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STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—XLVI[1]

CONSTITUENTS OF HOSTA SPECIES (3)[1] $\Delta^{25(27)}$-SAPOGENINS

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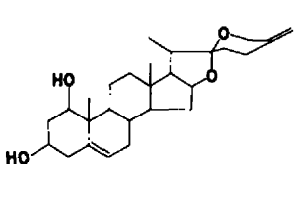
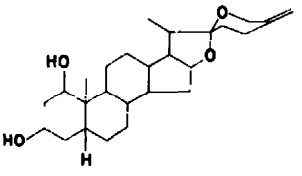
Abstract—Based on the isolation of neoruscogenin (I) and convallamarogenin (II), there may be many kinds of $\Delta^{25(27)}$-sapogenins in the vegetable kingdom. The steroidal constituents of *Hosta kiyosumiensis* F. Maek. have been investigated, and four $\Delta^{25(27)}$-sapogenins isolated—$\Delta^{25(27)}$-gitogenin (XIIa), $\Delta^{25(27)}$-manogenin (XIII), $\Delta^{25(27)}$-9-dehydromanogenin (XIV) and $\Delta^{25(27)}$-tigogenin (XV).

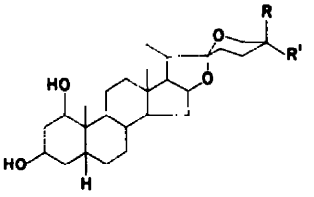
RECENTLY, neoruscogenin (I) and convallamarogenin (II) have been isolated by Robert,[2] Tschesche[3] and by our group,[4] and their structures established as steroidal sapogenins having a double bond at C-25(27). Robert hypothesized that neoruscogenin (I) exists as its 25-hydroxy derivative in the plant and may be obtained by dehydration of the hydroxyl group at C-25 on saponification of its saponin, and that neoruscogenin



I



II



III : R = CH_3, R' = OH
IV : R = OH, R' = CH_3

is not a natural product but an artifact. During structural studies on steroidal components of *Reineckia carnea* Kunth we isolated two steroidal sapogenins —reineckiagenin[4] (III) and isoreineckiagenin[4] (IV)—having an hydroxyl group at C-25. The hydroxyl group of III or IV at C-25 could not be dehydrated to give convallamarogenin (II) under the conditions of saponification of its saponin. Moreover, assuming that the hydroxyl group of III or IV at C-25 may exist as the ester of some fatty acid, the acetate at C-25 of III or IV was treated with acid or alkali. In this case, the acetate was hydrolysed and the starting material (III or IV) recovered. Dehydration of III or IV with thionyl chloride–pyridine or phosphorus oxychloride–pyridine always affords derivatives having an ethylenic double bond in the F-ring, as already reported.[4]

[1] Part XLV: K. Takeda, T. Okanishi, H. Minato and A. Shimaoka, *Chem. Pharm. Bull. Japan* **12**, 779 (1964).
[2] J. Robert, R. Vaupré and G. Poiget, *C.R. Acad. Sci., Paris* **250**, 3187 (1960).
[3] R. Tschesche, H. Schwarz and G. Snatzke, *Chem. Ber.* **94**, 1699 (1961).
[4] K. Takeda, T. Okanishi, H. Minato and A. Shimaoka, *Tetrahedron* **19**, 759 (1963).

As these results are contrary to Robert's hypothesis, the $\Delta^{25(27)}$-sapogenin is not a dehydration product of a 25-hydroxy sapogenin but a natural product. If this is the case, it is reasonable to assume that there are many kinds of $\Delta^{25(27)}$-sapogenins in the vegetable kingdom. From this point of view, a reinvestigation of steroidal sapogenins was undertaken and in particular as we were investigating the steroidal components of *Hosta*[1,5] species, the components of *Hosta kiyosumiensis* F. Maek. were examined.

TABLE 1. SAPOGENINS OBTAINED FROM THE CHLOROFORM EXTRACTS

	R_f value	Yield (mg) Hypogeous part	Epigeous part
Tigogenin (V)	0·58	*	2
Hecogenin (VI)	0·50	*	*
9-Dehydrohecogenin (VII)	0·48	*	*
Gitogenin fraction	0·33	1,520	184
Manogenin fraction	0·21	122	6
9-Dehydromanogenin fraction	0·19	280	18

($CHCl_3$–acetone-MeOH—15:5:1)

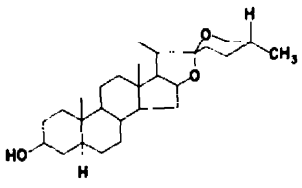
* Although the presence of these compounds were observed in the thin layer chromatogram, they were not isolated.

The isolation of the steroidal components was performed under conditions similar to those employed for other *Hosta* species.[1] From the hypogeous (1·2 kg) and the epigeous parts (800 g) of the dried plant, 10 g and 2 g of the chloroform extract were obtained respectively. As the thin layer chromatograms of these extracts are similar to that of *H. montana* F. Maekawa *var. liliiflora* F. Maekawa,[1] they were chromatographed on alumina and separated into the fractions as shown in the Table. The chloroform extract of the hypogeous part, yielded fractions corresponding to tigogenin (V), hecogenin (VI) and 9-dehydrohecogenin (VII) in very small quantities, the presence of these sapogenins was based on a comparison of the R_f values of thin layer chromatograms. The gitogenin fraction was rechromatographed on alumina to give the crude product (1·52 g), which was separated into gitogenin (VIIIa, 920 mg) and a compound (XIIb), m.p. 226–228°, $[\alpha]_D$ −97·1° (58 mg) by recrystallization and preparative thin layer chromatography of its acetate.

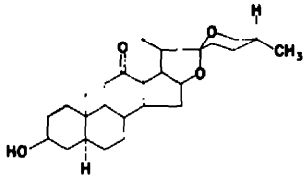
Compound XIIb was saponified with 5% sodium carbonate–methanol to give XIIa, $C_{27}H_{42}O_4$, m.p. 266–267°, $[\alpha]_D$ −80·1°, R_f value of 0·35 (35 mg). The IR spectrum of XIIa shows frequencies at 1658 and 878 cm^{-1} corresponding to the $>C{=}CH_2$ type double bond, and the shape of the absorption bands at 1100–800 cm^{-1} is very similar to that of convallamarogenin[6] (II, Fig. 1). Moreover, the NMR spectrum of XIIa shows a signal at 5·27 τ due to the hydrogen of the $>C{=}CH_2$ group. This value is in good agreement with the value of the same type proton of convallamarogenin[7] (II, 5·27 τ). Based on these results and the facts that XIIa is obtained together with gitogenin (VIIIa) and has a R_f value similar to that of gitogenin, it is reasonable to suppose that XIIa is $\Delta^{25(27)}$-gitogenin. Then, the diacetate (XIIb) was catalytically hydrogenated with 10% Pd–C in ethanol to give gitogenin diacetate (VIIIb) and neogitogenin diacetate (IXb) in almost the same yield. Compound XIIa, therefore, was established to be $\Delta^{25(27)}$-gitogenin.

[5] K. Takeda, T. Okanishi and A. Shimaoka, *Ann. Rept. Shionogi Res. Lab.* **5**, 633 (1955).
[6] K. Takeda, H. Minato, A. Shimaoka and Y. Matsui, *J. Chem. Soc.* 4815 (1963).
[7] H. Minato and A. Shimaoka, *Chem. Pharm. Bull. Japan* **11**, 876 (1963).

When the mother liquor of the crude acetate (XIIb) obtained by preparative thin layer chromatography was oxidized with osmium tetroxide and chromatographed on alumina to separate the oxidation product, neogitogenin acetate (lXb, 5 mg) was obtained. From this result, it is obvious that the gitogenin fraction is a mixture of



V



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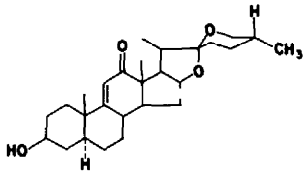
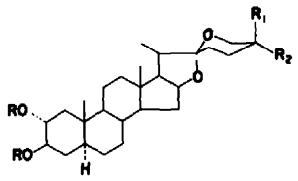
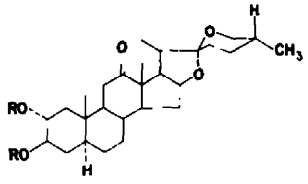


VII

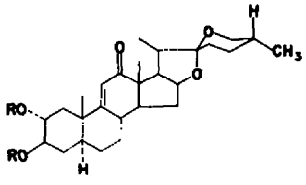


VIII a : R = H , R_1 = H , R_2 = CH_3
b : R = Ac , R_1 = H , R_2 = CH_3

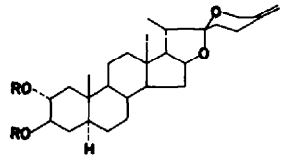
IX a : R = H , R_1 = CH_3 , R_2 = H
b : R = Ac , R_1 = CH_3 , R_2 = H



X a, b

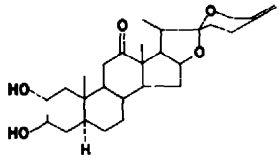


XI a, b

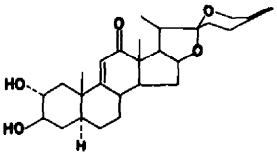


XII a, b

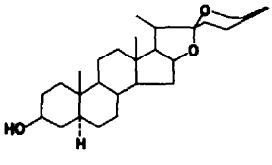
a : R = H
b : R = Ac



XIII



XIV



XV

gitogenin, neogitogenin and $\Delta^{25(27)}$-gitogenin. The fact that $\Delta^{25(27)}$-gitogenin (XIIa) exists together with the ordinary (25D- and 25L-)sapogenin in the gitogenin fraction prompted an investigation of other fractions.

The fraction (410 mg) containing manogenin (X) and 9-dehydromanogenin (XI) was refluxed with Girard reagent T in ethanol for 10 minutes to separate it into each fraction. The manogenin fraction (122 mg) afforded manogenin (X, 30 mg) and the crude compound (XIII, 7 mg) by alumina and preparative thin layer chromatography and recrystallization. The 9-dehydromanogenin fraction (280 mg) also gave 9-dehydromanogenin (XI, 60 mg) and crude XIV (12 mg) by the same treatment. As

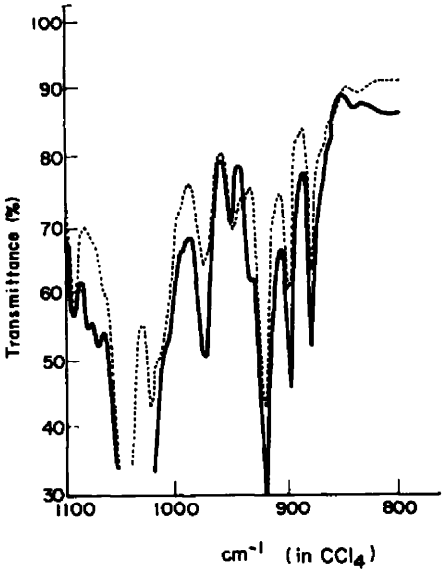
the IR spectrum of XIII or XIV shows a frequency at 878 cm^{-1} corresponding to the $>C{=}CH_2$ type double bond and characteristic bands of $\Delta^{25(27)}$-sapogenin at 1100–800 cm^{-1}, it is assumed to be $\Delta^{25(27)}$-manogenin or $\Delta^{25(27)}$-9-dehydromanogenin and combined with the corresponding fraction obtained from the epigeous part of the plant, and it is further purified as mentioned later.

The chloroform extract of the epigeous part was fractionated by a manner similar to that used for the hypogeous part and the resulting fractions are shown in the Table. The total yield of the sapogenins from the epigeous part was low in comparison with that of the hypogeous part, and especially the fractions other than the gitogenin were obtained in poor yield.

The gitogenin fraction was purified to give a crystalline substance (102 mg), which was a mixture (ca. 6:4) of $\Delta^{25(27)}$-gitogenin (XIIa) and gitogenin (VIIIa). The fraction containing manogenin and 9-dehydromanogenin was treated with Girard reagent T to afford the manogenin fractions (6 mg) and the 9-dehydromanogenin fraction (18 mg), which gave crude XIII (3 mg) and crude XIV (8 mg), respectively.

The crude XIV was recrystallized to give colourless needles, $C_{27}H_{38}O_5$, m.p. 230–232, $[\alpha]_D$ −36·1°, λ_{max} 237 mμ (ϵ 11,700), R_f value of 0·20. As its IR spectrum shows frequencies at 1658 and 878 cm^{-1} and the NMR spectrum a signal at 5·25 τ, XIV is confirmed as $\Delta^{25(27)}$-9-dehydromanogenin. The crude XIII was also recrystallized to give colourless needles, $C_{27}H_{40}O_5$, m.p. 238–240°, R_f value of 0·22, which was concluded to be $\Delta^{25(27)}$-manogenin by its IR spectrum. Moreover, although the tigogenin fraction (2 mg) could not be isolated in the pure state, it was assumed to be $\Delta^{25(27)}$-tigogenin (XV) by its IR spectrum.

As mentioned above, we could isolate the expected $\Delta^{25(27)}$-sapogenins from *H. kiyosumiensis* F. Maek. The biochemical interrelation between the $\Delta^{25(27)}$-sapogenin and the ordinary 25D- or 25L-sapogenin or 25-hydroxylated sapogenin is now under investigation in our laboratory.



EXPERIMENTAL

All m.ps were taken on a Kofler hot-stage apparatus. UV spectra were taken in 95% EtOH, IR spectra in $CHCl_3$ and NMR spectra in $CDCl_3$ with a Varian A-60 NMR spectrometer. Optical rotations are for $CHCl_3$ solutions. Thin-layer chromatography was carried out with "Merck", Kieselgel G and the solvent system of $CHCl_3$–acetone–MeOH (15:5:1).

Isolation and fractionation of sapogenins from the plant. As described in the preceding paper,[1] the dried and sliced plant was extracted with 80% hot MeOH giving a deep brown syrup, which was extracted with ether. The ether-insoluble residue was saponified with 5% H_2SO_4 in 50% EtOH and then with 5% KOH in MeOH, and extracted with $CHCl_3$. The $CHCl_3$-extract was chromatographed on alumina (Table).

$\Delta^{25(27)}$-*Gitogenin* (XIIa). Gitogenin fraction (2·15 g) was rechromatographed on alumina to give a crystalline substance (1·52 g), which was recrystallized from $CHCl_3$–MeOH affording gitogenin (VIIIa, 920 mg), m.p. 266–268°, and a mixture (200 mg) of gitogenin and $\Delta^{25(27)}$-gitogenin. This mixture was acetylated and gave crude $\Delta^{25(27)}$-*gitogenin diacetate* (XIIb, 70 mg) by preparative thin-layer chromatography, which was recrystallized from MeOH to give XIIb (58 mg), colourless prisms, m.p. 226–228°, $[\alpha]_D^{24}$ −97·1° ±6° (*c*, 0·337). (Found: C, 72·6; H, 9·25. $C_{31}H_{46}O_6$ requires: C, 72·35; H, 9·0%.) Compound XIIb (58 mg) was saponified with 5% Na_2CO_3–MeOH to give XIIa, colourless needles (35 mg, from MeOH), m.p. 266–267°, $[\alpha]_D^{25}$ −80·1° ±4° (*c*, 0·507), R_f 0·37, ν_{max} 1658 and 878 cm^{-1}, 5·27 τ. (Found: C, 75·4; H, 9·95. $C_{27}H_{42}O_4$ requires: C, 75·3; H, 9·85.) The mother liquor after recrystallization of XIIb was oxidized with O_3O_4 and chromatographed on alumina to separate XIIb as dihydroxygitogenin diacetate, giving neogitogenin diacetate (IXb, 5 mg), colourless needles (from hexane), m.p. 212–215°.

Hydrogenation of $\Delta^{25(27)}$-*gitogenin diacetate* (XIIb). Compound XIIb (50 mg) in EtOH (5 ml) was catalytically hydrogenated with 10% Pd–C and the product recrystallized from MeOH to give gitogenin diacetate (VIIIb, 15 mg), colourless needles, m.p. 242–244°. The mother liquor after recrystallization was separated by preparative thin layer chromatography giving neogitogenin diacetate (IXb, 13 mg), colourless needles, m.p. 213–214° (from hexane).

$\Delta^{25(27)}$-*Manogenin* (XIII) *and* $\Delta^{25(27)}$-*9-dehydromanogenin* (XIV). The fraction (410 mg) containing manogenin (X) and 9-dehydromanogenin (XI) was refluxed with Girard reagent T in EtOH for 10 min to separate into manogenin fraction (122 mg) and 9-dehydromanogenin fraction (280 mg) as already reported.[1] Each fraction was separated into manogenin (X), colourless needles (30 mg), m.p. 243–245° (from MeOH) and crude XIII (7 mg) or into 9-dehydromanogenin (XI), colourless prisms (60 mg), m.p. 235–237° (from MeOH–ethyl acetate) and crude XIV (12 mg) by the same manner as the gitogenin fraction. The crude XIV was recrystallized from MeOH–hexane to give $\Delta^{25(27)}$-9-dehydromanogenin (XIV), colourless needles, m.p. 230–232°, $[\alpha]_D^{27}$ −36·1° ±6°, (*c*, 0·382), R_f 0·20, λ_{max} 237 mμ (ε 11,700), ν_{max} 1673, 1658, 1601 and 878 cm^{-1}, 5·25 τ. (Found: C, 72·9; H, 8·8. $C_{27}H_{38}O_5$ requires: C, 73·25; H, 8·65%.) The diacetate crystallized as colourless prisms, m.p. 246–248°. (Found: C, 70·7; H, 8·4. $C_{31}H_{42}O_7$ requires: C, 70·7; H, 8·05%.) The crude XIII was recrystallized from acetone to give $\Delta^{25(27)}$-manogenin (XIII), colourless needles, m.p. 238–240°, R_f 0·22, ν_{max} 1657 and 878 cm^{-1}. (Found: C, 70·3; H, 9·2. $C_{27}H_{40}O_5 \cdot H_2O$ requires: C, 70·1; H, 9·15%.)