

ON THE GENUINE SAPOGENOL OF METEOGENIN, AN A-RING AROMATIZED  
SPIROSTANOL FROM METANARTHECIUM LUTEO-VIRIDE MAXIM.  
STRUCTURE ELUCIDATION BY MEANS OF SOIL BACTERIAL HYDROLYSIS

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Nine spirostanols (metagenin (1), 3-epi-metagenin (2), nogiragenin, neonogiragenin, narthogenin, isonarthogenin, meteogenin (3), neometeogenin (4), and luvigenin (5)) were elucidated as the sapogenols of *Metanartheccium luteo-viride* Maxim. (Liliaceae).<sup>1,2)</sup> They were obtained by acid hydrolysis of the total glycoside mixture. Among them, three minor sapogenols (meteogenin (3), neometeogenin (4), and luvigenin (5)) are remarkable in having an aromatized A-ring and have been presumed to be the artifacts which were formed secondarily from the precursory genuine sapogenols during the acid hydrolysis of the parent glycosides.<sup>1d,e)</sup>

Recently, by means of the soil bacterial hydrolysis of the same glycoside mixture, we elucidated the structures of two new type prosapogenols: 2-O-acetyl-11-O-tri-O-acetyl- $\alpha$ -L-arabinopyranosyl-3-epi-metagenin (6)<sup>2)</sup> and 11-O-galactopyranosyl-nogiragenin (7),<sup>3)</sup> and these findings have suggested that the glycoside fraction of the plant is rich in saponins having an O-glycoside linkage at C-11 of the steroidal sapogenol. In a continuing study, we have isolated another new prosapogenol (purified as the peracetate) which, on acid treatment, readily affords meteogenin (3) almost quantitatively and is considered to be an arabinoside of a precursory sapogenol of meteogenin. The structure elucidation is the subject of this paper.

A glycoside mixture obtained from the subterranean part was subjected to the soil bacterial hydrolysis<sup>2,4)</sup> and acetylation of the hydrolysate followed by repeated chromatographic separation has furnished a pentaacetate of a new prosapogenol (8a).<sup>5)</sup> The pentaacetate (8a),  $C_{42}H_{60}O_{14}$ <sup>6)</sup> gives, in the mass spectrum, a series of abundant fragment ion peaks:  $C_{11}H_{15}O_7$  (1a),  $C_9H_{11}O_5$ ,  $C_7H_9O_4$ ,  $C_7H_7O_3$ , which are derived from a fully acetylated pentoaldopyranose moiety,<sup>2,7)</sup> and a base peak of  $C_9H_{15}O$  (1i) derived from the E and F rings of the spirostane skeleton.<sup>2,8)</sup>

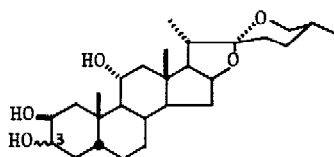
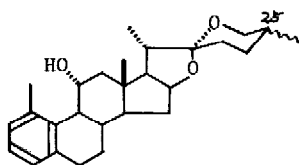
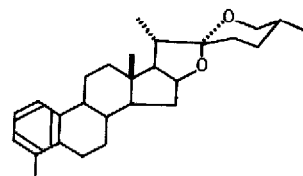
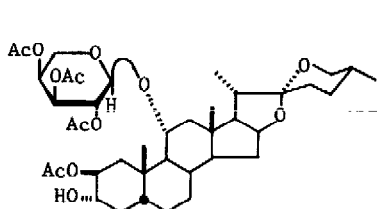
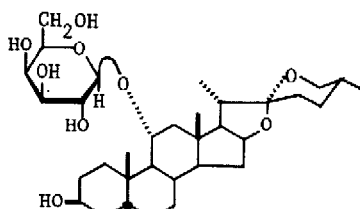
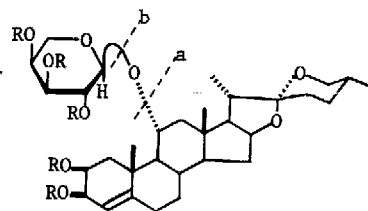
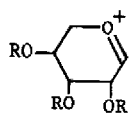
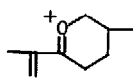
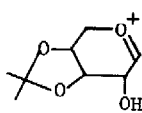
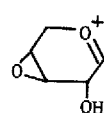
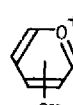
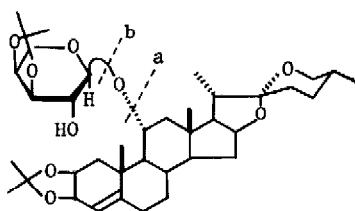
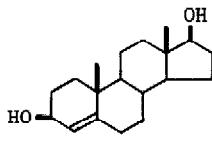
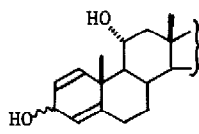
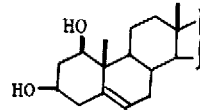
The IR spectrum ( $\text{CS}_2$ ) of 8a shows the acetoxy absorption bands but no hydroxyl band. It also shows absorption bands at 981, 920, 899, and  $864 \text{ cm}^{-1}$  (intensity at 899 > at  $920 \text{ cm}^{-1}$ ) suggesting the 25R spirostane structure,<sup>9)</sup> which is supported by the PMR signals (in  $\text{CDCl}_3$ ) at  $\delta 0.79$  (3H, s, 13-Me), 1.24 (3H, s, 10-Me), 0.83 (3H, d, 25-Me), and 0.99 (3H, d, 20-Me). The PMR spectrum of 8a also shows the signals due to five acetoxy groups among which three are, on the mass spectral basis, presumed to be present in the carbohydrate part and two in the aglycone part. The presence of one olefinic proton is assumed from the signal intensity between  $\delta 4.9$  and  $\delta 5.6$ . The anomeric proton signal is observed as a doublet at  $\delta 4.62$  ( $J = 7 \text{ Hz}$ ) in the PMR spectrum of 8a taken in  $\text{C}_6\text{D}_6$ - $(\text{CD}_3)_2\text{CO}$  (1:1).

Treatment of 8a with 0.5% NaOMe-MeOH gave a prosapogenol (8), mp  $265.5$ - $267^\circ$ ,  $\text{C}_{32}\text{H}_{50}\text{O}_9$ , which lacks the acetoxy function and possesses the resumed hydroxyls (IR:  $3385$  (br)  $\text{cm}^{-1}$ ). Here again, the presence of 25R spirostane skeleton in 8 is supported by the IR spectrum. The mass spectrum of 8 gives a rich fragment ion of  $\text{C}_5\text{H}_9\text{O}_4$  (i), a base peak of  $\text{C}_9\text{H}_{15}\text{O}$  (ii), and two fragment ions ( $\text{C}_{27}\text{H}_{41}\text{O}_4$  and  $\text{C}_{27}\text{H}_{41}\text{O}_5$ ) derived from the aglycone part (a and b fissions in 8), thus the molecular composition of the aglycone being suggested to be  $\text{C}_{27}\text{H}_{42}\text{O}_5$  (two hydrogen less than that of metagenin (1)). Acid hydrolysis (c.HCl-MeOH = 1:4) of 8 under reflux furnished metegenin (3)<sup>1d)</sup> and arabinose<sup>10)</sup> in the excellent yields.

The above accumulated evidence has led to an assumption that the prosapogenol is an  $\alpha$ -L-arabinopyranoside of a new 25R spirostanol (now named protometegenin) having three hydroxyls (one at  $11\alpha$ ) and one trisubstituted double bond (probably at C-4,5 or C-5,6).

Treatment of 8 with dry acetone- $\text{CuSO}_4$  gave a diacetonide (9),  $\text{C}_{38}\text{H}_{58}\text{O}_9$ , which gives the mass fragment ions of  $\text{C}_8\text{H}_{13}\text{O}_5$  (17%) (a fission in 9),  $\text{C}_8\text{H}_{13}\text{O}_4$  (21%) (iii),  $\text{C}_5\text{H}_7\text{O}_3$  (23%) (iv), and  $\text{C}_5\text{H}_5\text{O}_2$  (17%) (v) derived from the monoacetonide of arabinoside moiety<sup>11)</sup> and the fragment ions of  $\text{C}_{30}\text{H}_{45}\text{O}_5$  (1.4%) (b fission in 9),  $\text{C}_{30}\text{H}_{45}\text{O}_4$  (2.1%) (a fission in 9), and  $\text{C}_{27}\text{H}_{39}\text{O}_3$  (22%) (=  $\text{C}_{30}\text{H}_{45}\text{O}_4 - (\text{CH}_3)_2\text{CO}$ ),<sup>11)</sup> in addition to a base peak at  $m/e$  59 ( $(\text{CH}_3)_2\text{CO} + \text{H}$ ). These findings suggest that two hydroxyls in the aglycone part have suffered the acetonidation.

The allocation of two hydroxyls and one double bond in the aglycone part of 8 has been made on the basis of the CD examinations. The CD spectra of 8 and 8a show a strong negative maximum at 200 nm due to  $\pi_x \rightarrow \pi_x^*$  of C=C: 8:  $[\theta] -70700$ , 8a:  $[\theta] -88100$ , which are best explained by assigning the  $3\beta$ -hydroxy-4-ene structure for 8 (cf. 10:  $[\theta]_{197} -33000$ ).<sup>12,13)</sup> The location of another hydroxyl is consequently assigned as  $2\beta$  since i) the hydroxyl is involved in the ready acetonide formation and ii) 8 gives metegenin (3) on acid treatment (cf. mechanistic consider-

1 3β-OH: metagenin2 3α-OH: 3-epi-metagenin3 25R: meteogenin4 25S: neometeogenin5 luvigenin678 R= H 8a R= Aci R= Hia R= Aciiiiiivv9101112

ation<sup>1d,e</sup>). Consequently, the structure 8 has become reasonable for the new prosapogenol now designated as 11-O- $\alpha$ -L-arabinopyranosyl-protometeogenin. The molar ellipticity of 8 and 8a is increased in comparison with that of 10 presumably due to the presence of 2 $\beta$ -OH and 2 $\beta$ -OAc respectively as rationalized by the Dreiding model examinations in relation to the octant projection.<sup>12)</sup>

The structure of protometeogenin (aglycone of 8) corresponds to a hydrated form of a dienol-type structure (11) which was previously accounted on the chemical basis as one of the probable

precursory sapogenols of meteogenin (3).<sup>1d)</sup> As for the genuineness of the spirostane structure in 8, the problem is still open for the study since it has been known that a furostanol 26-O-glucoside is readily converted to a corresponding spirostanol aglycone even upon enzymatic hydrolysis<sup>14)</sup> and the presence of rich amount of the furostanol glycosides is suggested by the detailed TLC examinations (detection by Ehrlich reagent)<sup>15)</sup> of the total glycosides of the above plant.

Protometeogenin seems to be the first naturally occurring spirostanol having a 2 $\beta$ ,3 $\beta$ -di-hydroxy-4-ene moiety which is readily aromatized on acid treatment although ruscogenin (having a 1 $\beta$ ,3 $\beta$ -dihydroxy-5-ene moiety (12)) has been shown to give luvigenin (5) on acid treatment.<sup>16)</sup>

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#### References and Footnotes

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