Studies on the Sapogenins of Dioscorea tokoro Makino. IV[1]. *The Contents of Tokorogenin, Yonogenin and Diosgenin*

By Katsura MORITA

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Until recently, three steroids of natural origin possessing a hydroxyl group in position 1 had been announced. They are ouabagenin[2], acovenosigenin A[3], both being cardiac aglycones, and ruscogenin[4], a steroidal sapogenin isolated quite recently by C. Sannié et al. from *Ruscus aculeatus* L. and defined by Benn et al.[5] as 25D-spirost-5-en-1ξ, 3β-diol. Tokorogenin and rhodeasapogenin[6] are further additions to the examples of 1-hydroxylated steroids of natural origin. Tokorogenin is the only known steroid possessing a glycerol moiety in ring A, its structure having been defined by the author as 25D-5β-spirostan-1β, 2β, 3α-triol.

As described in a previous paper[7], tokorogenin was isolated from the rhizomes of *D. tokoro* Makino in a poor yield compared with diosgenin, which had been regarded as the sole steroidal sapogenin in the same plant. In the course of the present investigation, however, it was noticed that the rhizomes of *D. tokoro* Makino collected at Gotenba in Shizuoka Prefecture yielded extraordinarily large amounts of tokorogenin and a new sapogenin[8] and a minute quantity of diosgenin, compared with those collected in other places. No difference in appearance could be found between the rhizomes and the plants collected at Gotenba and those from other places.

In the light of these findings it was presumed that the rhizomes of *D. tokoro* Makino from certain districts other than Gotenba might be an excellent source of tokorogenin or other sapogenins, and the author started investigations on the sapogenin contents in the same plant species from various districts in Japan.

For this purpose, dried and sliced rhizomes collected at various sites were subjected to digestion with 1.2-N hydrochloric acid for six hours followed by drying and extraction with ether in a Soxhlet apparatus. The ether extract separated a voluminous jelly comprising tokorogenin, yonogenin and other unknown impurities. The jelly yielded rather impure tokorogenin and yonogenin by chromatographic purification on Florisil, while the mother liquor yielded almost pure diosgenin.

Since the above procedure was rather troublesome and unsuitable for rapid estimation with many samples, the following process was used: The ether extract was evaporated to dryness, and the residue was dissolved in methylene chloride and chromatographed on Florisil to give a diosgenin fraction and a yonogenin-tokorogenin fraction; both fractions were then evaporated and weighed to find roughly the amount of diosgenin and the total amount of other sapogenins. The results are summarized in Table I, which shows that the amount of diosgenin and that of other sapogenins vary markedly according to the site of collection of the rhizomes. It is especially noteworthy that the rhizomes collected even in neighboring villages (No. 21—No. 25) in Gotenba showed considerable differences in their sapogenin contents.

In order to obtain further information on these points, similar experiments were carried out with the rhizomes cultivated in the Experimental Farm of our company[9], each sample being prepared from one plant and by the same process.

A methanol extract of a rhizome was

1) This communication is Part XI of Nishikawa's paper entitled "Studies in Steroids". Part X; K. Morita, This Bulletin, **32**, 796 (1959).

2) C. Mannich and C. Siewert, *Ber.*, **75**, 737 (1942); Ch. Tamm, G. Volpp and C. Baumgartner, *Helv. Chim. Acta*, **40**, 1469 (1957).

3) W. Schlegel, Ch. Tamm and T. Reichstein, ibid., **38**, 1013 (1955).

4) C. Sannié and H. Lapin, *Bull. soc. chim. France*, **1955**, 1556.

5) W. R. Benn, F. Colton and R. Pappo, *J. Am. Chem. Soc.*, **79**, 3920 (1957).

6) The previous papar (Part III) of this series; H. Nawa, *Chem. & Pharm. Bull.*, **6**. 255 (1958).

7) M. Nishikawa, K. Morita, H. Hagiwara and M. Inoue, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **74**, 1165 (1954).

8) This sapogenin was thereafter shown to be identical with yonogenin: K. Takeda, T. Okanishi and A. Shimaoka, ibid., **77**, 822 (1957).

9) Cultivation of the plants in the Experimental Farm was started from the rhizomes collected in the neighborhood of Gotenba and other places, and has been continued for more than three years.

TABLE I

Expt. No.	Collection site	♀ ♂	Diosgenin (%)	Yonogenin & tokorogenin (%)	Expt. No.	Collection site	♀ ♂	Diosgenin (%)	Yonogenin & tokorogenin (%)
1	Kongosan (Osaka)	—	1.4	0.5	15	Takarazuka (Hyogo)	♀	1.2	0.6
2	Tachikawa (Chiba)	—	3.2	0.7	16	Takarazuka (Hyogo)	♂	1.3	1.7
3	Mishima (Shizuoka)	—	2.5	1.0	17	Shinjuku (Tokyo)	♀	3.8	1.4
4	Yamakita (Shizuoka)	—	1.0	1.0	18	Shinjuku (Tokyo)	♂	3.0	1.4
5	Nagano (Nagano)	—	2.8	0.8	19	Gotenba (Shizuoka)	♀	0.4	1.8
6	Jubuin (Kyushu)	—	2.0	0.6	20	Gotenba (Shizuoka)	♂	0.6	2.8
7	Miyanohara (Kyushu)	—	2.7	0.8	21	Gotenba-Nagahara (Shizuoka)	—	1.4	2.3
8	Mutobe (Kyoto)	—	2.7	0.7	22	Gotenba-Taniga (Shizuoka)	—	1.1	1.4
9	Sado (Niigata)	—	1.9	0.5	23	Gotenba-Oyama (Shizuoka)	—	0.2	2.6
10	Suginosawa (Niigata)	—	4.1	1.6	24	Gotenba-Ueno (Shizuoka)	—	0.3	2.9
11	Katsuyama (Fukui)	♀	2.4	1.6	25	Gotenba-Subashiri (Shizuoka)	—	2.5	1.9
12	Katsuyama (Fukui)	♂	2.0	0.6					
13	Hakusanshita (Ishikawa)	♀	2.0	0.7					
14	Hakusanshita (Ishikawa)	♂	2.2	0.6					

TABLE II

Expt. No.	Diosgenin (%)	Yonogenin (%)	Tokorogenin (%)	Expt. No.	Diosgenin (%)	Yonogenin (%)	Tokorogenin (%)
1	<0.1	0.7	0.8	26	1.1	0.2	0.3
2	<0.1	0.5	0.7	27	0.9	0.1	0.2
3	<0.1	0.6	0.8	28	0.1	0.5	0.6
4	<0.1	0.5	0.6	29	<0.1	0.7	0.8
5	<0.1	1.4	0.8	30	<0.1	0.4	0.6
6	<0.1	0.7	0.8	31	1.5	0.2	0.2
7	0.8	0.2	0.3	32	0.1	0.5	0.5
8	0.1	0.3	0.7	33	0.1	0.5	0.6
9	1.1	0.1	0.2	34	<0.1	0.6	0.7
10	0.7	0.2	0.1	35	<0.1	0.2	0.4
11	<0.1	0.9	0.6	36	<0.1	0.3	0.4
12	0.1	0.7	0.6	37	<0.1	0.5	0.7
13	0.1	0.7	0.7	38	<0.1	0.8	0.9
14	0.1	0.6	0.6	39	2.1	0.2	0.3
15	0.1	1.3	0.8	40	2.3	0.2	0.3
16	0.2	1.0	1.0	41	0.1	0.3	0.6
17	1.3	0.2	0.2	42	<0.1	1.0	0.7
18	0.2	0.6	0.7	43	1.9	0.2	0.3
19	1.1	0.1	0.3	44	1.3	0.2	0.3
20	1.7	0.2	0.3	45	2.1	0.2	0.3
21	1.0	0.2	0.3	46	<0.1	0.7	0.8
22	*1.1*	*0.1*	*0.2*	47	0.1	0.7	0.7
23	1.3	0.2	0.2	48	0.2	0.8	0.7
24	0.2	1.0	0.6	49	0.2	0.7	0.8
25	0.3	0.7	0.7	50	0.1	0.5	0.7

TABLE II (Continued)

Expt. No.	Diosgenin (%)	Yonogenin (%)	Tokorogenin (%)	Expt. No.	Diosgenin (%)	Yonogenin (%)	Tokorogenin (%)
51	<0.1	0.8	1.0	106	1.8	<0.1	<0.1
52	<0.1	0.2	1.1	107	0.8	<0.1	<0.1
53	0.1	1.0	1.0	108	1.2	<0.1	<0.1
54	<0.1	1.1	1.0	109	1.0	<0.1	<0.1
55	0.1	0.7	1.0	110	0.9	<0.1	<0.1
56	1.3	0.2	1.3	111	1.0	<0.1	<0.1
57	<0.1	0.5	1.8	112	0.6	<0.1	<0.1
58	<0.1	0.8	1.1	113	1.0	<0.1	<0.1
59	0.1	0.5	1.9	114	1.5	0.1	0.2
60	0.2	0.7	0.7	115	1.8	<0.1	0.2
61	1.2	0.2	0.3	116	0.2	1.0	0.6
62	0.1	0.5	0.8	117	2.3	0.2	0.3
63	0.2	0.9	0.8	118	0.2	0.5	0.6
64	0.2	0.7	0.8	119	0.2	0.6	0.7
65	0.1	0.8	0.8	120	2.0	0.1	0.2
66	0.2	0.7	0.8	121	0.3	0.7	0.6
67	0.1	0.7	0.8	122	2.3	0.3	0.2
68	0.2	0.6	0.6	123	1.7	0.1	0.2
69	0.1	0.6	0.6	124	2.1	0.2	0.3
70	0.1	0.8	0.6	125	1.2	0.1	0.2
71	<0.1	0.8	0.9	126	1.1	0.1	0.1
72	<0.1	0.7	1.1	127	2.1	0.2	0.2
73	<0.1	0.8	0.9	128	1.4	0.1	0.2
74	0.1	0.9	0.9	129	0.3	0.4	1.1
75	<0.1	0.6	1.0	130	0.2	0.4	1.0
76	0.1	0.6	0.9	131	0.3	0.8	0.8
77	0.2	0.8	1.5	132	0.1	0.4	1.2
78	<0.1	0.7	0.8	133	0.3	1.1	1.4
79	<0.1	1.1	1.0	134	0.3	0.9	1.7
80	0.3	0.5	0.8	135	0.2	0.8	1.6
81	0.3	1.0	1.2	136	0.2	0.9	1.3
82	0.2	1.6	1.1	137	0.2	1.2	1.4
83	0.1	0.3	1.1	138	0.2	0.8	1.0
84	0.2	1.3	1.1	139	1.6	0.1	0.2
85	2.2	0.2	0.4	140	0.2	0.6	0.7
86	<0.1	0.6	1.1	141	1.4	0.1	0.2
87	<0.1	1.2	1.1	142	0.2	0.9	0.7
88	0.4	0.1	<0.1	143	1.6	0.2	0.2
89	0.6	<0.1	<0.1	144	1.8	0.1	0.2
90	0.9	<0.1	<0.1	145	0.2	0.6	0.7
91	0.6	<0.1	<0.1	146	0.2	0.5	0.8
92	0.9	<0.1	<0.1	147	1.7	0.1	0.3
93	0.8	0.3	<0.1	148	0.2	0.7	1.0
94	0.7	<0.1	<0.1	149	1.3	0.1	0.2
95	1.1	<0.1	<0.1	150	1.4	0.1	0.3
96	1.1	0.1	<0.1	151	1.2	0.1	0.2
97	0.7	0.1	<0.1	152	0.3	1.0	0.9
98	0.8	0.1	<0.1	153	0.1	0.6	0.6
99	0.9	<0.1	<0.1	154	1.7	0.2	0.2
100	0.8	<0.1	<0.1	155	1.1	0.9	0.8
101	0.8	<0.1	<0.1	156	0.3	1.0	0.9
102	0.7	<0.1	<0.1	157	0.2	0.7	0.9
103	0.9	<0.1	<0.1	158	1.2	0.2	0.2
104	1.0	<0.1	<0.1	159	0.3	0.8	0.9
105	1.3	<0.1	<0.1	160	1.0	0.3	0.7

hydrolyzed with ethanolic hydrochloric acid[10], extracted with methylene chloride, chromatographed on Florisil and finally subjected to the colorimetric estimation of the three sapogenins by means of Yamagishi's method[11]. The results of the analyses tabulated in Table II reveal that the plants containing diosgenin as the major ingredient contain very small amounts of tokorogenin and yonogenin or lack them, while those containing large amounts of tokorogenin and yonogenin give only minute yields of disogenin or lack it. These findings are very interesting in view of the fact that the structures of tokorogenin and yonogenin are closely related (5β,2β-OH, 3α-OH), while diosgenin is considerably different from these two sapogenins (Δ^5, 3β-OH). In this connection it is also interesting to point out that the Shionogi's workers have isolated the fourth sapogenin, kogagenin, from the vines and leaves of the same plant[8] and suggested a structure of 25D-spirostan-1β, 2β,3α,5β-tetrol for the new sapogenin[12].

Experimental

Improved Isolation of Diosgenin, Yonogenine and Tokorogenin from the Rhizomes of *D. tokoro* Makino (Collected at Gotenba). —Dried rhizomes (1 kg.) collected at Gotenba was gently heated with 4.6 l. of 1,2-N hydrochloric acid for six hours and the hydrolyate was centrifuged, and the solid was washed with water and dried to give 186 g. of a dark brown cake. The cake was then extracted with petroleum benzine in a large Soxhlet apparatus for five hours, whereby diosgenin separated out as a white crystalline solid during the extraction. Ether was then substituted for petroleum benzine and extraction was continued for twenty more hours to give voluminous precipitation of dark brown jelly, which was filtered, washed with ether and dried. It weighed 18.7 g.

TABLE III

Sample	Content		
	Diosgenin (%)	Yonogenin (%)	Tokorogenin (%)
Dried rhizomes 1 kg.	0.82	0.63	0.50
Acid hydrolysate 186 g.	4.32	3.1	2.6
Jelly 18.7 g.	—	30	24

10) Hydrolysis was carried out by Marker's standard method.

11) M. Yamagishi and I. Nakamura, *Chem. & Pharm. Bull.*, **6**, 421 (1958).

12) At the Meeting of Organic Chemistry of Naturally Occurring Substances, October 18, 1958.

The analytical data shown in Table III[13] indicate that the procedure described above is satisfactory for the separation of diosgenin and a mixture of tokorogenin and yonogenin from the rhizomes.

For the separation of tokorogenin from yonogenin, the jelly (18.7 g.) was acetylated with pyridine-acetic anhydride and the acetylated mixture dissolved in ether was repeatedly washed with dilute aqueous alkali until washings became colorless. After the ether solution was dried and evaporated, the residue was redissolved in hot ethanol and treated with charcoal. Upon the solution was cooled tokorogenin triacetate was precipitated as almost colorless leaflets melting at 250~255°C. For analysis the crude material (m. p. 250~255°C) was dissolved in methylene chloride and the solution was passed through a column of Florisil, and concentration of the solution and recrystallization of the residue from ethanol raised the m. p. to 260~263°C. Yield, 4.7 g. The mother liquor, after hydrolysis with methanol and alkali, was poured into water and extracted with ether. The ether solution was washed with water, dried and evaporated to dryness. Chromatography of a portion of the residue on Florisil furnished yonogenin from methylene chloride-ethyl acetate (4 : 1) eluates and a small amount of tokorogenin from ethyl acetate eluates. After recrystallization from hot ethanol[14] the sample of yonogenin melted at 238~240°C and was identified with an authentic sample.

Yonogenin Acetate. — A small portion of yonogenin was acetylated with pyridine and acetic anhydried to give colorless needles of the diacetate, m. p. 213°C, $[\alpha]_D^{17}$ −17° (c=1.0%, $CHCl_3$). The sample showed no depression of m. p. on admixture with an authentic specimen.

Hydrolysis of the diacetate with alkali and methanol led to needles of the free sapogenin melting at 238~240°C.

Summary

(1) Improved isolation of tokorogenin, yonogenin and diosgenin from the rhizomes of *D. tokoro* Makino collected at Gotenba is described.

(2) The sapogenin contents in rhizomes of *D. tokoro* Makino collected in various districts in Japan were examined by means of chromatography.

(3) The sapogenin contents in rhizomes of cultivated plants of *D. tokoro* Makino were examined and it was found that the plants containing diosgenin as the major ingredient contain very small amounts of tokorogenin and yonogenin or lack them, while those containing large amounts of tokorogenin and yonogenin give only minute yields of diosgenin or lack it.

13) Analyses were carried out by Yamagishi's colorimetric method[7].

14) A solution of yonogenin in organic solvents showed strong tendency to form a gel.

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Takeda Research Laboratories
Juso, Higashiyodogawa-ku
Osaka