Chem. Pharm. Bull. 29(5)1299—1311(1981)

Saponin and Sapogenol. XXX.[1] Furostanol Glycosides from *Metanarthecium luteo-viride* Maxim.: Bisdesmosides of Furometagenin and Furometanarthogenin

Isao Kitagawa* and Tsutomu Nakanishi

Faculty of Pharmaceutical Sciences, Osaka University, 133-1, Yamada-kami, Suita, Osaka, 565, Japan

(Received November 8, 1980)

Bisdesmosides of two new furostanols (named furometagenin and furometanarthogenin) were isolated as their 22-O-methylated peracetates (designated as NF-1 and NF-2) from the subterranean part of *Metanarthecium luteo-viride* Maxim. (Liliaceae). The structures of NF-1 and NF-2 have been elucidated as 11-O-α-L-arabinopyranosyl-26-O-β-D-glucopyranosyl-22-O-methyl-furometagenin nonaacetate (**12**) and 11-O-α-L-arabinopyranosyl-26-O-β-D-glucopyranosyl-22-O-methyl-furometanarthogenin octaacetate (**15**) on the basis of chemical and physicochemical evidence. The 2β-acetoxy-4-en-3-one moiety included in the steroidal part of **15** has been demonstrated to undergo air oxidation during alkaline saponification followed by acidic hydrolysis in methanol and to give a new sapogenol: 2-methoxy-11α-hydroxy-25*R*-spirosta-1,4-dien-3-one (2-O-methyl-dehydrometanarthogenin) (**23**). This secondary conversion in the A-ring of sapogenol part has been corroborated by the examination of the model steroids: 2β- and 2α-acetoxy-17β-chloroacetyl-androst-4-en-3-ones (**25**, **28**).

Keywords——*Metanarthecium luteo-viride*; furostanol bisdesmoside; arabinosyl-glucosyl-furometagenin; arabinosyl-glucosyl-furometanarthogenin; furometagenin; furometanarthogenin; 2β-acetoxy-4-en-3-one steroid; air-oxidation; 2-O-methyl-dehydrometanarthogenin; CD

Since 1961, nine sapogenols, *i.e.*, metagenin (**1**), 3-epi-metagenin (**2**), nogiragenin (**3**), neonogiragenin, narthogenin (**4**), isonarthogenin, meteogenin (**5**), neometeogenin, and luvigenin (**6**), have been characterized from the hydrolysate obtained by acidic hydrolysis and subsequent alkaline treatment of the whole glycosidic fraction isolated from *Metanarthecium luteo-viride* Maxim. (nogiran in Japanese, Liliaceae).[2,3] Afterwards, by using the soil bacterial hydrolysis method,[4] we identified three prosapogenols, 11-O-α-L-arabinopyranosyl-3-epi-metagenin (**7**),[2f] 11-O-β-D-galactopyranosyl-nogiragenin (**9**),[5] and 11-O-α-L-arabinopyranosyl-protometeogenin (**10**),[6] as their peracetates. Among those prosapogenols, **10** was characteristic since it was an arabinoside of the genuine sapogenol of A-ring-aromatized meteogenin (**5**) and readily liberated **5** on acidic hydrolysis. All sapogenols and prosapogenols described above are spirostane-type derivatives.

In order to shed light on the glycosidic constituents of the plant, we then attempted to isolated the parent glycosides of those sapogenols and prosapogenols. In 1976, we isolated four spirostane-type glycosides from the less polar glycosidic portion of the epigeous part of the plant and elucidated their structures as **7**, 11-O-α-L-arabinopyranosyl-metagenin (**8**), and their monoacetyl derivatives.[7] However, we had been unable to isolate any other glycoside in pure form, although the glycosidic fraction was found to contain a large quantity of furostane-type glycosides as shown by thin-layer chromatographic (TLC) examination using the Ehrlich reagent for detection.[8]

As a continuation of these studies, we attempted to isolate the furostane-type glycosides from the subterranean part of the plant, and we have been able to isolate two major bisdesmosides. This paper deals with the isolation of these glycosides as their peracetates and with the structure elucidation of bisdesmosides of furometagenin[9] and furometanarthogenin.[10]

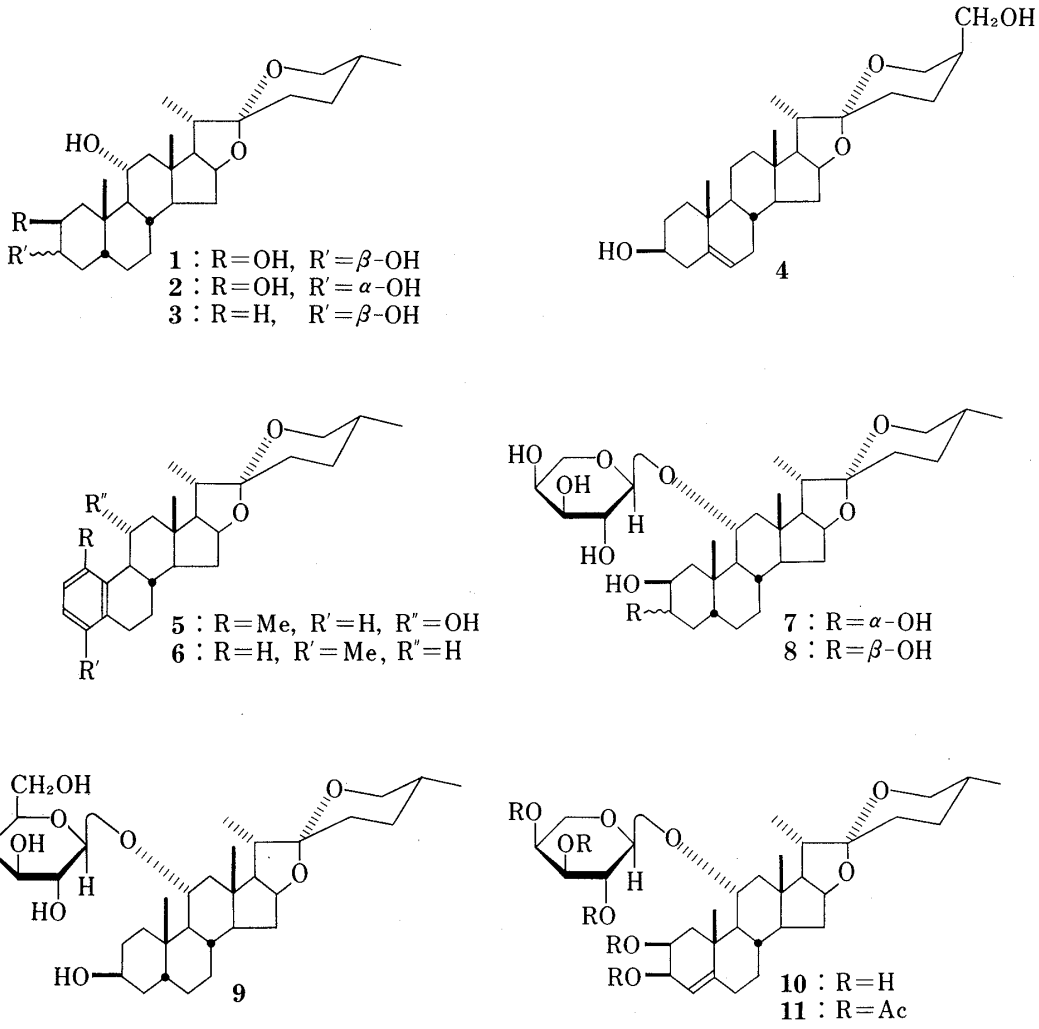


Chart 1

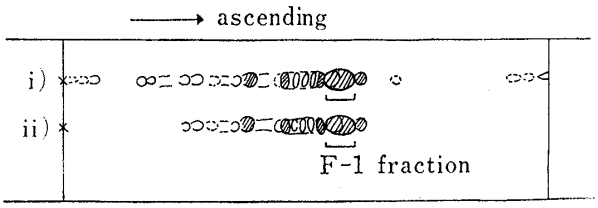


Fig. 1. TLC Diagrams of the Glycoside Mixture (=R–BE)

Solvent: chloroform–methanol–water (7: 3: 1, lower phase).
Detection: i) 1% ceric sulfate in 10% sulfuric acid.
ii) Ehrlich reagent.
Adsorbent: Kieselgel 60 F-254.

The whole glycoside mixture (R–BE), after being refluxed in dry methanol,[11] gave TLC diagrams (Fig. 1) which suggested that the mixture contains many furostane-type glycosides. The major part (F-1 fraction) of the mixture was separated by column chromatography and in order to facilitate isolation, the fraction was acetylated and refluxed in dry methanol again. After repeating the chromatographic separation (using a medium pressure column), we isolated two furostane-type bisdesmosides (positive to the Ehrlich reagent) in the fully acetylated form (designated NF-1 and NF-2).

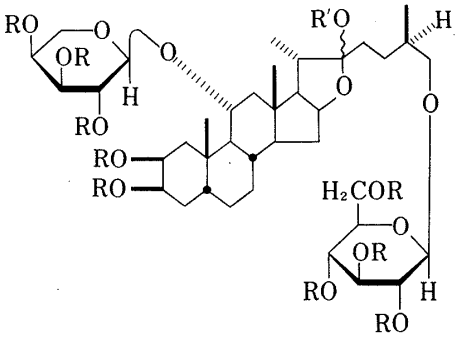
Furometagenin Bisdesmoside

The infrared (IR) spectrum of NF-1 (**12**) shows absorption bands due to the acetoxyl group but lacks a hydroxyl absorption band. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of NF-1, signals due to two *tert.* methyls, two *sec.* methyls, and nine acetoxyls together with a three-proton singlet at δ 3.10, ascribable to the methoxyl group at

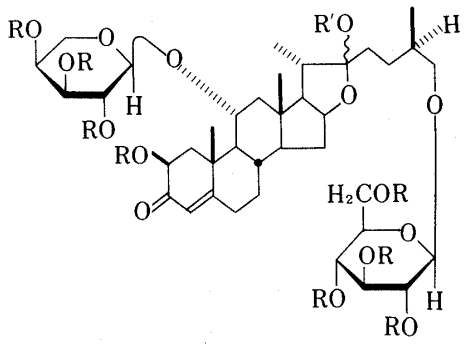
C-22,[12] are observed. The electron impact mass spectrum (MS) of NF-1 gives an ion peak at *m/z* 1121 (M^+-CH_3OH) as the highest ion, and this behavior is in good accord with that observed in the MS of 22-O-methyl-furostanol derivatives.[12] Based on these physicochemical results along with the molecular composition, $C_{57}H_{84}O_{24}$, NF-1 was presumed to be the nonaacetate of a furostane-type diglycoside.

When NF-1 was refluxed in aqueous acetone, it was converted to the furostanol glycoside nonaacetate (**14**) (as judged by IR and ^{1}H-NMR spectroscopy), which was reconverted to NF-1 by heating under reflux in dry methanol. These conversions[12] were both effected quantitatively. In the ^{1}H-NMR spectrum of **14**, two anomeric proton signals are observed at δ 4.29 (d, J=8 Hz) and 4.42 (d, J=7 Hz) supporting the view that **14** is a diglycoside.

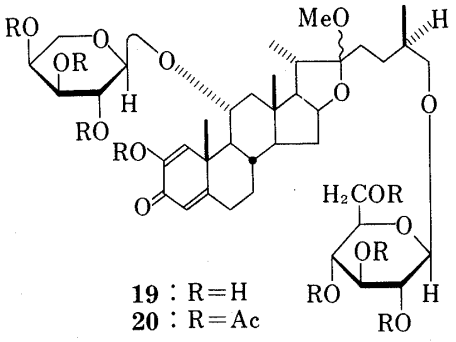
Treatment of NF-1 with sodium methoxide in dry methanol gave the desacetyl derivative (**13**), which lacks an acetoxyl absorption band but shows strong absorption bands due to hydroxyl groups in its IR spectrum. Enzymic hydrolysis of **13** with almond emulsin furnished a prosapogenol and glucose.[13] The prosapogenol thus obtained was found to be identical with 11-O-α-L-arabinopyranosyl-metagenin (**8**) (mixed mp determination, IR, and TLC). Furthermore, acidic hydrolysis of **13** with hydrochloric acid in methanol furnished metagenin (**1**),



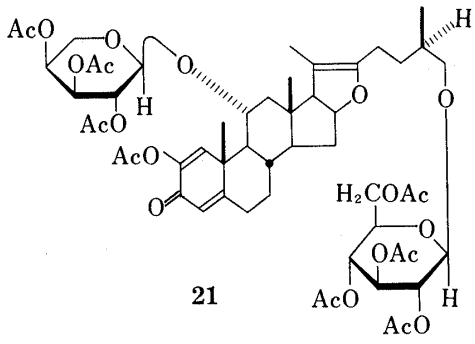
12 : R=Ac, R′=Me (NF-1)
13 : R=H, R′=Me
14 : R=Ac, R′=H



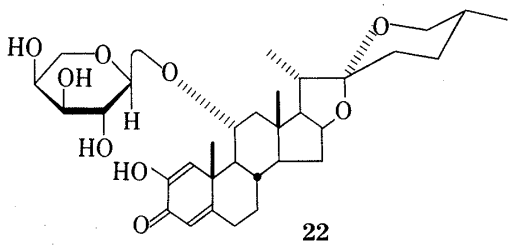
15 : R=Ac, R′=Me (NF-2)
16 : R=Ac, R′=H
17 : R=R′=H
18 : R=H, R′=Me



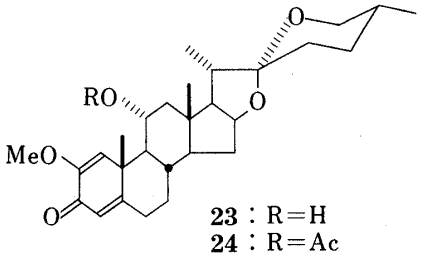
19 : R=H
20 : R=Ac



21



22



23 : R=H
24 : R=Ac

Chart 2

glucose, and arabinose. It was thus clear that NF-1 is the furostane-type counterpart of **8** with a glucosidic linkage at the C-26 hydroxyl group.[14] Since the anomeric proton signals in the ^{1}H-NMR spectrum of **14** are observed as two doublets of large J values (*vide supra*), the presence of a β-D-glucopyranoside linkage ($^4C_1$) in **14** is indicated.

Based on the above evidence, the structure of the desacetyl derivative (**13**) has been assigned as 11α-(α-L-arabinopyranosyl)-26-β-D-glucopyranosyl-22ξ-methoxy-5β,25*R*-furostan-2β,3β,11α,26-tetraol, and the structures **12** and **14** become reasonable. Although its isolation has not yet been effected, the furostanol analog of metagenin (**1**) is now named furometagenin, so the structure of NF-1 may be expressed as 11-O-α-L-arabinopyranosyl-26-O-β-D-glucopyranosyl-22-O-methyl-furometagenin nonaacetate (**12**).

Furometanarthogenin Bisdesmoside

NF-2 (**15**) is a fully acetylated octaacetate, since the IR spectrum lacks a hydroxyl absorption band. The IR and ultraviolet (UV) spectra of NF-2 suggest the presence of a six-membered α,β-unsaturated ketone moiety, while the ^{1}H-NMR spectrum shows signals assignable to two *tert.* methyls, two *sec.* methyls, eight acetoxyl groups, one methoxyl group, one olefinic proton (s, δ 5.73), and one methine proton (dd, δ 5.32)[15] which is geminal to an acetoxyl group (Tables I and II).

Table I. IR, UV, and CD Data for **15**, **25**, and **28**

Compd.	IR ν_{max}^{Nujol} cm^{-1}	UV λ_{max}^{EtOH} nm (ε)	CD (dioxane): $[\theta]_{max}$ (nm)
15	1697, 1618	240.5(13800)	+4000(324)
25	1690, 1618	242.5(15900)	+3200(322)
28	1683, 1613	240.0(16100)	−7000(324)

Table II. ^{1}H-NMR Data for **15**, **16**, and **25** (90 MHz, $CDCl_3$)

Compd.	13-Me	10-Me	2α-H	4-H	Others
15	0.84 (s)	1.30 (s)	5.32[15] (dd, J=4, 13)	5.73 (s)	3.14 (3H, s, 22-OMe) OAc×8
16[a]	0.79 (s)	1.13 (s)	5.53[15] (dd, J=4, 13)	5.77 (s)	4.31 (1H, d, J=8) (anom. H of glu.) 4.45 (1H, d, J=7) (anom. H of ara.)
25	0.84 (s)	1.20 (s)	5.26 (dd, J=5.5, 12)	5.74 (s)	

a) Measured in d_6-benzene.

Based on these spectral properties and bearing in mind the structures of hitherto isolated sapogenols (*e.g.* **1**—**6**), we assumed NF-2 to be an octaacetate of a 22-O-methyl-furostanol bisdesmoside having the 4-en-3-one chromophore. Since the circular dichroism (CD) spectrum of NF-2 shows a positive maximum at 324 nm ascribable to the n-π^* transition of the α,β-unsaturated ketone moiety, the presence of the 2β-acetoxyl group seems likely.[16] In order to substantiate this, we prepared several model compounds (**25**,[17,18] **26**,[17,18] **28**[18]) from testosterone, and examined in parallel studies their physicochemical properties (see "Experimental") and chemical behavior. As shown in Tables I and II, the IR, UV, CD, and ^{1}H-NMR data arising from the 2β-acetoxy-4-en-3-one moiety of **25** are in good accord with those for NF-2 (**15**), while the CD spectrum of **28** shows a negative maximum at 324 nm as expected. The paramagnetically shifted signal of 10-CH_3 in NF-2 as compared to that in **25** is presumably ascribable to the presence of the 11α-oxygen function in NF-2, as will be mentioned later.

When NF-2 was treated with aqueous acetone under reflux, the 22-hydroxy analog (**16**) was quantitatively formed. Reconversion from **16** to the parent 22-O-methyl derivative (NF-2)

was also smoothly effected by heating under reflux in dry methanol.[12] The ^{1}H-NMR spectrum of **16** shows two anomeric proton signals at δ 4.31 (1H, d, J=8 Hz) and δ 4.45 (1H, d, J=7 Hz) suggesting the bisdesmoside structure, but it lacks the signal due to the methoxyl group. The 2β-acetoxy-4-en-3-one moiety in NF-2 is retained in **16** (Table II).

The ^{1}H-NMR signal due to 2α-H in **25** is observed at δ 5.26 as a doublet of doublets (J= 5.5 and 12 Hz). However, the corresponding signals of **15** and **16** are unclear due to partial overlap with other signals. Therefore, the spectrum of **16** was examined by using a shift reagent (see "Experimental") and the signal patterns (dd, J=4 and 13 Hz) for **15** and **16** were found to be similar to that of **25** (Table II).

In order to prepare the desacetyl derivative, NF-2 was treated with sodium methoxide in dry methanol at room temperature, but the product was not the desired one (**18**). The IR spectrum of the product (**19**) lacks an acetoxyl absorption band, but shows strong absorption bands due to the hydroxyl groups. It shows absorption at 1636 cm^{-1} ascribable to a chelated conjugated carbonyl chromophore. The UV and ^{1}H-NMR spectra of the product suggest the formation of a 2-hydroxy-1,4-dien-3-one moiety, and this view is supported by the wine-red coloration with the ferric chloride reagent (Table III). The CD spectrum of the product also supports the presence of the dienone moiety, showing a positive maximum due to the n-π^* transition.[19]

Similar conversion from the steroidal 2β-acetoxy-4-en-3-one moiety to the 2-hydroxy-1,4-dien-3-one moiety under alkaline conditions has already been reported by Clark (**27**→**29**),[20] and we reexamined the conversion using modified procedures (**25**→**29**, **28**→**29**). The cross-dienone derivative (**29**)[20] thus formed was colored wine-red with the ferric chloride reagent and gives spectral data similar to those for **19** except for the chemical shift of a one-proton singlet due to 1-H (Table III). Although the solvents used while taking the ^{1}H-NMR spectra of **19** and **29** were different, 1-H in **19** is observed at a downfield position due to the anisotropic effect of the 11α-oxygen function.[2f,7] Similar downfield shifts are also observed for the 1-H signals of **20** and **21** (in deuterochloroform), as will be described later (Table V). For comparison, 2β-hydroxytestosterone (**26**), which has the same A-ring structure as **18**, was prepared by the reported method.[18] As shown in Table III, the spectral properties of **26** are significantly different from those of **19** and **29**, including the negative coloration of **26** with the ferric chloride reagent. Based on these results, the presence of the 2-hydroxy-1,4-dien-3-one moiety in the product (**19**) can be rationalized and its formation may be explained in terms of deacetylation (giving **18**) and subsequent air-oxidation under alkaline conditions (Chart 3).

Enzymic hydrolysis of **19** with almond emulsin furnished glucose[13] and a monoglycoside (**22**) which retains the same A-ring moiety as in **19**, as shown by the spectral data and the color

Table III. IR, UV, and ^{1}H-NMR Data and $FeCl_3$ Test for **19**, **29**, and **26**

Compd.	IR ν_{max}^{KBr} cm^{-1}	UV λ_{max}^{EtOH} nm (ε)	^{1}H-NMR (δ) 4-H	^{1}H-NMR (δ) 1-H	$FeCl_3$ Test
19	1636	249(11100), 293(2600)[a]	6.27(s)[b]	7.77(s)[b]	+(wine-red)
29	1639	254(13000), 290(3000)[a]	6.12(s)[c]	6.27(s)[c]	+(wine-red)
26	1680, 1613	243.5(14900)	5.78(s)[c]		−

a) Shoulder. *b*) Measured in d_5-pyridine-D_2O. *c*) Measured in $CDCl_3$.

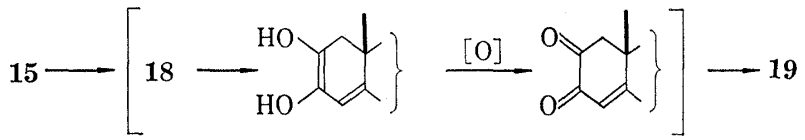


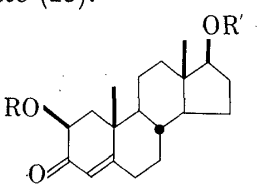
Chart 3

reaction. The IR spectrum of **22** shows absorption bands attributable to the 25R-spirostane moiety,[21] the presence of which is further supported by the base peak of *m/z* 139 (**i**) observed in the MS of **22**. The MS also gives the fragment ion peaks of *m/z* 149 and 133 (**iii**) which arise from the pentoaldopyranose moiety.[2f,6,22] The prominent fragment ion peak of *m/z* 424 (**iv**) presumably originates from the spirostane-type aglycone of **22** (Chart 4). It is thus likely that the structural relationship between **19** and **22** is similar to that between **13** ane **8** (*vide infra*).

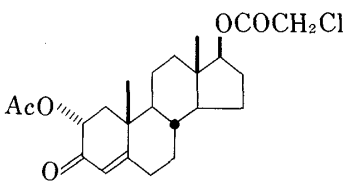
Treatment of **22** with sodium borohydride in dry isopropanol furnished three isomeric products, which were isolated after acetylation. The most polar acetate (one of the major products) has been found to be identical with 11-O-α-L-arabinopyranosyl-protometeogenin pentaacetate (**11**)[6] (IR, MS, and TLC), thus substantiating the structure **22**.

Based on the above-described evidence, the structures of NF-2 (**15**), the 22-hydroxyl analog (**16**), and the alkali-treated product (**19**) have been clarified.

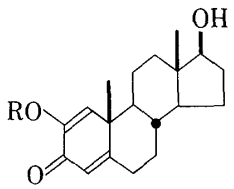
Since the furostane-type aglycone of **17** has not been reported from the title plant, we propose the trivial name furometanarthogenin for it, and therefore, NF-2 may be expressed as 11-O-α-L-arabinopyranosyl-26-O-β-D-glucopyranosyl-22-O-methyl-furometanarthogenin octaacetate (**15**).



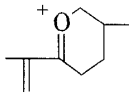
25 : R=Ac, R′ =$COCH_2Cl$
26 : R=R′ =H
27 : R=R′ =Ac



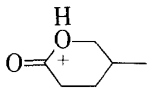
28



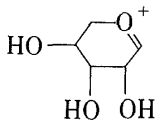
29 : R=H
30 : R=Me



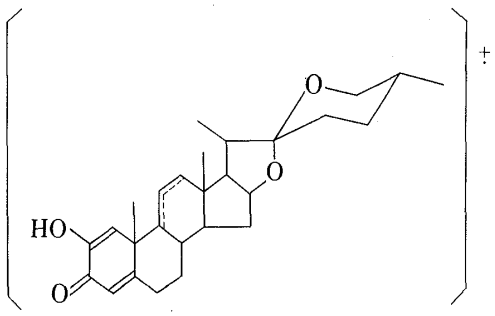
i



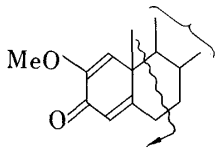
ii



iii



iv



fragment a

v : fragment a + 2H
vi : fragment a + H

Chart 4

Next, we examined the chemical responce of **19** to acidic hydrolysis and acetylation. When **19** was heated under reflux with hydrochloric acid in methanol, glucose, arabinose, and an aglycone (**23**) were obtained. The same aglycone was also liberated from **22** together with arabinose by similar acidic treatment. Acetylation of **23** furnished the monoacetate (**24**).

The spectral data for **23** and **24** (Table IV), as compared with those for **19**, **29** (Table III) and **20** (Table V), suggest the presence of the 2-methoxy-1,4-dien-3-one moiety in **23** and **24**. The MS of both compounds give two prominent ion peaks at *m/z* 152 (**v**) and 151 (**vi**),[23] together

with **i** and **ii**.[2f,6,22] The model compound (**30**), which was prepared from **29** through a procedure similar to that used to go from **19** to **23**, shows spectral properties closely similar to those of **23** except that the chemical shift of 1-H in **23** is shifted downfield by the anisotropic effect of the 11α-hydroxyl group (*cf.*, 1-H in **24** at δ 5.96) (Table IV).

Table IV. IR, UV, ^{1}H-NMR, and MS Data for **23**, **24**, and **30**

Compd.	IR $\nu_{max}^{CHCl_3}$ cm^{-1}	UV λ_{max}^{EtOH} nm (ε)	^{1}H-NMR ($CDCl_3$, δ)[c]	MS[d]: *m/z* (%)
23	1662, 1634 1611	252(9300) 290(2300)[a]	0.86 (13-Me), 1.33 (10-Me) 3.63 (2-OMe), 6.05 (4-H), 6.81 (1-H)	152(37), 151(83)
24	1667, 1638 1613	252(13000)[b] 290(2750)[a,b]	0.91 (13-Me), 1.31 (10-Me) 3.62 (2-OMe) 6.09 (4-H), 5.96 (1-H)	152(17), 151(88)
30	1660, 1635 1609	253(14200) 290(2700)[a]	0.81 (13-Me), 1.24 (10-Me) 3.64 (2-OMe) 6.04 (4-H), 5.91 (1-H)	152(72), 151(100)

a) Shoulder. *b*) Measured in methanol. *c*) All resonances listed here were singlets.
d) The molecular compositions $C_9H_{12}O_2$ and $C_9H_{11}O_2$ for *m/z* 152 and 151 were determined by high resolution MS.

The presence of the 11α-hydroxyl group in steroids having the 1,4-dien-3-one moiety has been reported to reverse the sign of the CD maximum due to the n-π^* transition of the chromophore. Thus, the steroidal 1,4-dien-3-ones show negative curves, while the corresponding 11α-hydroxyl (or acetoxyl) derivatives show positive ones.[19,23] In our examples, **23** gives a positive CD curve, but **30** gives a negative curve. The ^{1}H-NMR spectrum of **24** shows a doublet of triplets at δ 5.27 (J=5, 11 Hz) which is assignable to 11β-H geminal to the acetoxyl group (11β-H at δ *ca.* 4.0 for **23**).

Therefore, the structure of the aglycone has been demonstrated as **23** (now named 2-O-methyl-dehydrometanarthogenin), in which the 2-O-methyl group has been formed from the enolic hydroxyl group in **22** during the acidic hydrolysis in methanol.

Acetylation of **19** furnished two octaacetates. The major one is the ordinary product (**20**), which shows the characteristic IR absorption bands of the 2-acetoxy-1,4-dien-3-one moiety,[24] together with the other expected spectral data (Table V). The minor octaacetate presumably has the 20(22)-ene structure (**21**)[25] based on its spectral properties (Table V). The significant ^{1}H-NMR data for **21** are the presence of a three-proton singlet at δ 1.61 ascribable to 20-CH_3[12a] and the absence of the 22-O-methyl signal which is observed in **20**. The acetate (**21**) was recovered unchanged when it was treated with either dry methanol or aqueous acetone under reflux.

Table V. IR, UV, ^{1}H-NMR, and CD Data for **20** and **21**

Compd.	IR ν_{max}^{Nujol} cm^{-1}	UV λ_{max}^{EtOH} nm (ε)	^{1}H-NMR ($CDCl_3$, δ)[a]	CD (n-π^*)
20	1757(br.), 1222 (OAc) 1674, 1648, 1614 (dienone)	246(12500)	0.84 (s, 13-Me), 1.39 (s,10-Me) 6.06 (s, 4-H), 7.57 (s, 1-H) 3.11 (s, 22-OMe); OAc×8	Positive maximum
21	1757(br.), 1221 (OAc) 1675, 1648, 1613 (dienone)	246.5(11600)	0.74 (s, 13-Me), 1.38(s, 10-Me) 6.07 (s, 4-H), 7.59 (s, 1-H) 1.61 (s, 20-Me); OAc×8	Positive maximum

a) Chemical shifts for two *sec.* methyls of **20** and **21** were unclear.

As described above, we have identified two furostanol bisdesmosides from the subterranean part of the plant. However, since the glycosidic constitutents of the plant are a complex mixture of variously acetylated glycosides of many sapogenols,[6] the isolation of those bisdesmosides has been effected only as their peracetates [NF-1 (**12**), NF-2 (**15**)].[26]

Of those two furostanol bisdesmosides, the A-ring structure in the aglycone of NF-2 (**15**) is unprecedented among hitherto known sapogenols (*e.g.*, **1**—**6**) and prosapogenols (**7**—**10**).

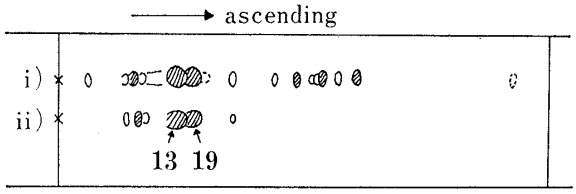


Fig. 2. TLC Diagrams of the Glycoside Mixture (R-BE) after Alkaline Treatment

Solvent: chloroform–methanol–water (7: 3: 1, lower phase).
Detection: i) 1% ceric sulfate in 10% sulfuric acid.
ii) Ehrlich reagent.
Adsorbent: Kieselgel 60 F-254.

In order to determine the content of these glycosides in the plant, we next examined the composition of the glycoside mixture after alkaline hydrolysis. As shown in Fig. 2, two major Ehrlich-positive spots on TLC coincided with the spots of **13** and **19**, the latter presumably being formed from the partially acetylated derivative (present in R-BE) of **18** during the alkaline treatment. This finding also suggests that partially acetylated bisdesmosides of furometagenin and furometanarthogenin may be the major glycosidic constituents of the subterranean part of the plant.

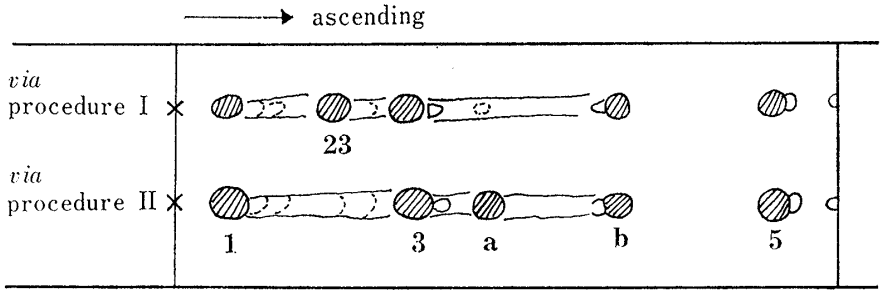


Fig. 3. TLC Diagrams of the Sapogenol Mixtures obtained from the Glycoside Mixture (R-BE) by Procedures I and II.

Solvent: benzene–acetone (4: 1).
Detection: 1% ceric sulfate in 10% sulfuric acid.
Adsorbent: Kieselgel 60 F-254.
a, **b**: unidentified sapogenols.

Finally, we examined the composition of the sapogenols which were obtained from the glycoside mixture (R–BE) by two different methods: i) methanolic sodium methoxide treatment followed by methanolic hydrochloric acid hydrolysis (procedure I), and ii) methanolic hydrochloric acid hydrolysis followed by potassium hydroxide saponification in aqueous methanol (procedure II).[27] As shown in Fig. 3, three major sapogenols [metagenin (**1**), nogiragenin (**3**), and meteogenin (**5**), previously isolated in 0.143%, 0.049%, and 0.056% yields, respectively[2c]] together with two unknown sapogenols were detected as distinct spots on TLC of the sapogenol mixture prepared by procedure II. On the other hand, TLC of the sapogenol mixture from procedure I showed major spots of a new sapogenol in addition to **1**, **3**, and **5**. The sapogenol, which was isolated in 0.165% yield from the plant, was found to be identical with 2-O-methyl-dehydrometanarthogenin (**23**), a sapogenol secondarily produced from NF-2 (**15**) *via* alkaline treatment and subsequent acidic hydrolysis in methanol.

Characterization of other sapogenols (*e.g.* those giving spots **a** and **b** in Fig. 3) requires further investigation.

Experimental[28]

Isolation of NF-1 (12) and NF-2 (15)——The air-dried and powdered subterranean part of the title plant (5.7 kg) was immersed in methanol (40 l) at room temperature for 19 days. The syrupy extract (875 g) obtained by removal of the solvent under reduced pressure was partitioned into *n*-BuOH–AcOEt (15: 2)/H_2O. The organic phase was separated and concentrated under reduced pressure to give the glycoside mixture (R-BE, 590 g). R-BE (140 g) was heated in dry MeOH (400 ml) under reflux for 4 hr, then the solvent was

removed under reduced pressure. The residue was subjected to column chromatography (SiO_2 3.35 kg, $CHCl_3$–MeOH=30: 1→20: 1→19: 1→9: 1→3: 1). Removal of the solvent from the eluate ($CHCl_3$–MeOH=19: 1) gave F-1 fraction (8.6 g). The fraction (2 g) was acetylated with Ac_2O (9 ml) and pyridine (12 ml) at room temperature for one day and the reaction mixture was treated again with Ac_2O (6 ml) and pyridine (8 ml) for a further one day. The reaction mixture was poured into ice-aq. $NaHCO_3$ and worked up in the usual manner. The product was then treated with dry MeOH under reflux for 12 hr, and 1.2 g of the peracetate mixture (2.4 g) obtained by removal of the solvent under reduced pressure was subjected twice to column chromatography under medium pressure (SiO_2 85 g, $CHCl_3$→$CHCl_3$–AcOEt=3: 1, 3 kg/cm^2) to afford NF-1 (**12**, 389 mg) and NF-2 (**15**, 302 mg).

NF-1 (**12**), mp 105—107° (colorless fine crystals from MeOH), $[\alpha]_D^{25}$ −21° (c=0.5, MeOH). *Anal.* Calcd for $C_{57}H_{84}O_{24}$: C, 59.36; H, 7.34. Found: C, 59.32; H, 7.23. IR ν_{max}^{Nujol} cm^{-1}: 1752, 1222 (OAc), 1045 (C–O–C). ^{1}H–NMR ($CDCl_3$, δ): 0.76 (3H, s, 13-Me), 0.87 (3H, d, J=6 Hz), 0.99 (3H, d, J=6 Hz), 1.12 (3H, s, 10-Me) (Me×4), 1.97 (9H, s), 2.00 (12H, s), 2.06 (3H, s), 2.11 (3H, s) (OAc×9), 3.10 (3H, s, 22-OMe), 3.8—4.5 (6H, unresolved m), 4.7—5.2 (7H, m, >CHOAc×7), 5.40 (1H, m, 3-H). MS: m/z 1121 (M^+−MeOH).

NF-2 (**15**), mp 110—111.5° (colorless plates from MeOH), $[\alpha]_D^{21}$ −45.2° (c=0.42, MeOH). *Anal.* Calcd for $C_{55}H_{78}O_{23}$: C, 59.66; H, 7.10. Found: C, 59.35; H, 7.28. IR ν_{max}^{Nujol} cm^{-1}: 1753, 1223 (OAc), and as given in Table I. UV λ_{max}^{EtOH}: as given in Table I; λ_{max}^{MeOH} 241 nm (ε 14100). ^{1}H-NMR ($CDCl_3$, δ): 0.88 (3H, d, J=7 Hz), 1.02 (3H, d, J=7 Hz) (*sec.* Me×2), 2.00 (9H, s), 2.02, 2.04, 2.08, 2.17, 2.20 (3H each, all s) (OAc×8), 3.2—4.6 (*ca.* 12H, m), 4.9—5.3 (6H, m, >CHOAc×6), and others as given in Table II. CD (c=0.1, dioxane) [θ] (nm): 0 (367), +3900 (334) (sh.), +4000 (324) (pos. max, Table I), 0 (290); (c=0.067, MeOH) [θ] (nm): +5300 (320) (pos. max), −62700 (241) (neg. max).

Treatment of NF-1 (12) with Aqueous Acetone giving 14——A solution of **12** (160 mg) in acetone–water (3: 1, 28 ml) was heated under reflux for 13 hr. Removal of the solvents gave **14** quantitatively. Recrystallization from acetone furnished **14** (colorless fine crystals) of mp 109—111°, $[\alpha]_D^{21}$ −11° (c=0.67, acetone). *Anal.* Calcd for $C_{56}H_{82}O_{24}$: C, 59.04; H, 7.26. Found: C, 59.26; H, 7.04. IR ν_{max}^{Nujol} cm^{-1}: 3475 (br., OH), 1751, 1222 (OAc). ^{1}H-NMR (benzene-d_6, δ): 0.79 (3H, s, 13-Me), 0.84 (3H, d, J=6 Hz), 0.89 (3H, d, J=6 Hz), 1.14 (3H, s, 10-Me) (Me×4), 1.70 (6H, s), 1.72 (3H, s), 1.74 (6H, s), 1.80, 1.86, 1.87, 2.12 (3H each, all s) (OAc×9), 3.9—4.2 (4H, m), 4.29 (1H, d, J=8 Hz, anom. H of glucoside), 4.42 (1H, d, J=7 Hz, anom. H of arabinoside), 5.0—5.5 (7H, m, >CHOAc×7), 5.88 (1H, m, 3-H).

12 was regenerated quantitatively from **14** by heating in dry MeOH under reflux for 4.5 hr.

Alkaline Hydrolysis of 12 giving 13——A solution of **12** (90 mg) in 0.35 N NaOMe–MeOH (10 ml) was stirred at room temp. (12°) for 3.5 hr. The reaction mixture was neutralized with Dowex 50 W×8 (3 g) and concentrated under reduced pressure to give a residue which was washed with n-hexane and benzene. Recrystallization from MeOH furnished **13** (colorless fine crystals), mp 167—169°, $[\alpha]_D^{16}$ −38° (c=0.54, MeOH). *Anal.* Calcd for $C_{39}H_{66}O_{15}\cdot 1/2H_2O$: C, 59.75; H, 8.61. Found: C, 59.52; H, 8.48. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH).

Enzymic Hydrolysis of 13——A suspension of **13** (27 mg) in dist. water (6 ml) was treated with almond emulsin (19 mg) and incubated with stirring at 37° for 3.5 hr. The resulting precipitate was collected by filtration, washed with water, and dried *in vacuo*. Recrystallization of the product from MeOH gave a prosapogenol, mp 299—301°, which was identical with **8**[7] as judged by mixed mp determination, TLC ($CHCl_3$–MeOH–H_2O=7: 3: 1, lower phase), and IR spectroscopy (KBr). The filtrate was washed with n-BuOH and concentrated under reduced pressure to give a residue which was identified as glucose by PPC (double development with iso-PrOH–n-BuOH–H_2O=7: 1: 2), TLC (AcOEt–pyridine–AcOH–H_2O=5: 5: 1: 3, n-BuOH–AcOH–H_2O=6: 2: 2), and GLC (as TMS deriv., column temp.: 140°; N_2 flow rate: 40 ml/min).

Acid Hydrolysis of 13——A solution of **13** (18 mg) in conc. HCl–MeOH (1: 4, 5 ml) was heated under reflux for 3 hr. The reaction mixture was poured into ice-water and extracted with ether. After usual work-up, a residue obtained by removal of the ether was purified by prep. TLC (benzene–acetone=1: 1) and recrystallized from $CHCl_3$–MeOH to furnish a sapogenol (6 mg), mp 258—262°, which was identical with metagenin (**1**) as judged by mixed mp determination, TLC (benzene–acetone=1: 1), and IR spectroscopy (KBr). The acidic aqueous layer was neutralized with Amberlite IR 45 (15 g) and concentrated under reduced pressure to give a residue, from which glucose and arabinose were identified by PPC (double development with iso-PrOH–n-BuOH–H_2O=7: 1: 2 or phenol–H_2O=5: 1, respectively).

Treatment of NF-2 (15) with Aqueous Acetone giving 16——A solution of **15** (400 mg) in acetone–water (3: 1, 20 ml) was heated under reflux for 15 hr. The product obtained by removal of the solvents was purified by prep. TLC [Kieselgel 60 F-254 (Merck), benzene–acetone=2: 1, detection with UV] to furnish **16** (370 mg) as colorless crystals of mp 107—109° (recryst. from acetone), $[\alpha]_D^{24}$ −26.6° (c=0.91, acetone). *Anal.* Calcd for $C_{54}H_{76}O_{23}\cdot 1/2H_2O$: C, 58.85; H, 7.04. Found: C, 58.74; H, 7.15. ^{1}H-NMR benzene-d_6, δ): 0.83 (3H, d, J=7 Hz), 0.87 (3H, d, J=6 Hz), 1.73 (3H, s), 1.77 (12H, s), 1.86, 1.89, 2.17 (3H each, all s) (OAc×8), 5.0—5.5 (6H, m, >CHOAc×6), and others as given in Table II.

15 was regenerated quantitatively from **16** by heating in dry MeOH under reflux for 4 hr.

^{1}H-NMR Measurement of 16 with Shift Reagent[15]——A solution of **16** (34 mg) in benzene-d_6 (0.4 ml) containing tetramethylsilane was treated with $Eu(fod)_3$ (3.83 mg) and subjected to ^{1}H-NMR spectroscopy. The signal ascribable to 2α-H was observed at δ 6.08 as a doublet of doublets with $J_{2\alpha,1\beta}$=13 Hz and $J_{2\alpha,1\alpha}$=4 Hz (Table II).

Alkaline Treatment of 15 giving 19——A solution of **15** (230 mg) in 0.3 N NaOMe–MeOH (54 ml) was stirred at room temp. (18°) for 5 hr. The reaction mixture was neutralized with Dowex 50 W×8 (24 g) and filtered. Removal of the solvent by evaporation gave a residue which was dried *in vacuo* and heated in dry MeOH under reflux for 5 hr. The product obtained by evaporation of the solvent was recrystallized from MeOH to furnish **19** (colorless fine crystals, 140 mg), mp 156—157°, $[\alpha]_D^{18}$ −15.4° (c=0.5, MeOH). *Anal.* Calcd for $C_{39}H_{60}O_{15}\cdot 2H_2O$: C, 58.20; H, 8.01. Found: C, 58.32; H, 7.85. IR ν_{max}^{KBr} cm^{-1}: 3380 (br., OH) and as given in Table III. UV λ_{max}^{EtOH}: as given in Table III; λ_{max}^{MeOH} nm (ε): 250 (10300), 293 (sh.) (2600). ^{1}H-NMR: as given in Table III. CD (c=0.14, dioxane) [θ] (nm): 0 (368), +7000 (337) (pos. max), +6800 (332) (sh.), 0 (311).

An ethanolic solution of **19** was colored wine-red with 2% $FeCl_3$–EtOH.

Enzymic Hydrolysis of 19——A suspension of **19** (110 mg) in dist. water (15 ml) was treated with almond emulsin (100 mg) and incubated with stirring at 37° for one day. Almond emulsin (50 mg) was added again and the whole mixture was stirred for a further one day. The precipitate was collected by filtration, washed with water, and dried *in vacuo* to give a product which was purified by prep. TLC [Kieselgel PF_{254} (Merck), $CHCl_3$–MeOH–H_2O=7: 3: 1, lower phase] to furnish **22** (50 mg) as a white powder (from acetone–*n*-hexane). High resolution MS: Found: 574.314, 442.271, 424.261, 149.048, 139.110, 133.052. Calcd for $C_{32}H_{46}O_9$ (M^+)=574.314, $C_{27}H_{38}O_5$=442.272, $C_{27}H_{36}O_4$ (**iv**)=424.261, $C_5H_9O_5$=149.045, $C_9H_{15}O$ (**i**)=139.112, $C_5H_9O_4$ (**iii**)=133.050. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1640 (br.), 980, 914, 897, 867 (intensity 897>914, 25*R*-spiroketal). UV λ_{max}^{EtOH} nm (ε): 250 (11600), 297 (sh.) (2500). CD (c=0.09, dioxane) [θ] (nm): 0 (370), +5500 (338) (pos. max), +5100 (328) (sh.), 0 (310). MS m/z (%): 574 (M^+, 1), 442 (6), 424 (**iv**, 24), 310 (15), 281 (15), 242 (31), 181 (55), 149 (15), 139 (**i**, 100), 133 (**iii**, 10), 115 (15), 97 (15).

An ethanolic solution of **22** was colored wine-red with 2% $FeCl_3$–EtOH.

The aqueous filtrate was concentrated under reduced pressure to give a residue which was identical with glucose upon PPC, TLC, and GLC as described above.

Conversion of 22 to 11——A solution of **22** (20 mg) in dry iso-PrOH (2 ml) was treated with $NaBH_4$ (2 mg) and the mixture was stirred at room temp. (28°) for 24 hr. The reaction mixture was treated again with $NaBH_4$ (2 mg) and stirred for a further 23 hr. The whole mixture was poured into ice-water and extracted with *n*-BuOH–AcOEt (1: 1). The product obtained by usual work-up of the extract was acetylated with Ac_2O (0.7 ml) and pyridine (1 ml) at room temp. (29—31°) for 50 hr. After usual work-up, the product was subjected to prep. TLC [silica gel (Camag D-5), double development with *n*-hexane–AcOEt=3: 2] to furnish product a (7 mg) (least polar), product b (3 mg) (medium), and product c (6 mg) (most polar). These three products gave the same molecular ion at m/z 788. Product c was crystallized from *n*-hexane–EtOH. It was identical with **11**[6] as judged by TLC (double development with *n*-hexane–AcOEt=3: 2), IR (CS_2), and mass spectroscopy.

Acid Hydrolysis of 19——A solution of **19** (22 mg) in conc. HCl–MeOH (13: 60, 8 ml) was heated under reflux for 7 hr. The reaction mixture was diluted with water and the methanol was removed by evaporation under reduced pressure. The aqueous mixture was then extracted with AcOEt and the extract was worked up in the usual manner. Purification of the product by prep. TLC [Kieselgel 60 F-254, benzene–acetone=2: 1] furnished **23** (9 mg). Colorless crystals of mp 234—236° (from acetone–*n*-hexane), $[\alpha]_D^{17}$ −90.3° (c=0.54, acetone). High resolution MS (m/z): Found 456.287, 152.085, 151.076, 139.113, 115.077. Calcd for $C_{28}H_{40}O_5$ (M^+)=456.288, $C_9H_{12}O_2$ (**v**)=152.084, $C_9H_{11}O_2$ (**vi**)=151.076, $C_9H_{15}O$ (**i**)=139.112, $C_6H_{11}O_2$ (**ii**)=115.077. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (OH), 980, 915, 897, 865 (intensity 897>915, 25*R*-spiroketal), and as given in Table IV. UV: as given in Table IV. ^{1}H-NMR ($CDCl_3$, δ): 0.78 (3H, d, J=5 Hz, 25-Me), 0.96 (3H, d, J=6 Hz, 20-Me), 3.40 (2H, m, 26-H_2), 4.00 (1H, m, 11β-H), 4.41 (1H, m, 16-H), and others as given in Table IV. CD (c=0.18, dioxane) [θ] (nm): 0 (382), +960 (368) (sh.), +2240 (352) (sh.), +2740 (339) (pos. max), +2240 (327) (sh.), 0 (306). MS m/z (%): 456 (M^+, 11), 438 (M^+−18, 3.5), 152 (**v**, 37, *cf.* Table IV), 151 (**vi**, 83, *cf.* Table IV), 139 (**i**, 100), 115 (**ii**, 39).

The aqueous phase, after extraction with AcOEt, was neutralized with Amberlite IR 45 (60 g) and concentrated under reduced pressure to give a residue, from which glucose and arabinose were identified by PPC as described above.

Acid Hydrolysis of 22——A solution of **22** (3 mg) in conc. HCl–MeOH (1: 5, 2 ml) was heated under reflux for 5 hr. Work-up of the reaction mixture as described above furnished **23** [identified by TLC (benzene–acetone=3: 1)] and arabinose (PPC, TLC).

Alkaline Treatment followed by Acid Hydrolysis of Glucoside Mixture (R-BE) (Procedure I)——A solution of R-BE (20 g) in 0.33 N NaOMe–MeOH (150 ml) was stirred at room temp. (20°) for 3.5 hr. The reaction mixture was diluted with MeOH (450 ml) and neutralized with Dowex 50 W×8 (50 g). Removal of the solvent afforded a mixture of desacetyl glycosides (16 g) which gave TLC diagrams as shown in Fig. 2. The mixture was dissolved in conc. HCl–MeOH (13: 60, 183 ml) and heated under reflux for 7 hr. The reaction mixture was allowed to cool to room temp., then the mixture was poured into ice-water and the precipitate was collected by filtration, washed with water, and dried *in vacuo* to furnish the sapogenol mixture. Extraction of the filtrate with $CHCl_3$ and work-up of the extract in the usual manner furnished an additional crop of the sapogenol mixture. The composition of the sapogenol mixture (8.8 g) was as shown in Fig. 3. Column chromatography of the mixture (SiO_2 500 g, elution with benzene–acetone=30: 1→15: 1→10: 1→6: 1→3: 1,

and MeOH) afforded **23** (312 mg, eluted with benzene–acetone=3: 1, 0.165% from the dried subterranean part of the plant). Purification with charcoal in MeOH and recrystallization from benzene–*n*-hexane and acetone–*n*-hexane furnished colorless crystals of mp 235—237°. This product was identical with the above described sapogenol obtained from **19** as judged by mixed mp determination, TLC, IR ($CHCl_3$), and mass spectroscopy.

Acetylation of 23 giving 24——A solution of **23** (100 mg) in Ac_2O (1.2 ml) and pyridine (3 ml) was allowed to stand at 31° for 23 hr. After usual work-up, the product was purified by prep. TLC (Kieselgel 60 F-254, benzene–acetone=4: 1) treated with acetone (or MeOH) to furnish **24** (70 mg, colorless glassy material; attempts at crystallization were unsuccessful), $[\alpha]_D^{17}$ −48.0° (c=0.54, acetone). High resolution MS: Found: 498.298, 152.082, 151.076, 139.114, 115.077. Calcd for $C_{30}H_{42}O_6$ (M^+)=498.298, $C_9H_{12}O_2$ (**v**)=152.084, $C_9H_{11}O_2$ (**vi**)=151.076, $C_9H_{15}O$ (**i**)=139.112, $C_6H_{11}O_2$ (**ii**)=115.076. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1240 (OAc), 980, 915, 896, 862 (intensity 896>915, 25*R*-spiroketal), and others as given in Table IV. UV: as given in Table IV. ^{1}H-NMR ($CDCl_3$, δ): 0.78 (3H, d, J=6 Hz, 25-Me), 0.94 (3H, d, J=5 Hz, 20-Me), 2.07 (3H, s, 11α-OAc), 4.40 (1H, m, 16-H), 5.27 (1H, t.d, J=11 and 5 Hz, 11β-H), and others as given in Table IV. CD (c=0.2, dioxane) [θ] (nm): 0 (388), +530 (372) (sh.), +1020 (356) (sh.), +1160 (342) (pos. max), +860 (330) (sh.), 0 (310). MS m/z (%): 498 (M^+, 6), 438 (M^+−60, 67), 152 (**v**, 17, *cf.* Table IV), 151 (**vi**, 88, *cf.* Table IV), 139 (**i**, 100), 115 (**ii**, 8).

Acetylation of 19 giving 20 and 21——Compound **19** (120 mg) was treated with Ac_2O (4 ml) and pyridine (6 ml) at room temp. (30°) for 2 days, and worked up as usual. The resulting material was heated in dry MeOH under reflux for 5 hr, and the product (152 mg) obtained by removal of the MeOH was subjected to prep. TLC (Kieselgel 60 F-254, $CHCl_3$–AcOEt=1: 1) to furnish **20** (71 mg, from the more polar fraction) and **21** (41 mg, from the less polar fraction). Since **20** was labile to moisture (partially convertible to the 22-OH deriv.), **20** was dissolved in dry MeOH (10 ml) and heated under reflux for 5 hr. Treatment with MeOH furnished **20** as a white powder. *Anal.* Calcd for $C_{55}H_{76}O_{23}\cdot H_2O$: C, 58.81; H, 7.00. Found: C, 58.99; H, 7.06. IR and UV: as given in Table V. ^{1}H-NMR ($CDCl_3$, δ): 2.00 (12H, s), 2.07 (6H, s), 2.17 (3H, s), 2.27 (3H, s) (OAc×8), and others as given in Table V. CD (c=0.18, dioxane) [θ] (nm): 0 (378), +1490 (350) (sh.), +1780 (340) (pos. max), +990 (320) (sh.), 0 (307). Treatment with MeOH gave **21** as a white powder.[30] IR and UV: as given in Table V. ^{1}H-NMR ($CDCl_3$, δ): 2.01 (12H, s), 2.08 (6H, s), 2.17 (3H, s), 2.28 (3H, s) (OAc×8), and others as given in Table V. CD (c=0.21, dioxane) [θ] (nm): 0 (387), +1840 (360) (sh.), +2670 (350) (sh.), +3010 (336) (pos. max), 0 (309). Heating under reflux of **21** either in dry MeOH (5 hr) or in acetone–water (1: 1) (7 hr) resulted in recovery of **21** as judged by ^{1}H-NMR and TLC.

Acid Hydrolysis followed by Alkaline Treatment of Glycoside Mixture (R-BE) (Procedure II)——A solution of R-BE (15 g) in conc. HCl–MeOH (13: 60, 146 ml) was heated under reflux for 7 hr. After cooling to room temp., the reaction mixture was poured into ice-water and the precipitated product (6 g) was collected by filtration and worked up as usual. A solution of the product (3 g) in KOH (3 g)–MeOH (100 ml)–H_2O (20 ml) was heated under reflux for 2 hr 15 min. The composition of the sapogenol mixture (2.5 g) obtained by usual work-up was as shown in Fig. 3.

2β- and 2α-Acetoxy-17β-chloroacetoxyandrost-4-en-3-ones (25 and 28)——**25** and **28** were prepared from testosterone 17β-chloroacetate[31] according to the reported method.[18] **25**, mp 194—196° (colorless plates from AcOEt) (lit. mp 190—191°,[17] 198—200°[18]). IR ν_{max}^{Nujol} cm^{-1}: 1758 (sh.), 1752, 1216, 1180 (ester), and as given in Table I. UV: as given in Table I. ^{1}H-NMR ($CDCl_3$, δ): 2.12 (3H, s, OAc), 4.03 (2H, s, $ClCH_2CO$), 4.67 (1H, m, 17α-H), and others as given in Table II. CD (c=0.17, dioxane) [θ] (nm): 0 (386), −340 (366) (neg. max), 0 (355), +2800 (330) (sh.), +3200 (322) (pos. max), 0 (276). These spectral properties coincided with the reported data (UV, ^{1}H-NMR, CD).[17,18] **28**, mp 183—186° (colorless needles from MeOH) (lit.[18] mp 189—192°). IR ν_{max}^{Nujol} cm^{-1}: 1758 (sh.), 1750, 1219, 1194 (ester), and as given in Table I. UV: as given in Table I. ^{1}H-NMR ($CDCl_3$, δ): 0.87 (3H, s, 13-Me), 1.33 (3H, s, 10-Me), 2.16 (3H, s, OAc), 4.06 (2H, s, $ClCH_2CO$), 4.68 (1H, m, 17α-H), 5.41 (1H, dd, J=5.5 and 13.5 Hz, 2β-H), 5.72 (1H, s, 4-H). CD (c=0.2, dioxane) [θ] (nm): 0 (370), −6600 (334) (sh.), −7000 (324) (neg. max), 0 (270). These spectral properties coincided with the reported data (UV, ^{1}H-NMR).[18]

Alkaline Treatment of 25 giving 29——A solution of **25** (127 mg) in 0.3 N NaOMe–MeOH (20 ml) was stirred at room temp. (28°) for 4.5 hr. After neutralization with Dowex 50 W×8 (10 g), the solvent was removed by evaporation to furnish **29** (86 mg), mp 205—207.5° (colorless crystals from *n*-hexane–AcOEt) and $[\alpha]_D^{24}$ −13.0° (c=0.6, $CHCl_3$). High resolution MS: Found: 302.189, 138.067, 137.059. Calcd for $C_{19}H_{26}O_3$ (M^+)=302.188, $C_8H_{10}O_2$=138.068, $C_8H_9O_2$=137.060. IR ν_{max}^{KBr} cm^{-1}: 3400 (br., OH) and as given in Table III. UV: as given in Table III. ^{1}H-NMR ($CDCl_3$, δ): 0.81 (3H, s, 13-Me), 1.23 (3H, s, 10-Me), 3.63 (1H, m, 17α-H), and others as given in Table III. CD (c=0.14, dioxane) [θ] (nm): 0 (415), −430 (376) (sh.), −500 (360) (neg. max), −430 (352) (neg. min), −500 (345) (neg. max), −370 (338) (neg. min), −470 (333) (neg. max), −370 (327) (neg. min), −510 (320). MS m/z: 302 (M^+, 30), 284 (M^+−18, 5), 147 (57), 138 (49), 137 (100). An ethanolic solution of **29** was colored wine-red with 2% $FeCl_3$–EtOH. The physical properties given here coincide with the reported data (mp, $[\alpha]_D$, IR, UV, and $FeCl_3$ coloration).[20,24]

Alkaline Treatment of 28 giving 29——Treatment of **28** (400 mg) with 0.3 N NaOMe–MeOH (40 ml) as described for **25** was shown to proceed with *ca.* 90—95% conversion. After 7 hr, the conversion was accomplished. Work-up of the reaction mixture in the usual manner furnished **29** (275 mg).

Acid Treatment of 29 giving 30——A solution of **29** (200 mg) in conc. HCl–MeOH (13: 60, 15 ml) was heated under reflux for 11 hr. TLC monitoring of the reaction mixture revealed that the composition no longer changed after 7 hr. After cooling, the reaction mixture was poured into ice-water and extracted with AcOEt. After usual work-up of the AcOEt extract, the product was purified by prep. TLC [Kieselgel 60 F-254, benzene–acetone=2: 1) to furnish **30** (135 mg), mp 222—224° (colorless crystals from acetone), $[\alpha]_D^{24}$ −30.0° (c=0.6, $CHCl_3$), High resolution MS: Found: 316.203, 152.082, 151.076. Calcd for $C_{20}H_{28}O_3$ (M^+)=316.204, $C_9H_{12}O_2$ (**v**)=152.084, $C_9H_{11}O_2$ (**vi**)=151.076. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH) and as given in Table IV. UV: as given in Table IV. ^{1}H-NMR ($CDCl_3$, δ): 3.61 (1H, m, 17α-H) and others as given in Table IV. CD (c=0.12, dioxane) [θ] (nm): 0 (398), −660 (356) (sh.), −760 (344) (neg. max), −690 (333) (sh.), −310 (306) (neg. min), −520 (300). MS m/z (%): 316 (M^+, 44), 164 (26), 152 (**v**, 72, *cf.* Table IV), 151 (**vi**, 100, *cf.* Table IV), 147 (33), 138 (33).

The physical properties of **30** coincide with the reported data (mp, $[\alpha]_D$, UV).[24]

Alkaline Hydrolysis of 25 under an Ar Atmosphere giving 26——A suspension of **25** (595 mg) in dry MeOH (40 ml) was heated with 1 N KOH–MeOH (1.7 ml) and stirred under an argon atmosphere at room temp. (13°) for 1.5 hr as reported elsewhere.[18] **26** (400 mg), mp 159—161° [colorless needles from acetone–light petroleum (bp 60—80°)] (lit. mp 157—159°,[17] mp 163—165°[18]). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH) and as given in Table III. UV: as given in Table III. ^{1}H-NMR ($CDCl_3$, δ): 0.79 (3H, s, 13-Me), 1.18 (3H, s, 10-Me), 3.64 (1H, m, 17α-H), 4.18 (1H, dd, J=5.5 and 13.5 Hz, 2α-H), 5.78 (1H, s, 4-H, Table III). CD (c=0.08, dioxane) [θ] (nm): 0 (384), −260 (366) (neg. max), 0 (354), +2800 (320) (pos. max), 0 (276). $FeCl_3$ test: negative.

The physical properties of **26** coincide with the reported data.[17,18]

Acknowledgement The authors are grateful to Drs. K. Takeda and I. Igarashi of Shionogi Res. Lab. for generous gifts of the plant material and the authentic specimens of sapogenols and to Miss K. Saiki of Kobe Women's College of Pharmacy for measuring the high resolution MS. This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Education, Science, and Culture (367381 for T.N.), for which the authors are very grateful.

References and Notes

1) Part XXIX: I. Kitagawa, T. Kamigauchi, H. Ohmori, and M. Yoshikawa, *Chem. Pharm. Bull.*, **28**, 3078 (1980).
2) *a*) K. Hamamoto, *Chem. Pharm. Bull.*, **9**, 32 (1961); *b*) K. Takeda, T. Okanishi, H. Osaka, A. Shimaoka, and N. Maezono, *ibid.*, **9**, 388 (1961); *c*) H. Minato and A. Shimaoka, *ibid.*, **11**, 876 (1963); *d*) K. Igarashi, *ibid.*, **9**, 729 (1961); *e*) K. Takeda, T. Okanishi, K. Igarashi, and A. Shimaoka, *Tetrahedron*, **15**, 183 (1961); *f*) I. Yosioka, K. Imai, Y. Morii, and I. Kitagawa, *ibid.*, **30**, 2283 (1974).
3) 3-epi-Metagenin (**2**) was obtained from the glycoside mixture by initial alkaline treatment followed by acidic hydrolysis.[2f]
4) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Lett.*, **1966**, 6303.
5) I. Yosioka, Y. Morii, and I. Kitagawa, *Chem. Pharm. Bull.*, **21**, 2092 (1973).
6) I. Kitagawa, T. Nakanishi, Y. Morii, and I. Yosioka, *Chem. Pharm. Bull.*, **25**, 2343 (1977).
7) I. Kitagawa, K.S. Im, and Y. Morii, *Chem. Pharm. Bull.*, **24**, 3114 (1976).
8) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968).
9) I. Kitagawa and T. Nakanishi, presented at the 24th Annual Meeting of the Japanese Society of Pharmacognosy, held at Tokyo, October 1977, Abstract Paper p. 29.
10) I. Kitagawa and T. Nakanishi, presented at the 28th Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, held at Nishinomiya, October 1978, Abstract Paper p. 61, 62.
11) This procedure was carried out to convert completely the furostane-type glycosides to the 22-O-methylfurostanol derivatives in order to simplify the composition.[12]
12) *a*) T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa, and K. Mihashi, *Chem. Pharm. Bull.*, **22**, 2164 (1974); *b*) T. Nohara, K. Miyahara, and T. Kawasaki, *ibid.*, **23**, 872 (1975).
13) Presumed to be D-glucose, although the optical rotation could not be measured due to shortage of the material.
14) H. Ripperger, H. Budzikiewicz, and K. Schreiber, *Chem. Ber.*, **100**, 1725 (1967).
15) The chemical shift and the coupling pattern were unclear until the shift reagent was employed (*vide infra*, see "Experimental").
16) The CD spectra of steroids having a 4-en-3-one or a 2α-acetoxy-4-en-3-one chromophore have been reported to show a negative maximum at 320—350 nm due to the n-π^* transition: G. Snatzke, *Tetrahedron*, **21**, 421 (1965) and references cited therein.
17) Y. Osawa and J.O. Gardner, *J. Org. Chem.*, **36**, 3246 (1971).
18) R.D. Burnett and D.N. Kirk, *J. Chem. Soc. Perkin I*, **1974**, 284 and references cited therein.
19) G. Snatzke, *Tetrahedron*, **21**, 439 (1965).
20) R.L. Clark, *J. Am. Chem. Soc.*, **82**, 4629 (1960).

21) *a*) M.E. Wall, C.R. Eddy, M.L. McClennan, and M.E. *Klumpp*, *Analyt. Chem.*, **24**, 1337 (1952); *b*) R.N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953).
22) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 113.
23) R. Tschesche, I. Mörner, and G. Snatzke, *Ann. Chem.*, **670**, 103 (1963).
24) J.S. Baran, *J. Am. Chem. Soc.*, **80**, 4629 (1958).
25) R. Tschesche, G. Lüdke, and G. Wulff, *Tetrahedron Lett.*, **1967**, 2785.
26) In order to define the origin of the 22-O-methyl group in **12** and **15**, we carried out a detailed comparative examination by TLC of the methanolic and dioxane extracts of the plant. However, we could not obtain a clear-cut result due to the complexity of the TLC patterns. We consider that the methoxyl function may be introduced during the methanolic extraction procedure.
27) All sapogenols except **2**[3] of the title plant were isolated by a method which was essentially identical with procedure II.
28) The instruments used to obtain physical data, and the experimental conditions for chromatography were the same as in our previous paper[29] unless otherwise specified. Paper partition chromatography (PPC) was carried out on Toyo filter paper No. 51 using aniline hydrogen phthalate for detection. The Ehrlich reagent, 1% ceric sulfate in 10% sulfuric acid, and UV irradiation (254 nm) were used for detection on TLC plates. TLC for sugar was carried out on Avicel cellulose and the reagent for detection was the same as for PPC.
29) I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, **26**, 1864 (1978).
30) Although the purity of the compound was confirmed by TLC and the spectral data were consistent with the proposed structure (**21**), attempts at crystallization failed and satisfactory elemental analysis data could not be obtained.
31) H.J. van der Molen, D. Groen, and J.H. van der Maas, *Steroids*, **6**, 195 (1965).