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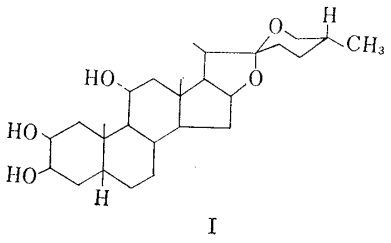
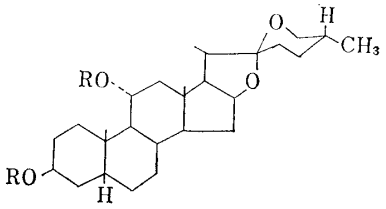
151. Hitoshi Minato and Ariyoshi Shimaoka : Studies on the Steroidal Components of Domestic Plants. XLII.[1] Narthogenin, Isonarthogenin and Neonogiragenin, Three New Sapogenins of *Metanarthecium luteo-viride* MAXIM.

(Shionogi Research Laboratory, Shionogi & Co., Ltd.[*1])

The steroidal components of *Metanarthecium luteo-viride* MAXIM. have already been investigated by our group. Metagenin[2] (I) and nogiragenin[3] (IIa), steroidal sapogenins having a hydroxyl group at C-11, and luvigenin[4] (III) and meteogenin[5] (IV), having an aromatic ring in the molecule, were isolated from this plant (see Chart 1).

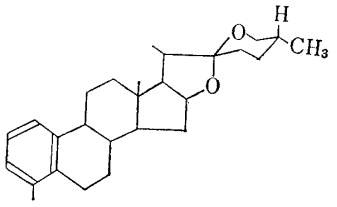


I

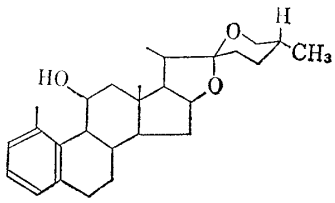


IIa, b

a: R=H
b: R=Ac



III



IV

Chart 1.

TABLE I. Paper Chromatogram of Extracts

	(°C)	Rf value	Yields (g.) From the rhizome (1.5 kg.)	Yields (g.) From the epigeous part (1.9 kg.)
Luvigenin (III)	183~184	0.98	—	0.26
β-Sitosterol	133~135	0.92	0.98	0.92
Meteogenin (IV)	157~158	0.85	0.84	0.02
Nogiragenin (IIa)	200~201	0.77	0.74	0.50
Unknown sapogenin A	245~246	0.70	0.08	—
Unknown sapogenin B	220~226	0.60, 0.58	0.02	0.315
Metagenin (I)	271~272	0.38	2.05	2.00

*1 Fukushima-ku, Osaka (湊 均, 島岡有昌).

1) Part XLI : K. Takeda, T. Okanishi, H. Minato, A. Shimaoka : Tetrahedron, **19**, 759 (1963).
2) K. Takeda, T, Okanishi, K. Hamamoto, A. Shimaoka, N. Maezono : Yakugaku Zasshi, **77**, 175 (1957). K. Takeda, K. Hamamoto : Tetrahedron Letters, No. 3, 1 (1960); This Bulletin, **8**, 1004 (1960), K. Hamamoto : This Bulletin, 8, 1099 (1960); **9**, 32 (1961).
3) K. Takeda, T. Okanishi, H. Osaka, A. Shimaoka, N. Maezono : This Bulletin, **9**, 388 (1961).
4) K. Takeda, T. Okanishi, K. Igarashi, A. Shimaoka : Tetrahedron, **15**, 183 (1961).
5) K. Igarashi : This Bulletin, **9**, 722, 729 (1961).

In addition to the spots of these four sapogenins, a few spots were found in the paper chromatogram of the methanol extract of the plant (Table I). One of these unknown spots was later confirmed to be that of β-sitosterol, while other spots were not investigated.

On the other hand, since 6% hydrochloric acid was used for saponification of the saponins, it may be reasonable to assume that luvigenin (III) and meteogenin (IV) having an aromatic ring in the molecule are not naturally occuring sapogenins but the artifacts result by partial aromatization during the course of saponification.

Especially in the case of luvigenin (III), the assumption of the presence of a proto-sapogenin[4] is more reasonable, because luvigenin has no hydroxyl group which would combine with sugar in the molecule. Therefore, the structural investigation of the unknown sapogenins from this plant was attempted in order to find the proto-sapogenins.

As shown in Table I, luvigenin (III) and an unknown sapogenin B were mainly isolated from the epigeous part of the plant, whereas meteogenin (IV) and an unknown sapogenin A were hardly found in the epigeous part and obtained from the rhizome of the plant. From this result, the unknown sapogenin B may be a proto-sapogenin of luvigenin. Thus we attempted the investigation on its structure.

The saponified methanol extract of the epigeous part of the plant (1.9 kg.) was extracted with chloroform. This extract was chromatographed on alumina to separate the fraction having a Rf value around 0.58, and this fraction afforded the unknown sapogenin B, m.p. 220~226° (315 mg.) by recrystallization from methanol. However, as this sapogenin showed two spots at Rf values of 0.58 and 0.60 in the paper chromatogram, it is obvious that the unknown sapogenin B is a mixture of two sapogenins. By the repetition of alumina chromatography and recrystallization two new sapogenins, m.p. 240~242° (Va, 147 mg.) and m.p. 214~216° (VIa, 14 mg.) were obtained. These have been named isonarthogenin and narthogenin, respectively.

Isonarthogenin (Va) and narthogenin (VIa), both of which were represented by the empirical formula, $C_{27}H_{42}O_4$, afforded the diacetates, m.p. 160~162° (Vb) and m.p. 144~146° (VIb), respectively on acetylation with acetic anhydride and pyridine at roon temperature; thus both sapogenins possess two hydroxyl groups, primary or secondary. The infrared spectra of Va and VIa showed that the characteristic bands[6] of the E- and F-rings had undergone a marked change similar to that seen in the cases of carneagenin and isocarneagenin.[1] Therefore, assuming that one of two hydroxyl groups is situated in the F-ring, isonarthogenin diacetate (Vb) was converted into a pregnene derivative by oxidative cleavage of the E- and F-rings by the usual methods.[7] Vb afforded 3β-acetoxypregna-5,16-dien-20-one (VII) in 80% yield.

This result established the structures of A, B, C, D and E-rings of Va and that the other hydroxyl group is situated in the F-ring. Moreover, tosylation of Va with 1.5 moles of tosyl chloride in pyridine afforded a mono-tosylate (VIII), m.p. 200~202°, which was converted into diosgenin (IX) by reduction with lithium aluminum hydride. Since the hydroxyl group in the F-ring was more easily tosylated than the 3β-hydroxyl group, it may be a primary one situated at C-27.

The nuclear magnetic resonance spectrum of Vb showed no characteristic signal of C-27 methyl group[8] in 25D sapogenins, which should appear as a doublet at 9.20~9.30 τ, in spite of the fact that isonarthogenin (Va) must be a 25D sapogenin because of

6) R.N. Jones : J. Amer. Chem. Soc., **75**, 158 (1953); M.E. Wall, C.R. Eddy, M.L. Meclenman, M.E. Klump : Anal. Chem., **24**, 1337 (1952).
7) W.G. Dauben, G.J. Fonken : J. Amer. Chem. Soc., **76**, 4618 (1954).
8) W.E. Rosen, J.B. Ziegler, A.C. Shabica, J.N. Shoolery : *Ibid.*, **81**, 1687 (1959).

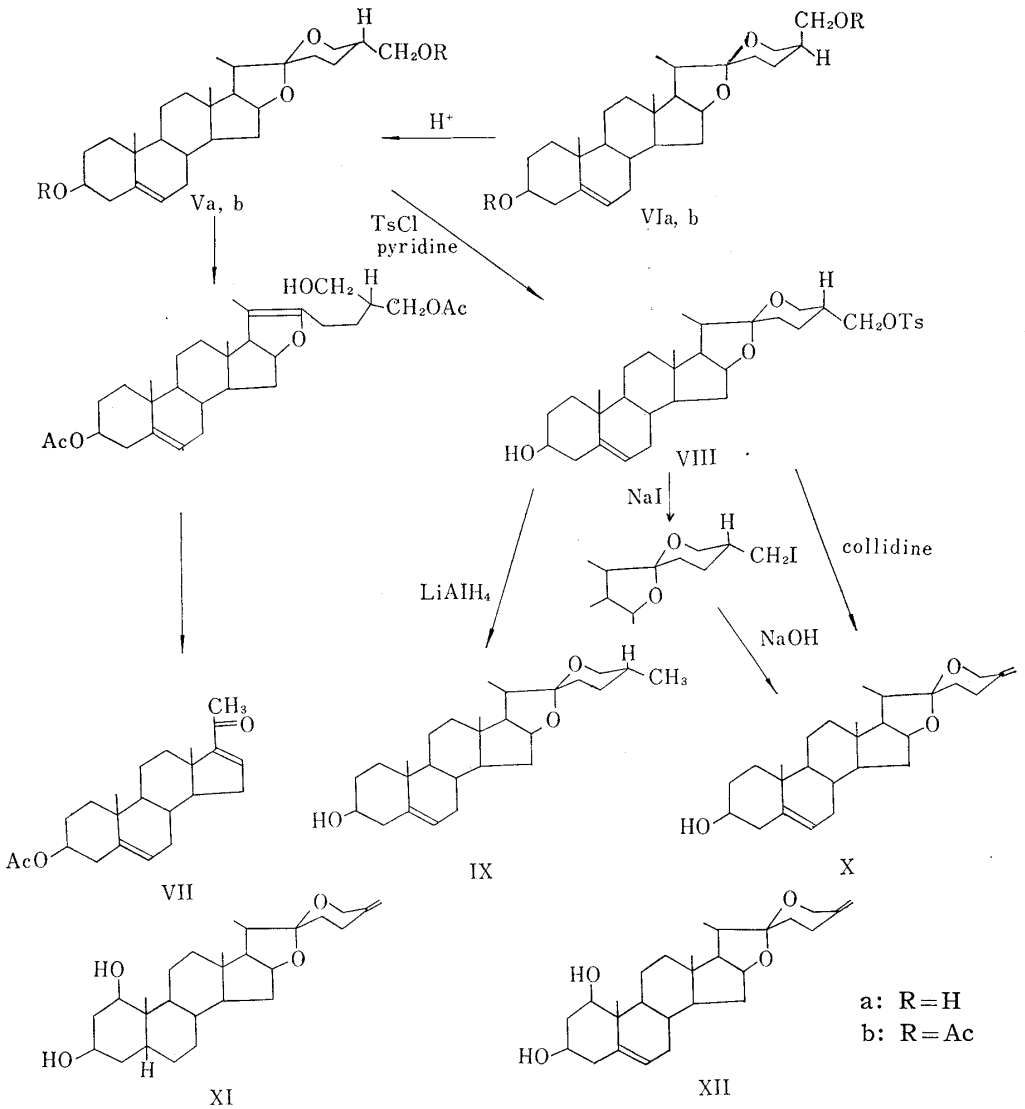


Chart 2.

the convertion of Va into diosgenin (IX). This provides further support for the presence of a hydroxyl group at C-27 in isonarthogenin (Va).

When the mono-tosylate (VIII) was refluxed in collidine or its iodide was heated with sodium hydroxide in methanol, an unsaturated derivative (X), m.p. 183~185° was obtained. The infrared spectrum of X showed frequencies at 1658 and 878 cm^{-1} corresponding to the $>C=CH_2$ type double bond. Moreover, the nuclear magnetic resonance spectrum showed a signal at 5.23 τ, which corresponded to the hydrogen of the $>C=CH_2$ group. This value, 5.23 τ, is in good agreement with the values of the same type protons of convallamarogenin[1,9] (XI, 5,27 τ) and neoruscogenin[10] (XII, 100 c.p.s.*[2]).

From these results, the constitution of isonarthogenin (Va) has been established as 25D-spirost-5-ene-3β,27-diol. Narthogenin (VIa), a stereoisomer of Va, was isomerized nearly quantitatively to isonarthogenin (Va) by treatment with dilute methanolic hydrochloric acid at room temperature for 2 hours. Thus narthogenin (VIa) was also established as 25L-spirost-5-ene-3β,27-diol in the same manner as carneagenin.[1]

*[2] The spectrum was measured in $CDCl_3$ solution at 40 mc./sec. A chloroform capillary is used as arbitrary reference zero.

9) R. Tschesche, H. Schwarz, G. Snatzke : Ber., **94**, 1699 (1961).
10) L. Mandll, A.L. Nussbaum, E.P. Oliveto : Tetrahedron Letters, No. 19, 25 (1960).

Consequently, it was established that the unknown sapogenin B was a mixture of isonarthogenin and narthogenin. However, as it is unreasonable that isonarthogenin or narthogenin is a proto-sapogenin of luvigenin (III), our presumption that the unknown sapogenin B may be a precursor of luvigenin is mistaken.

Confirmation of the structure of the unknown sapogenin A is now under investigation for the same purpose in our laboratory.

Complementarily, the fraction having Rf value of 0.77 isolated from the rhizome was reinvestigated (see Table I). This fraction was acetylated with acetic anhydride at 150~155° and the acetate fractionally crystallized from acetic anhydride to give nogiragenin diacetate (IIb), colorless prisms, m.p. 206~207° and colorless plates (XIIIb), m.p. 142~144°. This unknown acetate (XIIIb) was saponified with 3% potassium hydroxide in methanol to give colorless needles (XIIIa), m.p. 128~131°. Compound XIIIa corresponded to the empirical formula $C_{27}H_{44}O_4$ and afforded the diacetate (XIIIb). Thus it is evident that compound (XIIIa) possesses two hydroxyl groups. Moreover, compound (XIIIa) shows the same Rf value, 0.77, as that of nogiragenin (IIa) in the paper chromatogram and the characteristic bands of 25L sapogenins in the infrared spectrum. Therefore, it may be supposed that compound (XIIIa) is 5β,25L-spirostane-3β,11α-diol.

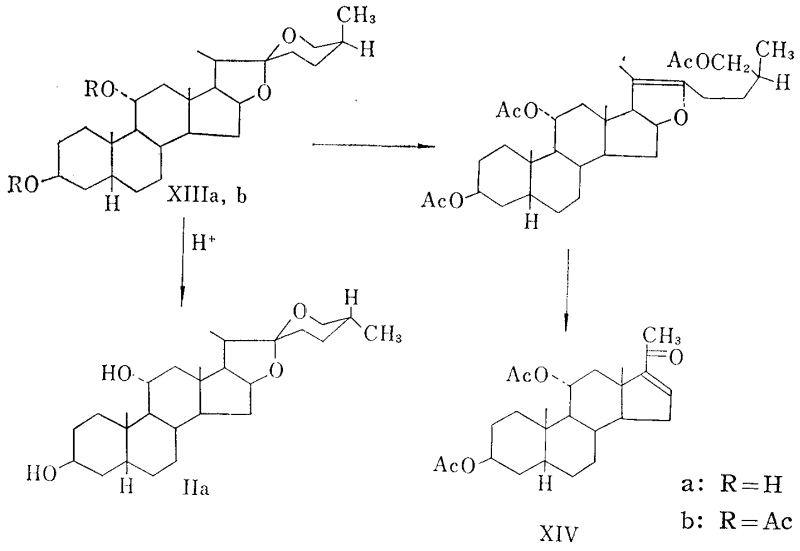


Chart 3.

The structure of XIIIa was further confirmed as follows. Compound (XIIIa) was isomerized to nogiragenin (IIa) under the conditions required for isomerization of 25L to 25D sapogenin, although the yield of nogiragenin (IIa) was poor. Furthermore, oxidative cleavage of the E- and F-rings of compound (XIIIb) afforded 3β,11α-diacetoxypregn-16-en-20-one (XIV), m.p. 183~185° in 63% yield.

From these results and the observation of the infrared spectrum, this compound (XIIIa) is a C-25 isomer of nogiragenin (IIa) and is named neonogiragenin.

Experimental*3

Isolation of Isonarthogenin (Va) and Narthogenin (VIa) from the Epigeous Part of the Plant——The dried and sliced epigeous part of the plant (1.9 kg.) was extracted with 90% hot MeOH giving a deep

*3 The NMR spectra were taken on deuterated chloroform solutions with a Varian A-60 NMR Spectrometer. All melting points were measured by use of Kofler block ("mono-scope" Hans Bock Co., Ltd., Frankfurt am Main, Germany) and uncorrected. Paper partition chromatography was carried out with the solvent system of toluene-acetic acid (50:3) by the ascending method.[11] Rotations were measured in $CHCl_3$ with Rudolph Photoelectric Polarimeter, Model 200.

11) T. Okanishi, A. Akahori, F. Yasuda : Ann. Rept. Shionogi Research Lab., 8, 927 (1958).

brown soiled syrup. The residue was dissolved in a solution of conc. HCl (700 ml.) in 50% EtOH (4 L.), refluxed for 6 hr. in a steam bath and poured into a great amount of H_2O. This mixture was filtered off and the insoluble sapogenins were extracted with MeOH giving a deep brown soiled syrup (200 g.). The syrup was refluxed with 4% KOH-EtOH (2 L.) for 1 hr. in a steam bath, poured into H_2O (2 L.) and extracted with $CHCl_3$ (2 L. × 3). The $CHCl_3$ extract (40.7 g.) was dissolved in Me_2CO to separate the Me_2CO-insoluble material (7.9 g.).. The Me_2CO solution was evaporated leaving a deep brown syrup (32 g.), which was dissolved in benzene (500 ml.) and chromatographed on Al_2O_3 (600 g.; see Table II).

Table II. Alumina Chromatogram of the $CHCl_3$ Extract

Fraction No.	Solvent	Rf value	Yield (g.)
1～3	benzene	0.98	2.43
4～7	〃	0.98	0.29
8～12	benzene-$CHCl_3$ (9:1)	0.98	0.41
13～20	〃 (7:3)	0.92, 0.85, 0.77	6.19
21～28	〃 (6:4)	0.92, 0.85, 0.77	3.29
29～35	〃 (1:1)	0.77	2.547
36～45	〃 (3:7)	0.70, 0.58	1.32
46～53	$CHCl_3$	0.70, 0.58	1.61
54	〃	0.58	1.89
55～56	$CHCl_3$-MeOH (9:1)	0.38	5.6
57	〃 (1:1)	0.38	2.5

Fractions 1～3 were crystallized from MeOH-$CHCl_3$ giving luvigenin (III, 260 mg.), m.p. 183～184°. Fractions 13～20 afforded β-sitosterol (920 mg.), m.p. 133～135° and meteogenin (IV, 20 mg.), m.p. 157～158°. Fractions 29～35 were crystallized from $CHCl_3$-light petroleum giving nogiragenin (IIa, 900 mg.), m.p. 201°. Fractions 55～57 were crystallized from MeOH-$CHCl_3$ giving metagenin (I, 2.0 g.), m.p. 272°. Fraction 54 was crystallized from MeOH giving the unknown sapogenin B (315 mg.), m.p. 220～226°, which showed two spots at Rf values of 0.60 and 0.58. This unknown sapogenin B was dissolved in benzene-$CHCl_3$ (4:1, 30 ml.) and rechromatographed on Al_2O_3 (10 g.) as shown in Table III.

Table III. Alumina Chromatogram of the Unknown Sapogenin B

Fraction No.	Solvent	Rf value	Yield (mg.)
1～5	benzene-$CHCl_3$ (4:1)	—	—
6～7	〃 (1:1)	—	—
8～10	〃 (1:1)	0.60, 0.58	26.0
11～14	$CHCl_3$	0.60, 0.58	215.0
15	$CHCl_3$-MeOH (9:1)	0.58	63.0

Fractions 11～15 were recrystallized from MeOH giving isonarthogenin (Va, 147 mg.) as a colorless prisms, m.p. 240～242°, $[\alpha]_D^{23}$ −110.2 ±4° (c=0.65), Rf value 0.58. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found : C, 75.25; H, 9.91. Diacetate (Vb), colorless plates, m.p. 160～162°, $[\alpha]_D^{23.5}$ −93.7 ±3° (c=0.778). *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found : C, 72.23; H, 8.98. Fractions 8～10 were rechromatographed on Al_2O_3 and recrystallized from MeOH giving narthogenin (VIa, 14 mg.) as a colorless prisms, m.p. 214～216°, $[\alpha]_D^{24}$ −112.3 ±4° (c=0.432), Rf value 0.60. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found : C, 75.04; H, 9.94. Diacetate (VIb), colorless plates, m.p. 144～146°, $[\alpha]_D^{25.5}$ −97.6 ±5° (c=0.242). *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found : C, 72.01; H, 9.17.

3β-Acetoxypregna-5,16-dien-20-one (VII) from Isonarthogenin diacetate (Vb)——A mixture of Vb (100 mg.) and pyridine hydrochloride (100 mg.) in Ac_2O (1 ml.) was refluxed in an oil bath for 3 hr. The mixture was cooled to 20°, diluted with AcOH (0.1 ml.) and H_2O (0.2 ml.) and stirred while adding chromic anhydride (60 mg.) in 90% AcOH (0.6 ml.) at room temperature. After stirring for 3 hr., to this solution were added 37% formaldehyde (0.1 ml.) and then AcONa (100 mg.). The mixture was heated in a steam bath for 1 hr., poured into ice water and extracted with Et_2O. The Et_2O extract was washed with 2*N* Na_2CO_3, dried over anhyd. Na_2SO_4 and evaporated. The residue was chromatographed on Al_2O_3 giving VII (53 mg.). VII was recrystallized from MeOH to give colorless plates, m.p. 169～172°, which was identical with the authentic sample by mixed melting point and IR comparison.

Tosylation of Isonarthogenin (Va)——A solution of tosyl chloride (100 mg., 1.5 mole.) and Va (180 mg.) in pyridine (2 ml.) was left for 26 hr. at room temperature, and the crude tosylate (VIII, 233 mg.) was obtained. The crude tosylate was chromatographed on Al_2O_3 and recrystallized from MeOH giving the starting material (Va, 92 mg.) and isonarthogenin monotosylate (VIII, 105 mg.), colorless prisms, m.p. 200～202°, $[\alpha]_D^{23.5}$ −86.3° ±2° (c=0.812). *Anal.* Calcd. for $C_{34}H_{48}O_6S$: C, 69.83; H, 8.27; S, 5.48. Found: C, 70.27; H, 8.38; S, 5.68.

Conversion of Isonarthogenin (Va) into Diosgenin (IX)——A solution of Ⅷ (17 mg.) in dry tetrahydrofuran (2 ml.) was added to a suspension of $LiAlH_4$ (30 mg.) in dry tetrahydrofuran (1 ml.), refluxed with stirring for 2 hr., decomposed by addition of H_2O and $2N$ H_2SO_4, extracted with $CHCl_3$, washed with $2N$ Na_2CO_3, dried over Na_2SO_4 and evaporated leaving a crystalline substance (12 mg.). The residue was recrystallized from MeOH giving colorless prisms (10 mg.), m.p. 204～207°, which was identical with diosgenin (Ⅸ) by mixed melting point and IR comparison.

X from Isonarthogenin (Va)——i) A solution of Ⅷ (60 mg.) and NaI (50 mg.) in Me_2CO (2 ml.) was refluxed for 8 hr., diluted with H_2O (10 ml.) and extracted with Et_2O. The Et_2O extract was washed with 1% $Na_2S_2O_3$, dried over anhyd. Na_2SO_4 and evaporated. The residue was dissolved in 25% NaOH-MeOH (4 ml.), refluxed for 3 hr., diluted with H_2O (20 ml.) and extracted with benzene. The benzene extract was washed with H_2O, dried over Na_2SO_4 and evaporated leaving a syrup (48 mg.). The residue was chromatographed on Al_2O_3 giving crude X (37 mg.), m.p. 175～183°. Recrystallization from MeOH gave X (22 mg.) as colorless plates, m.p. 183～185°, $[\alpha]_D^{23.5}$ $-120.8\pm4°$ ($c=0.475$), IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3450, 1658, 878, NMR : 5.23 τ. *Anal.* Calcd. for $C_{27}H_{40}O_3$: C, 78.59; H, 9.77. Found : C, 78.20; H, 9.78. Acetate, colorless plates, m.p. 192～194°, $[\alpha]_D^{23.5}$ $-112.3\pm4°$ ($c=0.422$). *Anal.* Calcd. for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found : C, 76.40; H, 9.39.

ii) A solution of Ⅷ (30 mg.) in γ-collidine (2 ml.) was refluxed for 6 hr., poured onto ice water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with $2N$ H_2SO_4, H_2O and $2N$ Na_2CO_3, dried over anhyd. Na_2SO_4 and evaporated. The residue (13 mg.) was chromatographed on Al_2O_3 giving X (5.5 mg.), m.p. 178～181°.

Isomerization of Narthogenin (VIa) to Isonarthogenin (Va)——A solution of narthogenin (VIa, 5 mg.) in 4% HCl-MeOH (3 ml.) was allowed to stand for 2 hr. at room temperature, neutralized with $2N$ Na_2CO_3, extracted with $CHCl_3$, washed with H_2O, dried over anhyd. Na_2SO_4 and evaporated leaving a crystalline substance (5 mg.). The residue was recrystallized from MeOH giving isonarthogenin (Va), colorless prisms, m.p. 238～240°,

Isolation of Neonogiragenin (XIIIa)——The saponified MeOH extract (51 g.) of the rhizome of the plant (1.5 kg.) was chromatographed on Al_2O_3 (550 g.). Elution with benzene-$CHCl_3$ (4:1, 3:1 and 1:1) afforded a syrup (15.0 g.) having Rf value of 0.77. This syrup was crystallized from $CHCl_3$-light petroleum giving colorless needles (1.4 g.), m.p. 110～118°. The crystalline substance was dissolved in Ac_2O (9 ml.), refluxed in an oil bath for 30 min. and allowed to stand at room temperature. After a while, the separation of colorless crystals occured. The crystalline substance was collected, washed with Me_2CO and H_2O, and recrystallized from MeOH giving nogiragenin diacetate (Ⅱb, 740 mg.) as a colorless needles, m.p. 206～207°. The mother liquid of the separation of Ⅱb and the Me_2CO washings were lumped, evaporated into about one-tenth *in vacuo* and allowed to stand at room temperature to give a mixture (220 mg.) of Ⅱb and neonogiragenin diacetate (XIIIb), colorless needles, m.p. 140～151°. The mother liquid was diluted with H_2O, and the precipitated crystals (480 mg.), m.p. 125～127° were collected. Recrystallization from MeOH afforded neonogiragenin diacetate (XIIIb, 260 mg.) colorless plates m.p. 142～144°, $[\alpha]_D^{26}$ $-77.7\pm4°$ ($c=0.539$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found : C, 72.02; H, 9.45. The acetate (XIIIb, 60 mg.) was saponified with 3% KOH-MeOH giving a crystalline substance (48 mg.), which was recrystallized from Me_2CO-light petroleum to give neonogiragenin (XIIIa, 43 mg.) as colorless needles, m.p. 128～131°, $[\alpha]_D^{22}$ $-75.0\pm4°$ ($c=0.412$), Rf value 0.77, IR ν_{max}^{Nujol} cm^{-1} : 987, 917, 895, 848 (F-ring : 917>895). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found : C, 74.82; H, 10.23.

Isomerization of Neonogiragenin (XIIIa) to Nogiragenin (IIa)——A solution of XIIIa (45 mg.) in 10% HCl-EtOH (20 ml.) was refluxed for 92 hr. and diluted with H_2O (10 ml.). The mixture was evaporated *in vacuo*, extracted with Et_2O, washed with $2N$ Na_2CO_3, dried over Na_2SO_4 and evaporated leaving a syrup (44 mg.). The residue was chromatographed on Al_2O_3 and recrystallized from $CHCl_3$-light petroleum giving colorless needles (4 mg.), m.p. 193～196°, which was identical with nogiragenin (Ⅱa) by comparisons of IR spectra and Rf values.

3β,11α-Diacetoxypregn-16-en-20-one (XIV) from Neonogiragenin diacetate (XIIIb)——Under the same conditions as oxidative cleavage of Vb, XIIIb (90 mg.) was oxidized with chromic anhydride to give XIV (40 mg., 63% yield), colorless prisms, m.p. 182～185°, which was identical with the authentic sample by mixed melting point and IR comparison.

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Summary

In order to find a proto-sapogenin of luvigenin (Ⅲ), the structural investigation of the unknown sapogenins from *Metanarthecium luteo-viride* Maxim was attempted. Isonarthogenin (Va), narthogenin (VIa) and neonogiragenin (XIIIa) were isolated, and the former two sapogenins were established to be steroidal sapogenins having a hydroxyl group at C-27.

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