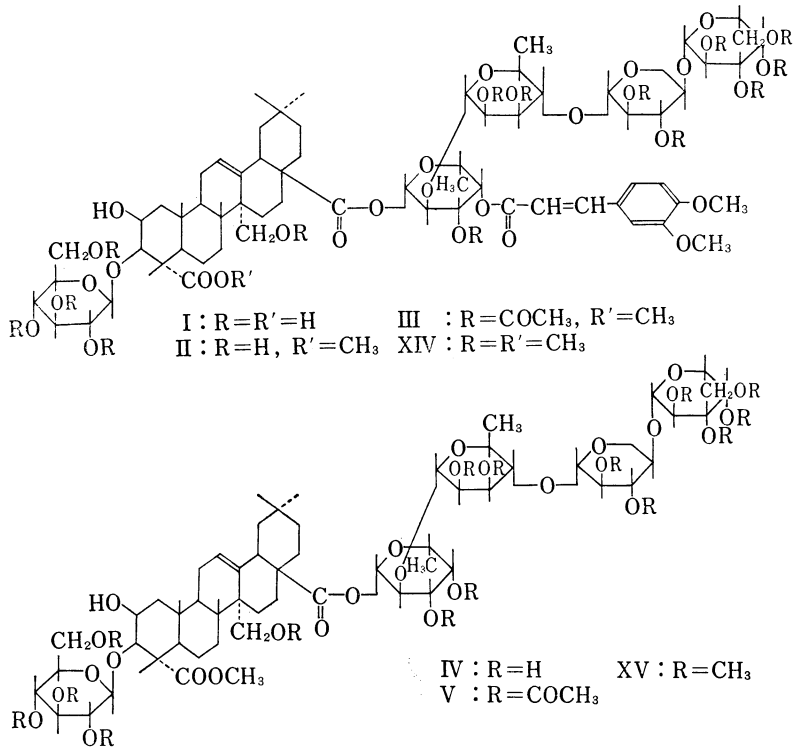


Chart 1

On methylation with diazomethane in methanol, I gave a hygroscopic monomethyl ester (II), which was characterized by acetylation with acetic anhydride and pyridine to afford senegin-II monomethyl ester tetradecaacetate (III), $C_{99}H_{134}O_{46}\cdot 2H_2O$, as a white powder, but on treatment with excess of diazomethane in methanol, I gave des-3,4-dimethoxycinnamoylsenegin-II monomethyl ester (IV), $C_{60}H_{96}O_{29}\cdot 3H_2O$, $[\alpha]_D^{15}+17°$ (in water), which was acetylated with acetic anhydride and pyridine to give the pentadecaacetate (V), $C_{90}H_{126}O_{44}$, $[\alpha]_D^{20}+10°$ (in methanol), as colorless needles.

As the presence of two kinds of ester groups has been suggested by IR spectrum of senegin-II, hydrolysis of compound II with 1N potassium hydroxide was carried out and the products were purified on silica gel column to afford (VI), $C_{37}H_{56}O_{12}\cdot 2H_2O$, $[\alpha]_D^{20}+60°$ (in ethanol), as colorless needles. The IR spectrum of compound VI indicates the absorption bands corresponding to hydroxyl groups (3500—3400 cm^{-1}), ester group (1725 cm^{-1}), carboxyl group (1690 cm^{-1}) and double bond (1620 cm^{-1}), and the nuclear magnetic resonance (NMR) spectrum shows the presence of one methyl ester (δ=3.8 ppm, 3H (s)). After acetylation of VI with acetic anhydride and pyridine, the resulted pentaacetate (VII), $C_{47}H_{68}O_{17}\cdot H_2O$, colorless needles, was treated with diazomethane to afford a pentaacetate monomethyl ester (VIII), colorless needles, $C_{48}H_{70}O_{17}\cdot 1/2H_2O$. The physical properties of compound VIII suggest that it should be presenegenin 3-O-glucoside dimethyl ester pentaacetate (=tenuifolin dimethyl-

ester pentaacetate) which was reported by S.W. Pelletier, *et al.*,[5] and compound VIII was identified with an authentic sample by mixed fusion and comparison of IR spectra.

To confirm the location of methyl ester group in compound VI, VII was treated with bromine to give monobromolactone (IX),[7] $C_{47}H_{67}O_{17}Br$, which shows the absorption band of γ-lactone at 1780 cm^{-1} in IR spectrum. The result suggests that the structure of VI must be 2β,27-dihydroxy-23-methoxycarbonyloleanolic acid 3β-D-glucopyranoside and the oligosaccharide moiety of I is assumed to link to the carboxyl group at C-17 of presenegenin in ester form.

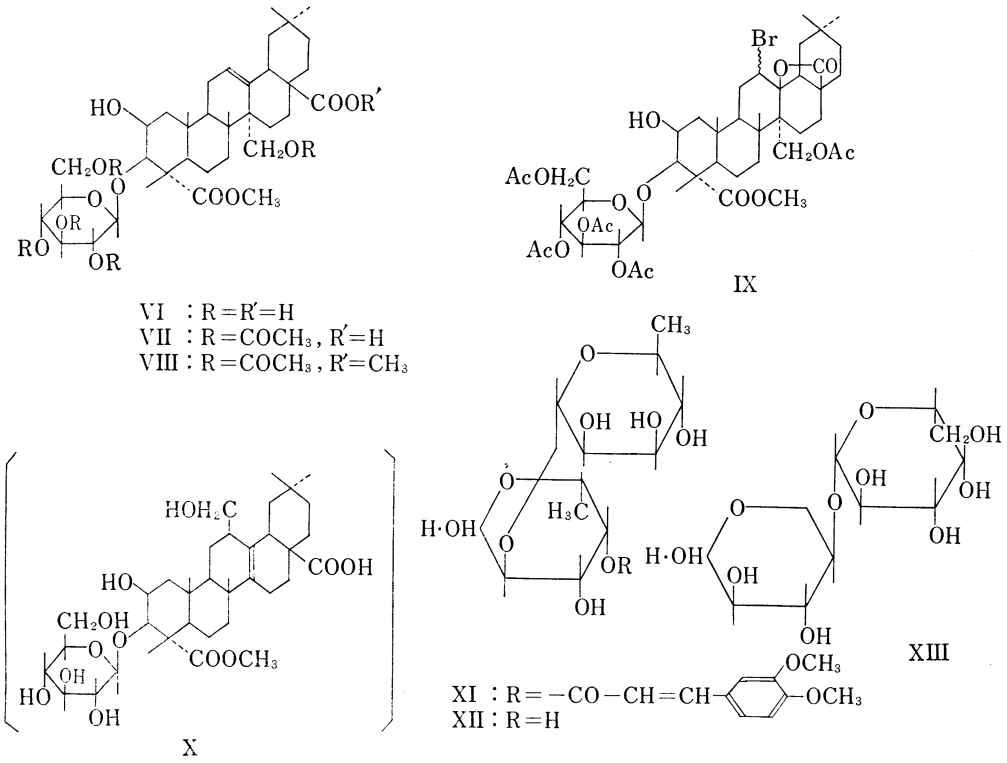


Chart 2

The hydrolysate obtained by heating II for 2 hr on a water bath with 0.03N sulfuric acid was separated into two fractions, namely a butanol soluble fraction and a water soluble fraction (butanol insoluble fraction). The butanol soluble fraction was revealed to contain more than six degradation products by thin-layer chromatography (TLC: solvent B; *Rf* 0.47 (product A), 0.41 (product B), 0.38 (product C), 0.33 (product D), 0.25 (product E) and 0.17 (product F)). These products were isolated by column chromatography and each product was examined by acid hydrolysis. The results are summarized in Table I.

The structure of product D, $C_{37}H_{58}O_{12}\cdot 2H_2O$, was established to be 2β,27-dihydroxy-23-methoxycarbonyloleanolic acid 3β-D-glucopyranoside (=tenuifolin monomethyl ester), VI, by comparing IR spectra and by mixed fusion with an authentic sample. Furthermore, product C, $C_{37}H_{58}O_{12}\cdot 2H_2O$, mp 216—218°, $[\alpha]_D^{20}$+30° (in methanol), was assumed to be hydroxysenegenin monomethyl ester 3β-O-glucoside[8] (X), but further examination has

7) S.W. Pelletier, N. Adityachaudhury, M. Tomaz, and J.J. Raynald, *J. Org. Chem.*, **30**, 4234 (1965).
8) Y. Shimizu and S.W. Pelletier, *J. Am. Chem. Soc.*, **88**, 1544 (1966).

Table I

Compounds	Hydrolysis products				
	Presenegenin monomethyl ester or its modified genin	Glucose	Fucose	Rhamnose	3,4-Dimethoxy-cinnamic acid
A			+		+
B	+	+	+		+
C (X)	+	+			
D (VI)	+	+			
E (XI)			+	+	+
F	+	+			

not yet carried out. A part of the butanol extract was treated with 0.5% potassium hydroxide in 50% ethanol to give a biose-A (XII), $C_{12}H_{22}O_9 \cdot 1/4H_2O$, $[\alpha]_D^{20}+5.7°$ (in water), as colorless needles, which gave fucose and rhamnose on acid hydrolysis. The biose-A was assumed to be a new bioside and the structure was confirmed by further experiments described later.

On the other hand, the water soluble fraction contains biose-B (XIII), $C_{11}H_{20}O_{10}$, mp 173°, $[\alpha]_D^{20}+18.7°$ (in water), which affords xylose and galactose on acidic hydrolysis. After methylation of XIII by Kuhn's method,[9] the resulted per-O-methyl ether was methanolyzed to afford methyl 2,3-di-O-methylxyloside and methyl 2,3,4,6-tetra-O-methylgalactoside. Therefore, the structure of biose-B is assumed to be 4(β-D-galactopyranoside)-D-xylose which has been reported by Srivastava, *et al.*[10] As the results of foregoing experiments, it is suggested (a) one of the carboxyl group of I is present in free form at C-4 and the other at C-17 in ester form. (b) 3,4-dimethoxycinnamic acid of I is attached to fucose.

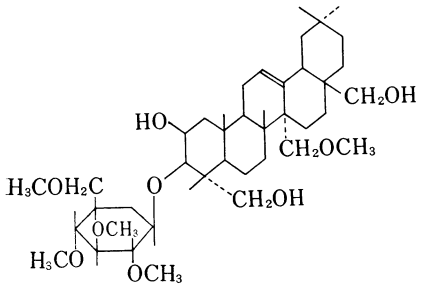
To establish the position of 3,4-dimethoxycinnamic acid attached to fucose, the following studies have been carried out. Permethylation of I and IV by the Hakomori's method[11] gave per-O-methyl-senegin-II (XIV), $C_{85}H_{134}O_{32}$, a white powder, and des-3,4-dimethoxycinnamoylsenegin-II monomethyl ester pentadeca-O-methyl ether (XV), $C_{75}H_{126}O_{29}$, a white powder, respectively. On methanolysis with 2N hydrogen chloride in dried methanol both compound XIV and XV gave common methylated sugars, namely methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside and methyl 2,3,4,6-tetra-O-methylglucoside, but only the difference between the constitutions of each methanolyzate is the presence of methyl 3-O-methylfucoside in the former and that of methyl 3,4-di-O-methylfucoside in the latter.

Consequently, it is proved that 3,4-dimethoxycinnamic acid is attached to the C-4 hydroxyl group of fucose which is a member of oligosaccharide moiety of senegin-II.

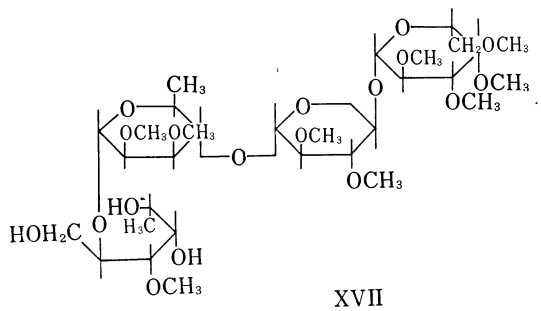
On reduction with lithium aluminium hydride in tetrahydrofuran, compound XIV afforded XVI, $C_{41}H_{68}O_{10}$, $[\alpha]_D^{20}+62.5°$, from the ether extract of the reaction mixture and compound XVII, $C_{32}H_{60}O_{18} \cdot 1/2H_2O$, $[\alpha]_D^{20}-86.4°$, from the chloroform extract. The NMR spectrum of compound XVI shows the presence of five O-methyl groups, one anomeric proton and one vinyl proton, while the IR spectrum indicates the absence of carbonyl group in this compound. Based on the physical properties of compound XVI and its methanolysis experment which affords methyl 2,3,4,6-tetra-O-methylglucopyranoside, the structure of XVI has suggested to be olean-12-ene-27-O-methyl-3β,23,28-trihydroxy-3β-tetra-O-methyl-β-D-glucopyranoside. The another product, XVII, shows the presence of two secondary methyl groups (δ=1.25, 3H (d) J=8 cps, δ=1.55 3H (broad)), nine O-methyl groups (δ=3.2—3.67) and three anomeric

9) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).
10) H.C. Srivastava and F. Smith, *J. Am. Chem. Soc.*, **79**, 982 (1957).
11) S. Hakomori, *J. Biochem.*, (Tokyo), **55**, 205 (1964).

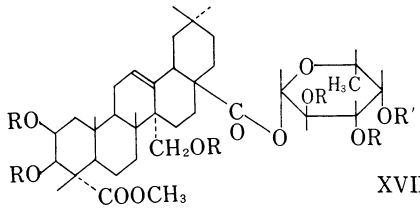
protons (δ=4.17 1H(d) J=7 cps, galactose, δ=4.83 1H(d) J=7 cps, xylose; δ=5.2 1H (broad s) rhamnose) in NMR spectrum. On methanolysis with 2N hydrogen chloride in methanol XVII gave methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside and mono-O-methylfucitol which was deduced to be 3-O-methylfucitol from the result of degradation experiment of XIV described above, but the identification with the authentic sample has not carried out.



XVI



XVII



XVIII : R=H, R′=—CO—CH=CH—(3,4-dimethoxyphenyl)

XIX : R=—COCH$_3$, R′=—CO—CH=CH—(3,4-dimethoxyphenyl)

XX : R=R′=H

XXI : R=R′=—COCH$_3$

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From the results of the foregoing experiments the structure of oligosaccharide of senegin-II was suggested to be D-galactopyranosyl ($1_{gal}\rightarrow4_{xyl}$)-D-xylopyranosyl($1_{xyl}\rightarrow4_{rham}$)-L-rhamnopyranosyl($1_{rham}\rightarrow2_{fuc}$)-4-(3′,4′-dimethoxycinnamoyl)-D-fucopyranoside. The configuration of each monosaccharide was assigned as follows. The configurations of D-galactose and xylose were assigned to be all β form from the value of coupling constants (J=7 cps) in NMR spectrum of compound XVII, while that of L-rhamnose was assigned to be α form from the comparison of molecular optical rotation of D-fucopyranoside ($[M]_D$+123°), compound XII ($[M]_D$+17.6°) and methyl α-rhamnopyranoside ($[M]_D$—111°). Furthermore, the configuration of D-fucose was deduced as follows. After oxidation of compound II with sodium metaperiodate, the product was reduced with sodium borohydride and then hydrolyzed with 0.05N hydrogen chloride in 50% methanol to afford a compound XVIII, which is composed of 3,4-dimethoxycinnamic acid, fucose and presenegenin monomethyl ester. The compound XVIII was treated with 0.5% potassium hydroxide in 50% methanol to give presenegenin D-fucopyranoside monomethyl ester (XX), $C_{37}H_{58}O_{11}\cdot H_2O$, $[\alpha]_D^{14}$+71.3°. The usual acetylation of XVIII afforded XIX, $C_{58}H_{78}O_{19}$, a white powder. The NMR spectrum of XIX shows the signal of anomeric proton (δ=5.55 1H(d) J=10 cps) and the value of coupling constant reveals that the D-fucose in the saponin is linked with β configuration.

From these experimental data, the structure of senegin-II has established to be presenegenin-(3)-[β-D-glucopyranosyl]-(28)-[β-D-galactopyranosyl($1_{gal}\rightarrow4_{xyl}$)-$\beta$-D-xylopyranosyl($1_{xyl}\rightarrow$

4_{rham})-α-L-rhamnopyranosyl($1_{rham}\rightarrow 2_{fuc}$)-4-(3′,4′-dimethoxycinnamoyl)-β-D-fucopyranoside] formulated as I.

The study on the structures of senegin-III and -IV will be published in the near future.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and uncorrected. IR absorption spectra were obtained with a Hitachi Model 215. NMR spectra were measured with a Hitachi Model R-20 High Resolution NMR spectrometer and a Hitachi Model R-22 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvents used are indicated. Gas chromatograph used was a Japan Electron Co., JGC Model 810 with a hydrogen flame ionization detector. Molecular weight was determined using a Hitachi Perkin-Elmer molecular weight apparatus Model 115. The *Rf* values were determined by thin-layer chromatography on silica gel H using Solvent A: ethyl acetate, Solvent B: $CHCl_3$–MeOH (5: 1), Solvent C: $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase) and 10% H_2SO_4 (spraying followed by heating) as a staining agent.

Isolation of Senegin-II(I)——As we reported in the previous paper, the crushed material was extracted with hot MeOH. After evaporation of the solvent, the syrupy brown residue was dissolved in water and extracted with benzene. The aqueous layer was extracted with *n*-BuOH saturated with water. The *n*-BuOH soluble fraction was submitted to column chromatography on silica gel using AcOEt saturated with water and then with the same solvent containing 5—100% MeOH. The eluate containing senegin-I, -II, -III, and -IV was repeatedly purified by column chromatography on silicic acid (Mallinckrodt) with $CHCl_3$–MeOH–H_2O (65: 35: 10, the lower phase) and finally senegin-II was isolated.

Senegin-II(I)——Senegin-II was recrystallized from *n*-BuOH–AcOH–H_2O (4: 1: 5, the upper phase) as colorless needles, mp 247—248°, $[\alpha]_D^{20}$ −6.8° (c=2.0 MeOH), *Anal.* Calcd. for $C_{70}H_{104}O_{32}\cdot 4H_2O$: C, 54.89; H, 7.37. Found: C, 54.71; H, 7.38. UV λ_{max}^{EtOH} mμ (log ε): 317 (4.28). Mol. wt. (osmotic vapour pressure method in MeOH) Calcd. for $C_{70}H_{104}O_{32}$.: 1456. Found: 1383. IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 1750 (COOR), 1730 (COOR), 1710 (COOH), 1635 (C=C), 1610, 1515 (benzenoid). H.I. (haemolytic index): 43478.

Senegin-II Monomethyl Ester(II) and Its Tetradecaacetate (III)——The solution of senegin-II in MeOH was treated with ethereal diazomethane to yield a hygroscopic non-crystalline monomethyl ester (II). Senegin-II monomethyl ester (1 g) was dissolved in Ac_2O (10 ml) and pyridine (10 ml), and the solution was allowed to stand for 48 hr at room temperature. The reaction mixture was worked up as usual and the product was purified with EtOH to give a tetradecaacetate (III) as a white powder, (mp 164—166°), $[\alpha]_D^{20}$ +23° (c=1.07 MeOH). *Anal.* Calcd. for $C_{99}H_{134}O_{46}\cdot 2H_2O$: C, 56.73; H, 6.59. Found: C, 56.62; H, 6.40. IR ν_{max}^{Nujol} cm^{-1}: 3600—3500 (OH), 1740 (COOR), 1630 (C=C), 1600, 1515 (benzenoid), 1230, 1030 (C–O–C).

Des-3,4-dimethoxycinnamoyl Senegin-II Monomethyl Ester (IV)——To a MeOH solution of I (1 g) was added excess ethereal diazomethane and the reaction mixture was allowed to stand overnight in refrigerator. After the decomposition of an excess diazomethane with AcOH, the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel column eluted with solvent C. The main product was reprecipitated from EtOH to give des-3,4-dimethoxycinnamoyl senegin-II monomethyl ester (IV) (500 mg) as a white powder, mp 230—233° (decomp.), $[\alpha]_D^{20}$ +17° (c=2.8 H_2O). *Anal.* Calcd. for $C_{60}H_{96}O_{29}\cdot 3H_2O$: C, 53.97; H, 7.64. Found: C, 53.90; H, 7.43.

Des-3,4-dimethoxycinnamoyl Senegin-II Monomethyl Ester Pentadecaacetate (V)——A solution of IV (200 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand for 48 hr at room temperature. The reaction mixture was worked up as usual and the product was recrystallized from EtOH to give pentadecaacetate of IV as colorless needles, mp 168—170°, $[\alpha]_D^{20}$ +10° (c=1.0 MeOH), *Anal.* Calcd. for $C_{91}H_{122}O_{42}\cdot 2H_2O$: C, 55.49; H, 6.68. Found: C, 55.68; H, 6.58. IR ν_{max}^{Nujol} cm^{-1}: 3600—3500 (OH), 1750 (COOR), 1630 (C=C), 1240, 1050 (C–O–C). NMR (in $CDCl_3$) δ: 2.0—2.4 (15x $COC\underline{H}_3$), 3.8 (3H(s) $COOC\underline{H}_3$).

Presenegenin 3β-D-Glucoside Monomethyl Ester (Tenuifolin Monomethyl Ester) (VI) from II——The compound II (1 g) was dissolved in 1N KOH (200 ml) and the solution was heated under nitrogen flow on a water bath for 1 hr. The reaction mixture was neutralized with 1N HCl and extracted with *n*-BuOH.
The *n*-BuOH solution were combined, washed with water and evaporated to dryness *in vacuo*. The residue was submitted to column chromatography on silica gel using AcOEt saturated with water to give crude product (96 mg). On crystallization from AcOEt saturated with water the compound VI was obtained as colorless needles, mp 231—232°, $[\alpha]_D^{20}$ +60° (c=1.0 EtOH), *Anal.* Calcd. for $C_{37}H_{58}O_{12}\cdot 2H_2O$: C, 60.82; H, 8.49. Found: C, 61.21; H, 8.52. IR ν_{max}^{Nujol} cm^{-1}: 3500—3400 (OH), 1725 (COOR), 1690 (COOH), 1630 (C=C). NMR (in $CDCl_3$) δ: 3.8 (3H(s) $COOC\underline{H}_3$).

Presenegenin 3β-D-Glucoside Monomethyl Ester Pentaacetate (VII)——The compound VI (50 mg) was acetylated with Ac_2O and pyridine to give a pentaacetate as colorless needles from MeOH. mp 215—217°, $[\alpha]_D^{20}$ +71.4° (c=1.4 acetone), *Anal.* Calcd. for $C_{47}H_{68}O_{17}\cdot H_2O$: C, 61.17; H, 7.59. Found: C, 61.10; H, 7.52. IR ν_{max}^{Nujol} cm^{-1}: 3560 (OH), 1740 (1760 shoulder COOR), 1690 (COOH), 1250 (C–O–C). NMR (in $CDCl_3$) δ: 1.97 (3H(s) 3×$OCOC\underline{H}_3$), 2.0 (3H(s) $OCOC\underline{H}_3$), 2.01 (3H(s) $OCOC\underline{H}_3$), 3.7 (3H(s) $COOC\underline{H}_3$), 5.53 (1H(q) $-\dot{C}=\dot{C}\underline{H}$).

Presenegenin 3β-D-Glucoside Dimethyl Ester Pentaacetate (VIII)——A solution of VII (50 mg) in MeOH (5 ml) was treated with ethereal diazomethane. Removal of the solvent gave a crystalline powder, which was recrystallized from $CHCl_3$–hexane to afford a methyl ester of VII as colorless needles. This product was identified with an authentic sample of presenegenin 3β-D-glucoside dimethyl ester pentaacetate, which was kindly given to us from Prof. S.W. Pelletier, by mixed fusion and the comparison of IR spectra. mp 220—222°, $[\alpha]_D^{20}$ +71.4° (c=1.4 $CHCl_3$) (lit. +67.7°).[5] *Anal.* Calcd. for $C_{48}H_{70}O_{17}\cdot 1/2H_2O$: C, 62.13; H, 7.65. Found: C, 62.34; H, 7.55. IR ν_{max}^{Nujol} cm^{-1}: 3580 (OH), 1760 (COOR), 1740 (COOR). NMR (in $CDCl_3$) δ: 2.00 (9H(s) 3× $OCOCH_3$), 2.01 (3H(s) $OCOCH_3$), 2.02 (3H(s) $OCOCH_3$), 3.63 (3H(s) $COOCH_3$), 3.70 (3H(s) $COOCH_3$), 5.55 (1H(q) $-\dot{C}=C\dot{H}$).

Presenegenin 3β-D-glucoside Monomethyl Ester Pentaacetate Bromolactone (IX)——To a solution of VII (50 mg) and $AcONa\cdot 3H_2O$ (90 mg) in 90% AcOH (10 ml) was added dropwise 0.1 ml of bromine solution (0.5 ml bromine in 10 ml of glacial acetic acid) with stirring. The reaction mixture was poured into water containing a little of sodium bisulfite and the resulted precipitate was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and evaporated to give a solid which was recrystallized from $CHCl_3$–hexane to afford a bromolactone (30 mg) as white powder. (mp 121—123°), IR ν_{max}^{Nujol} cm^{-1}: 3650 (OH), 1780 (γ-lactone), 1750 (COOR), 1240, 1040 (C–O–C). *Anal.* Calcd. for $C_{47}H_{67}O_{17}Br$: C, 57.37; H, 6.81. Found: C, 57.62; H, 7.00.

Partial Hydrolysis of II with 0.03N H_2SO_4——A solution of compound II (2 g) in 0.03N H_2SO_4 (200 ml) and dioxane (20 ml) was refluxed for 2 hr on a water bath. The reaction mixture was cooled and extracted twice with *n*-BuOH.

1) The Butanol Soluble Fraction: The *n*-BuOH layer were combined, washed with water and evaporated *in vacuo* to give a yellow powder, which was examined by TLC (solvent B, *Rf* 0.47 (product A), 0.41 (product B), 0.38 (product C), 0.33 (product D), 0.25 (product E), 0.17 (product F) to reveal the presence of more than six components. The studies about these products were carried out as follows.

1a) A part of the product was placed on a silica gel column eluted with $CHCl_3$–MeOH (20: 1–5: 1 gradient) to isolate the six products. These products were hydrolyzed with 4N H_2SO_4–dioxane on a water bath for 4 hr and the hydrolyzates were examined by TLC (solvent B) and PPC (Toyo-Roshi No. 50; solvent, *n*-BuOH–AcOH–H_2O (4: 1: 5, the upper layer), respectively. The results were summarized in Table I.

Properties of Product C(X); The product C was recrystallized from ethyl acetate saturated with water to give colorless needles, mp 216—218°, $[\alpha]_D^{20}$ +30° (c=1.1 MeOH). *Anal.* Calcd. for $C_{37}H_{58}O_{12}\cdot 2H_2O$: C, 60.82; H, 8.49. Found: C, 60.52; H, 8.46. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1725 (COOR), 1695 (COOH), 1245, 1075 (C–O–C). NMR (in $CDCl_3$) δ: 3.83 (3H(s) $COOCH_3$), 4.85 (1H,(d) J=7 cps, anomeric H).

Properties of Product D(VI): The product D was recrystallized from ethyl acetate saturated with water to afford colorless needles, mp 230—231°, IR ν_{max}^{Nujol} cm^{-1}: 3500—3400 (OH), 1725 (COOR), 1690 (COOH), 1630 (C=C). This product was identified with tenuifolin monomethyl ester (VI), which was obtained by basic hydrolysis of II, by mixed fusion and comparison of IR spectrum with that of an authentic sample.

1b) A part of the product (1 g) was dissolved in 0.5% KOH–50% EtOH (100 ml) and the solution was allowed to stand overnight. The solution was neutralized with ion exchange resin (Amberlite IR-MB), evaporated under reduced pressure to remove EtOH. The residual aqueous solution was diluted with water (100 ml) and extracted with *n*-BuOH saturated with water. The aqueous layer was concentrated *in vacuo* and the residue was submitted to column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (7: 3: 1, the lower phase). The biose-A (XII) was recrystallized from acetone to give colorless needles (64 mg), mp 144.4—145.5°, $[\alpha]_D^{20}$ +5.7° (c=1.7 H_2O). *Anal.* Calcd. for $C_{12}H_{22}O_9\cdot 1/4H_2O$: C, 45.78; H, 7.15. Found: C, 45.65; H, 7.16. On hydrolysis with 4N HCl biose-A gave fucose and rhamnose, which were characterized by PPC and GLC as described above.

2) The Aqueous Fraction: The aqueous layer was neutralized with ion exchange resin (Amberlite IR-MB) and evaporated *in vacuo*. The residue (810 mg) was dissolved in a small amount of water and the solution was passed through the column of activated charcoal (3 g). The column was washed with water and eluated with 5% EtOH (1 liter). The eluate was evaporated under reduced pressure to give a white powder (78 mg) which was purified by column chromatography on silica gel using solvent C. Recrystallization from EtOH gave biose-B(XIII) as colorless cubics (38 mg), which afforded xylose and galactose on hydrolysis with 4N HCl. mp 173°, $[\alpha]_D^{20}$ +18.7° (c=0.8 H_2O equiv. after three days). *Anal.* Calcd. for $C_{11}H_{20}O_{10}$: C, 42.31; H, 6.46. Found: C, 41.99; H, 6.65. Compound XIII was dissolved in 2N HCl–MeOH and the solution was allowed to stand overnight. After neutralization with Ag_2CO_3 and filtration, the filtrate was evaporated *in vacuo*. The residue was permethylated with Ag_2O and CH_3I in dimethylformamide (DMF) according to the Kuhn's method. The reaction mixture was worked up as usual and the product was purified by preparative TLC on silica gel using benzene–acetone (3: 1, *Rf* 0.16). The permethylate obtained was methanolyzed with 2N HCl–MeOH for 2 hr under refluxing. The solution was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was examined by TLC (solvent B) and GLC. Methyl 2,3,4,6-tetra-O-methylgalactoside and methyl 2,3-di-O-methylxyloside were identified.

Senegin-II Tetradeca-O-methyl Monomethyl Ester (XIV)——According to the Kuhn's method, 20 ml

of CH_3I and 20 g of freshly prepared Ag_2O were added to a solution of II (2 g) in DMF (60 ml) and the solution was kept for 48 hr at room temperature with stirring. The reaction mixture was filtered and to the filtrate was added CH_3I (20 ml) and Ag_2O (20 g). The reaction mixture was kept for 48 hr at room temperature with stirring. After filtration the reaction mixture was poured into a large amount of water and the product was extracted with $CHCl_3$ for several times. The $CHCl_3$ solutions were combined, washed with water, dried over Na_2SO_4. The solvent was removed *in vacuo* to give a yellow powder. The product was purified by column chromatography on silica gel eluated with ethyl acetate to afford a white powder reprecipitated from hexane (600 mg). (mp 140—142°), $[\alpha]_D^{20}$ +15° (c=1.0 $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1750 (shoulder), 1730 (shoulder), 1710 (COOR), 1630 (C=C), 1600, 1510 (benzenoid), 1050 (C–O–C). *Anal.* Calcd. for $C_{85}H_{134}O_{32}$: C, 61.22; H, 8.04. Found: C, 60.97; H, 8.25.

Des-3,4-dimethoxycinnamoyl Senegin-II Pentadeca-O-methyl Monomethyl Ester (XV)——According to the Hakomori's method, NaH (300 mg) was warmed with dimethylsulfoxide (3 ml) at 65° for 1 hr with stirring under N_2 gas flow. To this reagent a solution of IV (500 mg) in DMSO (3 ml) was added and the mixture was kept at 65° for 15 min with stirring under N_2 gas flow. Then CH_3I (3 ml) was added to the solution and the mixture was allowed to stand at room temperature for 2 hr with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and evaporated. The residue was placed on silica gel column and eluted with ethyl acetate to give a pentadeca-O-methyl ether (XV) as a white powder (100 mg) reprecipitated from hexane. (mp 125—127°), $[\alpha]_D^{20}$ +12.5° c=0.8 $CHCl_3$). *Anal.* Calcd. for $C_{75}H_{126}O_{29}$: C, 60.40; H, 8.45. Found: C, 60.35; H, 8.56. IR ν_{max}^{Nujol} cm^{-1}: 3600 (OH), 1740 (1720 shoulder COOR), 1250, 1100 (C–O–C).

Methanolysis of XIV and XV with 2N HCl–MeOH——Compound XIV (100 mg) was refluxed with 2N HCl–MeOH (5 ml) for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo* and the residue was fractionated by column chromatography on silica gel with $CHCl_3$–MeOH (50: 1–20: 1). Compound XV (100 mg) was also worked up in the same way. Each fractions were further separated by preparative TLC using solvent A to afford five O-methylated sugars, that is, methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside and methyl 3-O-methylfucoside in the methanolyzate of XIV, while methyl 2,3,4,6-tetra-O-methylglucoside, methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3,-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside and methyl 3,4-di-O-methylfucoside in that of XV.

TLC i) Solvent A: *Rf* 0.46 (α-methyl 2,3,4,6-tetra-O-methylglucoside), 0.35 (β-methyl 2,3,4,6-tetra-O-methylglucoside), 0.25 (α-methyl 2,3,4,6-tetra-O-methylgalactoside), 0.21 (β-methyl 2,3,4,6-tetra-O-methylgalactoside), 0.32 (α-methyl 2,3-di-O-methylxyloside), 0.25 (β-methyl 2,3-di-O-methylxyloside), 0.31 (methyl 2,3-di-O-methylrhamnoside), 0.11 (methyl 3-O-methylfucoside), 0.18 (methyl 3,4-di-O-methylfucoside).

ii) Solvent B: *Rf* 0.83 (methyl 2,3,4,6-tetra-O-methylglucoside), 0.77 (methyl 2,3,4,6-tetra-O-methylgalactoside), 0.61 (α-methyl 2,3-di-O-methylxyloside), 0.54 (β-methyl 2,3-di-O-methylxyloside), 0.57 (methyl 2,3-di-O-methylrhamnoside), 0.33 (methyl 3-O-methylfucoside), 0.57 (α-methyl 3,4-di-O-methylfucoside), 0.50 (β-methyl 3,4-di-O-methylfucoside).

GLC: Column: 1,4-butane diol succinate (5%) on Shimalite w (60—80 mesh), 3 mm×2 m, column temperature 175°, carrier gas N_2 35 ml/min, t_R (min) 2.5 (α- and β-methyl 2,3,4,6-tetra-O-methylglucoside), 5.5 (methyl 2,3,4,6-tetra-O-methylgalactoside), 4.0, 4.6 (α- and β-methyl 2,3-di-O-methylxyloside), 4.6 (methyl 2,3-di-O-methylrhamnoside), 8.2 (methyl 3-O-methylfucoside), 5.0, 7.8 (α- and β-methyl 3,4-di-O-methylfucoside).

Reductive Cleavage of XIV with $LiAlH_4$——To a solution of compound XIV (1.5 g) in tetrahydrofuran (THF 100 mg) was added 400 mg of $LiAlH_4$ and the reaction mixture was refluxed for 2 hr. After the decomposition of excess $LiAlH_4$ with AcOEt the reaction mixture was poured into a large amount of water and the aqueous solution was extracted with ether and then with $CHCl_3$. The ether solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue (700 mg) was purified by chromatography on silica gel with $CHCl_3$ to afford XVI as a white powder from hexane. (mp 125—126.5°), $[\alpha]_D^{20}$ +62.5° (c=0.8 $CHCl_3$), *Anal.* Calcd. for $C_{41}H_{68}O_{10}$: C, 68.30; H, 9.51. Found: C, 68.21; H, 9.61. IR ν_{max}^{Nujol} cm^{-1}: 3500—3400 (OH), 1630 (C=C), 1090 (C–O–C). NMR (in $CDCl_3$) δ: 3.2 (3H(s) $-OC\underline{H}_3$), 3.28 (3H(s) $-OC\underline{H}_3$), 3.3 (3H(s) $-OC\underline{H}_3$), 3.48 (3H(s) $-OC\underline{H}_3$), 3.59 (3H(s) $-OC\underline{H}_3$), 4.2 (1H(d) J=7 cps, anomeric $\underline{H}$), 5.4 (1H(m) $-\dot{C}=\dot{C}\underline{H}-$). On methanolysis with 2N HCl–MeOH, compound XVI gave methyl 2,3,4,6-tetra-O-methylglucoside and the corresponding genin, which were identified by TLC and GLC, respectively.

The $CHCl_3$ solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The residue (340 mg) was chromatographed on a silica gel column using $CHCl_3$–MeOH (50: 1) and the eluate was purified by reprecipitation from acetone–hexane to give a white powder, (XVII), (mp 59—60°), $[\alpha]_D^{20}$ −86.4° (c=2.2 $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{60}O_{18}\cdot 1/2H_2O$: C, 51.81; H, 8.23. Found: C, 52.00; H, 8.16. NMR (in benzene) δ: 1.25 (3H(d) J=7 cps $-\dot{C}H-C\underline{H}_3$), 1.55 (3H(d, broad) J=6 cps $-\dot{C}H-C\underline{H}_3$), 3.2 (3H(s) $OC\underline{H}_3$), 3.3 (3H(s) $OC\underline{H}_3$), 3.38 (3H(s) $OC\underline{H}_3$), 3.42 (3H(s) $OC\underline{H}_3$), 3.48 (3H(s) $OC\underline{H}_3$), 3.49 (3H(s) $OC\underline{H}_3$), 3.55 (3H(s) $OC\underline{H}_3$), 3.67 (3H(s) $OC\underline{H}_3$), 4.17 (1H(d) J=7 cps anomeric $\underline{H}$), 4.83 (1H, (d) J=7 cps anomeric $\underline{H}$), 5.2 (1H(s) anomeric $\underline{H}$).

Methanolysis of XVII with 2N HCl–MeOH——Compound XVII was refluxed with 2N HCl–MeOH for 2 hr and the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evapotraed *in vacuo* and the residue was examined by TLC (solvent B) and GLC. Methyl 2,3,4,6-tetra-O-methylgalactoside, methyl 2,3-di-O-methylxyloside, methyl 2,3-di-O-methylrhamnoside and partially O-methylated fucitol assumed to be 3-O-methylfucitol (*Rf* 0.04) were characterized.

Oxidative Degradation of II with $NaIO_4$——To a solution of II (1 g) in 95% EtOH (500 ml) was added a solution of $NaIO_4$ (2 g) in H_2O (20 ml). The reaction mixture was kept overnight at 4° with stirring. The precipitate was filtered off and the filtrate was evaporated *in vacuo* at 50° to remove EtOH. The residual solution was diluted with water (200 ml) and extracted with *n*-BuOH. The *n*-BuOH solution was washed with water and evaporated *in vacuo*. The residue was dissolved in 95% MeOH (100 ml) and then $NaBH_4$ (700 mg) was added portionwise at room temperature with stirring. After stirring for 2 hr, the reaction mixture was neutralized with 5% AcOH. The solution was evaporated *in vacuo* at 50° to remove MeOH and the residue was extracted with *n*-BuOH. The *n*-BuOH solution was washed with water and evaporated *in vacuo*. The residue (785 mg) was refluxed with 0.03N H_2SO_4 in 50% MeOH (200 ml) for 30 min. The hydrolysis mixture was concentrated *in vacuo* and the residual solution was extracted with *n*-BuOH. The *n*-BuOH solution was washed with water and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH (50: 1) to afford XVIII. The product XVIII was purified by usual acetylation to give an acetate XIX, a white powder from acetone–hexane. (mp 143—146° (decomp.)), IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1740 (COOR), 1620 (C=C), 1600, 1510 (benzenoid), 1240, 1050 (C–O–C). *Anal.* Calcd. for $C_{58}H_{78}O_{19}$: C, 64.57; H, 7.23. Found: C, 65.04; H, 7.28.

Hydrolysis of XVIII with Acid——Compound XVIII (150 mg) was hydrolyzed with 4N HCl–dioxane–benzene (3: 1: 2) under refluxing for 4 hr. The reaction mixture was treated as usual and 3,4-dimethoxycinnamic acid, fucose and presenegenin derivatives were detected by TLC, PPC, and GLC.

Hydrolysis of XVIII with 0.5% KOH——Compound XVIII (100 mg) was dissolved in alcoholic 0.5% KOH (50 ml) and the solution was allowed to stand overnight. After neutralization with ion exchange resin (Amberlite IR-MB) the solvent was evaporated *in vacuo*. The residue was purified by reprecipitation from hexane–acetone to afford presenegenin fucoside monomethyl ester (XX) as a white powder (40 mg), (mp 196—198° (decomp.)), $[\alpha]_D^{14}$ +71.3° (c=1.53 EtOH). *Anal.* Calcd. for $C_{37}H_{58}O_{11}\cdot H_2O$: C, 63.79; H, 8.62. Found: C, 63.59; H, 8.33. IR ν_{max}^{Nujol} cm^{-1}: 3300 (broad, OH), 1750—1710 (COOR), 1640 (C=C), 1250, 1160, 1060 (broad, C–O–C). NMR (in $CDCl_3$) δ: 3.60 (3H(s) $COO\underline{H}_3$), 5.55 (1H(d) J=10 cps, anomeric $\underline{H}$), 5.87 (1H(m) $-\dot{C}=\dot{C}\underline{H}-$).

Acetylation of XX——Compound XX (30 mg) was acetylated with Ac_2O and pyridine and the product was purified by reprecipitation from $CHCl_3$–hexane to afford a hexaacetate (XXI) as a white powder, (mp 133—135° (decomp.)), $[\alpha]_D^{20}$ +85.2° (c=1.76 $CHCl_3$). *Anal.* Calcd. for $C_{49}H_{70}O_{17}$: C, 63.22; H, 7.52. Found: C, 63.17; H, 7.27. IR ν_{max}^{Nujol} cm^{-1}: 1740 (COOR), 1240, 1050 (C–O–C).

Acknowledgement The authors express their gratitude to Prof. T. Kawasaki, University of Kyushu, Prof. S. W. Pelletier, The University of Georgia, Dr. E. E. Percival, University of London, and Associate Prof. E. G. Gros, Universdad De Buenos Aires for their kind supply of the authentic samples. Thanks are also due to Mr. H. Ishizone for his co-operation in this work, to the members of Analytical Laboratory of Showa University for elemental analyses.