UDC 547.918 : 582.572.7

66. Tatsuo Yamauchi: Saponins of Japanese *Dioscoreaceae*. IX.[1b] Hydrolysis of Diosgenin Glycosides.

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In the previous papers of this series,[1] it was reported that $\Delta^{3,5}$-desoxytigogenin (25D-spirosta-3,5-diene) (I) was isolated, besides diosgenin, from the hydrolysates of crude saponins or methanolic extracts of Dioscorea rhizomes with hydrochloric acid. Since pure dioscin and diosgenin itself also afforded (I) under similar conditions, (I) seemed to be an artifact formed during the hydrolysis of diosgenin glycosides. Almost the same time, Peal[2] reported the same findings and he investigated quantitatively (gravimetrically) the formation of (I) from diosgenin and Dioscorea saponin extracts.

This study was started with an intention of finding the optimum hydrolytic condition for diosgenin glycosides which minimizes the formation of (I).

$\Delta^{3,5}$-Steroids, like (I), cholesta-3,5-diene, or solanida-3,5-diene, show characteristic UV absorption at 235 mμ due to 3,5-diene system, this absorbance following the Beer's law (Fig. 1) and scarcely influenced by co-existence of corresponding Δ^{5}-3-ol such as diosgenin, cholesterol, or solanidine (Fig. 2). The amount of (I) in the binary mixture

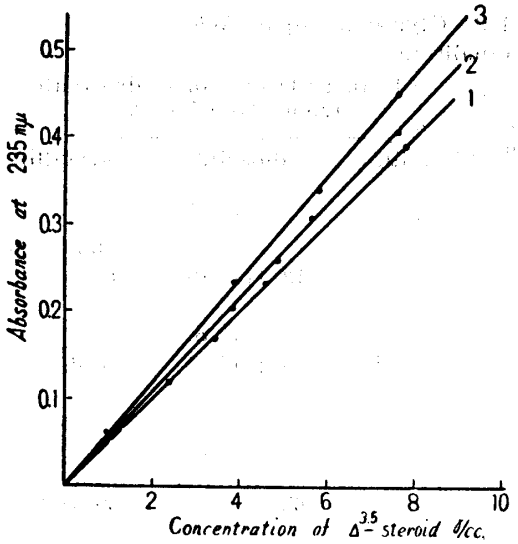

Fig. 1. Absorbance of $\Delta^{3,5}$-Steroid (in EtOH)
1 25D-Spirosta-3,5-diene
2 Cholesta-3,5-diene
3 Solanida-3,5-diene

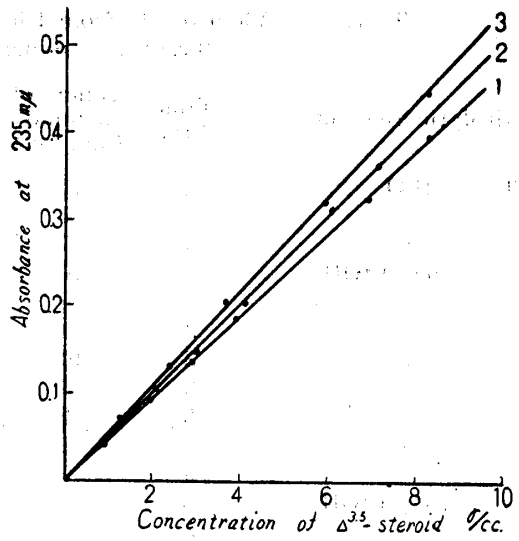

Fig. 2. Absorbance of $\Delta^{3,5}$-Steroid in a Binary Mixture of $\Delta^{3,5}$-Steroid and Δ^{5}-Steroid (in EtOH)
1 25D-Spirosta-3,5-diene (I) mixed with diosgenin
2 Cholesta-3,5-diene mixed with cholesterol
3 Solanida-3,5-diene mixed with solanidine
(Concentration of the binary mixture, 10 γ/cc.)

of diosgenin and (I) was reproducible with reasonably good accuracy using this absorption curve after the procedure of ether extraction (Table I). Glucose and rhamnose were partly decomposed by the hydrolytic reagent, and the ether-soluble portion of the decomposition products showed slight absorbance at 235 mμ, whose effect on the estima-

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1) a) Part VII: T. Tsukamoto, T. Kawasaki, Y. Shimauchi: Yakugaku Zasshi, 77, 1221(1957).
b) Part VIII: T. Tsukamoto, T. Kawasaki, T. Yamauchi: *Ibid.*, 77, 1225(1957); T. Tsukamoto, T. Kawasaki, T. Yamauchi, Y. Shimauchi: This Bulletin, 5, 492(1957).
2) W. J. Peal: Chem. & Ind. (London), 1957, 1451.

Table I. Estimation of 25D-Spirosta-3,5-diene (I) in the Mixture of (I) and Diosgenin

Original				Found	
Mixture of (I) and diosgenin (mg.)			Yield of (I) (%)	Ether extract (mg.)	Yield of (I) (%)
41.4	(I)	10.8	26.1	40.6	26.6
	diosgenin	30.6			
37.6	(I)	19.9	52.9	37.6	53.2
	diosgenin	17.7			
40.3	(I)	28.3	70.2	40.5	72.9
	diosgenin	12.0			

Table II. Yield of Ether-soluble Product from Sugar Decomposition

acid[a]	2N-HCl-75% EtOH	2N-HCl-75% EtOH	2N-HCl-50% EtOH	2N-HCl-50% EtOH	2N-HCl	4N-H_2SO_4-50% EtOH	4N-H_2SO_4
Glucose (mg.)	100		48.7	92.7	44.3	46.6	43.0
Rhamnose (mg.)		100	100.5	40.0	92.2	91.2	95.2
Ether-soluble portion of the decomposition products (mg.)	1.0	2.0	1.7	1.3	9.2	1.3	2.5
Calcd. for (I)[b] (mg.)	0.2	0.8	0.1	0.1	2.6	0.1	0.6

a) Boiled with 10 cc. of acid for 3 hrs.
b) The standard curve in Fig. 2 was used for estimation.

Table III. Yield of (I) from Diosgenin and its Glycosides upon Acid Treatment under Various Conditions

Hydrolytic reagent		Time (hrs.)	Concn. of sample (mg./cc.)	Yield of (I) from diosgenin (%)	(I) in Et_2O ext. of hydrolysate from glycoside (%)		
					trillin	dioscin*	gracillin
2N HCl	H_2O	2	10			4.1 (47.0)	
〃	〃	2	4			5.2 (47.3)	
〃	〃	3	5				4.7 (45.6)
〃	50% EtOH	2	10			12.4 (47.0)	15.4
〃	〃	2	5	4.2			
〃	〃	3	20			11.9 (45.8)	
〃	〃	3	10		12.9	18.4 (48.1)	27.0
〃	〃	3	5	8.3			
〃	〃	3	2	29.4			
〃	〃	3	1	36.0			
〃	〃	5	5	9.8			
〃	75% EtOH	3	10			63.0	69.4 (46.0)
〃	〃	3	5	21.5			
〃	〃	3	2	37.2			
〃	〃	3	1	42.8			
〃	100% EtOH	3	5	17.9			
4N HCl	50% EtOH	5	10			67.0	
〃	〃	5	5	61.5			
〃	50% EtOH + benzene (1:1)	5	20			12.0 (47.1)	
〃	75% EtOH	5	5	64.2			
2N H_2SO_4	50% EtOH	17	5	7.4			
4N H_2SO_4	H_2O	2	10			2.9 (47.2)	
〃	〃	2	4			5.1 (46.3)	
〃	50% EtOH	3	10			13.8	15.9 (45.5)
〃	〃	5	5	7.5			
〃	75% EtOH	5	5	64.2			
〃	100% EtOH	5	5	45.3			
2N HBr	50% EtOH	3	5	21.2			
4N HBr	50% EtOH	5	5	75.2			

* Figures in parentheses indicate the yield of ether-soluble portion of the hydrolysate (theoretical yield of aglycone : 46.3% of dioscin, 45.5% of gracillin)

tion of (I) seemed to be negligible (Table II). Therefore, $\Delta^{3,5}$-steroid in the hydrolysate was found to be assayed in a micro-scale by extracting steroid with ether followed by photometric estimation.

Yields of (I), main by-product formed from diosgenin, and its glycosides upon acid treatment in various conditions were determined with this method.

Concerning the formation of (I) from diosgenin, as shown in Table III, sulfuric acid was the mildest reagent of three acids, and hydrochloric acid and hydrobromic acid followed it. The yield of (I) was increased from 4.2% to 64.2% under the conditions of boiling with hydrochloric acid, with increased concentration of the acid, and of ethanol, dilution of sample, and time of heating.

In consideration of the above results, yields of (I) from diosgenin glycosides, dioscin,*1 gracillin,*2 and trillin*3 were then examined in order to find any effect of the sugar moiety (Table III). Influence of various hydrolytic conditions on the formation of (I) from glycosides, that is, those of kind and concentration of acid, concentration of ethanol, dilution of samples, and time of heating, were nearly parallel to the case of diosgenin. Generally it seemed that the glycosides were more liable than diosgenin to yield (I). The yield of (I) on the hydrolysis of dioscin varied from 2.9% to 67.0% under the conditions examined.

On the other hand, it was clarified, from the yield of the ether-soluble portion of the hydrolysate, that complete hydrolysis of dioscin occurred on boiling with 2*N* hydrochloric acid or 4*N* sulfuric acid for 2 hours, and of gracillin with 2*N* hydrochloric acid for 3 hours as well as under the conditions reported previously.[3,4]

Taking into account both results in Table III and those of complete hydrolysis described above, it seemed to be most suitable, as a condition for obtaining diosgenin from the glycosides, to reflux dioscin with 4*N* sulfuric acid (10 mg./cc.) for 2 hours (yield of (I) : 2.9%), and to reflux gracillin with 2*N* hydrochloric acid (5 mg./cc.) for 3 hours (yield of (I) : 4.7%).

In contrast to diosgenin and its glycosides, the formation of $\Delta^{3,5}$-diene compound from corresponding Δ^{5}-3-ol was examined in the same way (Table IV). The samples used were *epi*-diosgenin, 25D-spirost-4-en-3-ol,[5] cholesterol, cholesterol glucoside,[6] *epi*-cholesterol,[7] solanidine, and solanin. Concerning two Δ^{5}-3β-ols which have different C-17 side chain, solanidine was less and cholesterol was more stable than diosgenin upon acid treatment. Two Δ^{5}-3α-ols were much easier to form $\Delta^{3,5}$-diene compounds in comparison with their 3β-isomers, and 25D-spirost-4-en-3-ol was quantitatively converted into $\Delta^{3,5}$-compound under a milder condition such as in the case of cholest-4-en-3-ol.[8] Similar to diosgenin glycosides, cholesterol glucoside and solanin were somewhat more sensitive than their aglycones.

Since 3-hydroxy group in dehydro-*epi*-androsterone[9] was reported to be replaced by chlorine atom by hydrochloric acid solution as well as by thionyl chloride or phosphorus pentachloride, 3-chloro compound also seemed possible to be formed on hydrolysis of diosgenin glycoside with hydrochloric acid. Diosgenin boiled with 4*N*

*1 Saponin composed of 1 mole each of diosgenin and glucose, and two of rhamnose.
*2 Saponin composed of 1 mole each of diosgenin and rhamnose, and two of glucose.
*3 Diosgenin α-glucoside.

3) T. Tsukamoto, T. Kawasaki, T. Yamauchi : This Bulletin, **4**, 35(1956).
4) T. Tsukamoto, T. Kawasaki : *Ibid.*, **4**, 104(1956).
5) F. Sondheimer, C. Amendolla, G. Rosenkranz : J. Am. Chem. Soc., **75**, 5930(1953).
6) C. Meystre, K. Miescher : Helv. Chim. Acta, **27**, 234(1944).
7) P. A. Plattner, *et al.* : *Ibid.*, **27**, 1872(1944); **31**, 1455(1958).
8) J. C. Eck, R. L. Van Peusem, E. W. Hollingsworth : J. Am. Chem. Soc., **61**, 171(1939).
9) A. Butenandt, H. Dannenbaum : Z. physiol. Chem., **229**, 192(1934).

Table IV. Yield of $\Delta^{3,5}$-Diene Compounds from corresponding Δ^5-3-ols and Their Glycosides

Condition: Acid Reagent		Time (hr.)	Concn. of sample (mg./cc.)	Sample: *epi*-Diosgenin (%)	25D-Spirost-4-en-3-ol (%)	Cholesterol (%)	Cholesterol glucoside* (%)	*epi*-Cholesterol (%)	Solanidine (%)	Solanin (%)
1*N* HCl	75% EtOH	1	5		94.0					
〃	H_2O	1	10							26.8
〃	50% EtOH	1	5	11.9	102.4					
〃	〃	3	5	20.4				4.2		
2*N* HCl	50% EtOH	3	10				5.2			46.4
〃	〃	3	5	30.5		3.7		28.6		
〃	〃	3	1			15.0			28.6	
〃	75% EtOH	3	10				34.0			
〃	〃	3	5	53.5		14.7		51.2		
〃	〃	3	1			21.3				
〃	100% EtOH	3	5	43.3		21.6		67.5		
4*N* HCl	50% EtOH	5	5			19.6				
〃	75% EtOH	5	5			69.0				
〃	100% EtOH	5	5			29.8				
1*N* H_2SO_4	H_2O	2	10							24.8
4*N* H_2SO_4	50% EtOH	5	5			2.7				
〃	75% EtOH	5	5			20.0				

* Figures indicate percentage of $\Delta^{3,5}$-diene compound in the ether extract of the hydrolysate.

hydrochloric acid in 50% ethanol for 5 hours, followed by repeated alumina chromatographic separation, gave 3-chloro-25D-spirost-5-ene*4 (II) in 9% yield. The yield was much less than that of (I) (63%), and the milder the condition, the less the yield of (II). Influence of (II) to the yield of diosgenin on the hydrolysis of the glycosides, therefore, would probably be negligible.

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Experimental

Paper Chromatography—Filter paper: Toyo Roshi No. 50. Solvent: MeOH (paraffin-impregnated paper),[10] ascending method. Spray reagent: $SbCl_3$ in $CHCl_3$.

Quantitative Determination of $\Delta^{3,5}$-Steroids—1) Standard Material: 25D-Spirosta-3,5-diene was prepared according to the method of Wall, *et al.*[11] m.p. 164°, $[\alpha]_D^{11}$ −177°(c=0.52, $CHCl_3$); Rf 0.18; UV λ_{max}^{EtOH} mμ (log ε): 228 (4.28), 235 (4.31), 243 (4.09). *Anal.* Calcd. for $C_{27}H_{40}O_2$: C, 81.76; H, 10.17. Found: C, 81.50; H, 10.25.

Cholesta-3,5-diene was prepared according to the method of Fudge, *et al.*[12] m.p. 79°, $[\alpha]_D^{15}$ −106° (c=0.35, $CHCl_3$); Rf 0.04; UV λ_{max}^{EtOH} mμ (log ε): 228 (4.29), 235 (4.32), 243 (4.21). *Anal.* Calcd. for $C_{27}H_{44}$: C, 87.97; H, 12.03. Found: C, 87.50; H, 11.94.

Solanida-3,5-diene was prepared by heating solanine with *N* HCl for 1 hr. on a water bath,[13] followed by alumina chromatography and recrystallization from Me_2CO. m.p. 167°, $[\alpha]_D^{13}$ −81°(c=0.40, $CHCl_3$); Rf 0.12; UV λ_{max}^{EtOH} mμ (log ε): 228 (4.34), 235 (4.60), 243 (4.17). *Anal.* Calcd. for $C_{27}H_{41}N$: C, 85.40; H, 10.92. Found: C, 85.57; H, 10.93.

2) Absorbance of $\Delta^{3,5}$-Steroid in Various Concentrations—The absorbances of three $\Delta^{3,5}$-steroids at 235 mμ were measured in the concentration of 1~10 γ/cc. of EtOH, using Hitachi spectrophoto-

*4 According to Shoppee's description, configuration of C_3-Cl should be β (C. W. Shoppee: J. Chem. Soc., **1946**, 1147).

10) I. Nishioka: Yakugaku Zasshi, **78**, 1428(1958).
11) M. E. Wall, S. Serota: J. Am. Chem. Soc., **78**, 1747(1956).
12) A. J. Fudge, C. W. Shoppee, G. H. R. Summers: J. Chem. Soc., **1954**, 958.
13) S. Soltys: Ber., **66**, 762(1933); F. Bergel, R. Wagner: *Ibid.*, **66**, 1093(1933).

meter EPU-2. The absorbance of $\Delta^{3,5}$-steroid in the binary mixture of $\Delta^{3,5}$-steroid and genuine Δ^{5}-steroid in EtOH (10 γ of the mixture per cc.), was measured in the same way. The results are shown in Figs. 1 and 2.

3) Reproducibility of (I) by the Absorption Curve—A known amount of (I) and diosgenin (total, 40 mg.) was suspended in 2*N* HCl (8 cc.) and extracted 3 times with Et_2O (total, 30 cc.). The Et_2O solution was washed once with water, dried over Na_2SO_4, and evaporated. The residue was dried over $CaCl_2$ *in vacuo* and weighed. The residue was dissolved in EtOH (10 γ/cc.) for measuring absorbance at 235 mμ. The amount of (I) in the residue, found using the standard curve in Fig. 2, is shown in Table I.

4) Influence of Sugar Decomposition Products on the Estimation of (I)—A known amount of glucose, rhamnose, or a mixture of both, was boiled with acid reagent, EtOH in the reagent was removed, and 10 cc. of water was added. The solution was extracted with Et_2O, the Et_2O extract was dissolved in EtOH, and absorbance at 235 mμ was measured. The value calculated for (I) therefrom is shown in Table II.

5) Samples—Diosgenin, m.p. 207°; dioscin, m.p. 270°(decomp.) (cf. Part II[14] of this series); gracillin, m.p. 283°(decomp.) (cf. Part IV[15]); trillin, m.p. 260°(decomp.), prepared by partial hydrolysis of dioscin (cf. Part V[3]); *epi*-diosgenin, m.p. 237°, prepared by the procedure described below; 25D-spirost-4-en-3-ol, prepared by the method of Sondheimer, *et al.*,[5] m.p. 178°, $[\alpha]_D^{14}$ −39°(c=0.66, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.26; H, 10.14. Found: C, 77.87; H, 10.51; cholesterol, m.p. 149°, $[\alpha]_D^{20}$ −39°(c=1.35, $CHCl_3$); cholesterol glucoside, prepared by the method of Meystre, *et al.*,[6] m.p. 241°(decomp.); *epi*-cholesterol, prepared by the method of Plattner, *et al.*,[7] m.p. 143°, $[\alpha]_D^{13}$ −43° (c=0.41, $CHCl_3$); solanidine, m.p. 241°, $[\alpha]_D^{9}$ −56°(c=0.42, $CHCl_3$); solanine, extracted from the sprout of *Solanum tuberosum*, and purified by alumina chromatography and recrystallization; m.p. 241° (decomp.), $[\alpha]_D^{13}$ −60°(c=0.81, pyridine).

6) Procedure for Assay—About 20 mg. of sapogenin or sterol was boiled with acid reagent.*5 EtOH in the reagent was removed *in vacuo* and water (10 cc.) was added to the residue. The solution was extracted 3 times with Et_2O (total, 30 cc.) and Et_2O solution was treated as in (3). In glycosides, 30~100 mg. of the sample was used. The yield of $\Delta^{3,5}$-steroid, found from the standard curve in Fig. 2, is shown in Tables III and IV.

Determination of Complete Hydrolysis—Dioscin or gracillin (50~100 mg.) was boiled with acid reagent. When EtOH was used, it was removed *in vacuo* and water was added to the residue. The solution was extracted with Et_2O and Et_2O layer was treated as mentioned above. Yield of the ether extract is shown in Table III with figures in parentheses.

***epi*-Diosgenin**—*epi*-Diosgenin was synthesized from diosgenin 5,6-epoxide (25D-spirostan-3β-ol 5,6α-epoxide) according to the procedure of Plattner, *et al.*[7] who synthesized *epi*-cholesterol from cholesterol via cholestane-3β,5-diol.

Diosgenin 5,6-epoxide [reported, m.p. 190°, $[\alpha]_D^{29}$ −120°(c=1.35, $CHCl_3$)[16]] was acetylated and the 3-acetate [m.p. 230°, $[\alpha]_D^{29}$ −126°(c=0.63, $CHCl_3$)] was hydrogenated with $LiAlH_4$ and 25D-spirostane-3β,5α-diol, m.p. 260°(decomp.), $[\alpha]_D^{20}$ −65°(c=0.45, $CHCl_3$), was obtained. *Anal.* Calcd. for $C_{27}H_{44}O_4 \cdot H_2O$: C, 71.96; H, 10.29. Found: C, 72.52; H, 10.51.

3-Tosylate of the 3β,5α-diol was refluxed for 4.5 hr. with $CHCl_3$, diethylaniline, and AcCl, followed by alumina chromatography and saponification with methanolic KOH. Crude crystals, chromatographed, and recrystallized from MeOH, gave *epi*-diosgenin, m.p. 237°, $[\alpha]_D^{13}$ −111°(c=0.41, $CHCl_3$); IR λ_{max}^{Nujol} μ: 10.20(s), 10.90(m), 11.10(s), 11.55(w) (25D-spiroketal); Rf 0.70 (diosgenin 0.75). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.26; H, 10.14. Found: C, 78.54; H, 10.16.

Liebermann-Burchard reaction, positive; Kariyone-Hashimoto reaction, wine red (40°); digitonin reaction, no precipitation occurred after standing for 48 hr.

Acetylation of *epi*-diosgenin with Ac_2O and pyridine at room temperature, followed by recrystal lization from MeOH, gave *epi*-diosgenin acetate, m.p. 191°, $[\alpha]_D^{13}$ −74°(c=0.36, $CHCl_3$); Rf 0.48 (diosgenin acetate, 0.43). *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.30; H, 9.64. Found: C, 76.31; H, 9.60.

3-Chloro-25D-spirost-5-ene (II) from Diosgenin—1) Diosgenin (0.6 g.) was refluxed with EtOH (60 cc.) and 8*N* HCl (60 cc.) for 5 hr. The reaction mixture was evaporated to one-half the volume *in vacuo*, diluted with water, and extracted with Et_2O. Et_2O extract (555 mg.) was dissolved in petr. ether and chromatographed on alumina. Majority of the first fraction (petr. ether 115 cc., eluant

*5 The sample was previously dissolved in EtOH and then conc. acid was added.

*6 When insoluble substance (partially hydrolysed saponin) remained as intermediate layer in ether extraction and the yield of ether extract was found less than that of the theoretical, the hydrolytic cleavage was regarded as incomplete.

14) T. Tsukamoto, T. Kawasaki, A. Naraki, T. Yamauchi: Yakugaku Zasshi, **74**, 984(1954).
15) T. Tsukamoto, T. Kawasaki: *Ibid.*, **74**, 1127(1954).
16) T. Tsukamoto, Y. Ueno, T. Ota: *Ibid.*, **57**, 985(1937).

405 mg.) was identified as (I) by mixed fusion with the authentic specimen (yield, 350 mg., 63%). The intermediate fraction between (I) and diosgenin, which was eluted by a large quantity of petr. ether, was collected and purified by alumina chromatography. Repeated crystallization of the eluant (50 mg.) from Me_2CO afforded fine needles, m.p. 213°, $[\alpha]_D^{14}$ −101°(c=0.48, $CHCl_3$). Liebermann-Burchard and Beilstein reactions, positive; IR λ_{max}^{Nujol} μ: 10.23(s), 10.92(m), 11.14(s), 11.55(m) (25D-spiroketal). *Anal.* Calcd. for $C_{27}H_{41}O_2Cl$: C, 74.88; H, 9.54. Found: C, 75.04; H, 9.64. A mixed m.p. with authentic 3-chloro-25D-spirost-5-ene,[17] prepared from diosgenin and PCl_5 [m.p. 214°, $[\alpha]_D^9$ −99°(c=0.39, $CHCl_3$); IR λ_{max}^{Nujol} μ: 10.20, 10.92, 11.11, 11.53. *Anal.* Calcd. for $C_{27}H_{41}O_2Cl$: C, 74.88; H, 9.54. Found: C, 74.77; H, 9.52], gave no depression and IR spectra of the two were quite identical.

2) Diosgenin (0.4 g.) was refluxed with EtOH (40 cc.) and 4*N* HCl (40 cc.) for 3 hr., and treated as above. The Et_2O extract (428 mg.) was dissolved in petr. ether:benzene (1:1) mixture and poured into alumina column. The first fraction (1:1 solvent mixture 70 cc.; eluant, 48 mg.) was rechromatographed and the second fraction (petr. ether 5 cc., 4 mg.) in the second run was purified by alumina chromatography. The eluant was recrystallized from $CHCl_3$:MeOH (1:1) mixture to fine needles, m.p. 212° (1 mg.,), which was identified as (II) by mixed fusion with the synthetic specimen.

Summary

1) 25D-Spirosta-3,5-diene (I), cholesta-3,5-diene, and solanida-3,5-diene were found to be photometrically assayed on a micro-scale and accurately using a characteristic extinction at 235 mμ due to 3,5-diene system in the molecules. With the aid of this method, the yields of (I), the main by-product accompanying diosgenin, from diosgenin and its glycosides upon acid treatment under various conditions were determined in order to find the optimal hydrolytic condition of diosgenin glycosides to minimize the yield of (I).

2) In comparison with diosgenin and its glycosides, the formation of corresponding $\Delta^{3,5}$-steroid by acid treatment from *epi*-diosgenin, 25D-spirost-4-en-3-ol, cholesterol, cholesterol glucoside, *epi*-cholesterol, solanidine, and solanine was examined in the same way.

3) A minor amount of 3-chloro-25D-spirost-5-ene was obtained besides (I) on boiling diosgenin with 2~4*N* hydrochloric acid in 50% ethanol.

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17) K. Fujii, M. Matsukawa: Yakugaku Zasshi, **57**, 114(1937).