Die Fraktion 1 wurde wieder fraktioniert.

a)	12 mm	~75°	einige Tropfen
b)	12 mm	75~100°	einige Tropfen
c)	12 mm	100~115°	Gelbes Öl 1 ccm
d)	7 mm	~115°	wenig

Die Fraktion (a) wurde bromiert und aus MeOH umkristallisiert. Farblose Nadeln vom Schmp. 200~201°. Es war identisch mit Dibrom-1,2,3,4-tetramethylbenzol. $C_{10}H_{12}Br_2$—Ber.: C, 41.13; H, 4.14. Gef.: C, 41.24; H, 4.03.

Die Fraktion(3)wurde zum Pikrat geführt. Orange-gelbe Nadeln vom Schmp. 128~129° (aus EtOH). Es war mit Sapotalin-pikrat identisch. $O_{19}H_{17}O_7N_3$—Ber.: N, 10.5. Gef.: N, 10.71.

Zusammenfassung

Thea-sapogenol, dessen provisorische Zusammensetzung als $C_{30}H_{48\sim46}O_6$ angesehen wurde, hat in sich mindestens ein Carbonyl, eine α-Glykolgruppe und eine schwer hydrierbare Doppelbindung. Da das Sapogenol Hexaacetylderivate (Schmp. 272~274°) lieferte und das Acetat noch an sich die Färbung mit $C(NO_2)_4$ intakt behielt, ist das Carbonyl wahrscheinlich enolisierbar. Beim Behandeln mit Natrium und Amylalkohol wurde ein Reduktionsprodukt, Dihydrotheasapogenol (Schmp. 326~327°), erhalten. Bei der Destillation des Thea-sapogenols mit Hilfe von Selen wurden 1,2,3,4-Tetramethylbenzol und Sapotalin isoliert. So scheint das Sapogenol ein neues Triterpen von Amyrin-typus zu sein.

(Eingegangen am 18. März 1954)

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40. Ken'ichi Takeda, Tameto Okanishi, and Ariyoshi Shimaoka: Studies on the Steroidal Components of Domestic Plants. IV[1]. Constituents of Yucca Species. (1).

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Plants of the Yucca species, together with those of Agave and Dioscorea spp., have widely been studied as the material for steroidal sapogenins. Marker and others[2,3] have isolated 10 kinds of sapogenins from 42 kinds of the Yucca spp. growing in Mexico and the United States, including sarsasapogenin (from 19 plants), smilagenin (from 18), gitogenin (from 5), tigogenin (from 3), yuccagenin (from 3), kammogenin (from 3), and furcogenin, mexogenin, samogenin, and texogenin (from 1 plant each). In general, however, it is known that the quality and the amount of a steroidal sapogenin in a plant differ according to the age of the plant, position, and the season of collection, as well as the habitat[4]. Examination of the sapogenin contained in the domestic Yucca plants was therefore carried out and the results obtained were markedly different from that reported by Marker, *et al.*

The Yucca plants used for the present series of experiments are shown in Table I.

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1) Part III: J. Pham. Soc. Japan, **73**, 84 (1953).
2) R. E. Marker, *et al.*: A. D. I. Document 23, 8, 4.
3) R. E. Marker, *et al.*: J. Am. Chem. Soc., **69**, 2167 (1947).
4) R. E. Marker, *et al.*: J. Am. Chem. Soc., **69**, 2221 (1947).

Table I.

Plant	Part used	Habitat	Date collected
Yucca gloriosa L.	Rhizome	Hyogo Pref.	May, 1952
	Flower	〃	May 30, 1953
	Flower stalk	〃	〃
Y. recurvifolia Salisb.	Rhizome	〃	June, 1953
Y. aloifolia L. f. *tricolor* Bak.	Rhizome	〃	Oct. 21, 1953 / Feb. 16, 1954
	Leaves	〃	Oct. 21, 1953
Y. aloifolia L.	Leaves	Osaka Pref.	Nov. 5, 1953
Y. filamentosa L. var. *flaccida* Bak.	Rhizome	Kyoto Pref.	Sept. 14, 1953
Y. treculeana Carr.	Leaves	Osaka Pref.	Nov. 5, 1953

The isolation and purification of the sapogenin were similar to those described in the previous paper[1], except that the dried samples were prepared into coarse powder by a grinder and a chromatographic method was used for the isolation of a genin. Wall and others[5] proposed the treatment with butanol for the extraction of crude saponin but such complicated treatment is not necessary in the case of yucca. However, for the chromatographic separation of sapogenin, consecutive elution with three kinds of mixed solvents, chloroform : benzene = 2 : 98, chloroform : benzene = 2 : 8, and ethanol : benzene = 2 : 8, were used as recommended by Wall, *et al.* For the estimation, melting point admixture, specific rotation, and elementary analyses of the genin and its acetate, as well as the measurement of infrared absorption spectrum[6] of the acetate, for comparison of the finger print region (7~15 μ) bands with values given in literature, were made.

The experimental results obtained by the present writers, compared with those reported by Marker, *et al.* are shown in Table II.

Table II.

Plant	Part used	Sapogenin: Identified	m.p.°C	Sapogenin: Unidentified, m.p.°C	Sapogenin reported
Yucca gloriosa L.	Rhizome	Gitogenin	272		(Marker, et al.[2,3]) Smilagenin
		Tigogenin	204~206		
	Flower	Gitogenin	272		
		Tigogenin	202~205		
	Flower stalk			266[a] / 180~185[b]	
Y. recurvifolia Salisb.	Rhizome	Gitogenin	268	198~204[c]	Smilagenin
Y. aloifolia L. f. *tricolor* Bak.	Rhizome	Smilagenin	182		Smilagenin (var. *Naudin*)
		Tigogenin	205		
	Leaves	Tigogenin	205		
Y. aloifolia L.	Leaves	Tigogenin	205	181~182[d]	Smilagenin
Y. filamentosa L. var. *flaccida* Bak.	Rhizome	Sarsasapogenin	195		Gitogenin (*Y. filamentosa*)
Y. Treculeana Carr.	Leaves	(−)		120	Sarsasapogenin (var. *succulenta*) (var. *caniculata*)

a) Assumed to be gitogenin. *b*) Assumed to be tigogenin.
c) Admixture with tigogenin showed no depression in m.p.
d) Admixture with smilagenin showed no depression in m.p.

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5) M. E. Wall, M. M. Krider, E. S. Rothman, C. R. Eddy : J. Biol. Chem., **198**, 533(1952).
6) Parkin-Elmer Model 12-C (single beam), 10 mg./cc. CS_2, 1.0 mm. cell.

Experimental

Rhizome of *Yucca gloriosa* **L.**—The rhizome of the plant growing in the compound of the Laboratory was dug out, ground up, and thoroughly dried in the air, inside the room. The well-dried rhizome (250 g.) was digested twice with warm methanol and the methanol extract was evaporated under a reduced pressure. The residue was treated with ether to remove ether-soluble portion, dissolved in a small amount of water, and made into a 50% alcoholic solution. To this was added hydrochloric acid to make a 5% solution and the mixture was warmed on a water bath for 6 hours to effect hydrolysis. The precipitate thereby formed was extracted with ether and the ether extract was consecutively washed with 5% NaOH solution and two portions of water. After evaporation of ether, the residue was recrystallized from a mixture of methanol and acetone (1 : 1) and 1.3 g. (0.52%) of crude genin was obtained. This was separated into acetone-soluble and -insoluble portions and the crystals of m.p. 250~260° obtained from the acetone-insoluble portion was chromatographed through alumina column as a chloroform solution. The products were white needles, m.p. 264°, and white needles, m.p. 198°. The former recrystallized from acetone as white needles (I), m.p. 272°. This substance is insoluble in water and petroleum ether, sparingly soluble in ether, acetone, and methanol, and soluble in chloroform. The Liebermann-Burchard reaction of this substance changes from red to reddish brown color, and finally to dusky brown with fluorescence. No depression of the melting point occurred on admixture with gitogenin. $[\alpha]_D^{16}$: −63°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 75.00; H, 10.18. Found : C, 74.97; H, 10.29.

Acetate : m.p. 236~238°. The infrared absorption spectrum of the acetate was identical with that of gitogenin diacetate. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.09; H, 9.30. Found : C, 72.26; H, 8.97.

Recrystallization of the crystals melting at 198° yielded white needles (II) of m.p. 204~206°, undepressed on mixed fusion with tigogenin. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.88; H, 10.58. Found : C, 77.81; H, 10.17.

Acetate : m.p. 205~206°. The infrared absorption spectrum of the acetate was identical with that of tigogenin acetate. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.99; H, 10.04. Found : C, 76.46; H, 10.00.

The crystals obtained from the cold acetone-soluble portion melted at 204°, undepressed on admixture with (II).

Flower of *Yucca gloriosa* **L.**—a) Fresh flowers (600 g.) were extracted with warm methanol, and the residue was treated with chloroform. The ether-soluble substances were removed from this residue, and crude saponin, m.p. 250~260°, was obtained. A part of the methanolic extract was treated and hydrolyzed as in the case of the rhizome and 0.2 g. (0.03%) of crude genin, m.p. 263°, was obtained. Recrystallization raised the melting point to m.p. 272°, undepressed on admixture with (I).

b) The flowers and buds were dried in air, inside the room, for 45 days (loss on drying, 94.73%) and 150 g. of such dried substance was treated and hydrolyzed as in the foregoing case. Chromatographic separation through alumina yielded crystals (IIa), m.p. 202~205°, from a mixed solvent (i) of $CHCl_3$: benzene (2 : 98) and crystals (Ia), m.p. 268°, from a mixed solvent (ii) of $CHCl_3$: benzene (2 : 8). Yield of (IIa), 0.2 g. (0.13%). No depression of the melting point occurred on admixture with tigogenin. Yield of (Ia), 0.9 g. (0.6%). No depression of the melting point occurred on admixture with gitogenin. *Anal.* Calcd. for $C_{27}H_{44}O_4$(Gitogenin) : C, 75.00; H, 10.18. Found : C, 74.57; H, 10.59.

c) The flower stalk from which all the flowers and buds were removed was dried (loss on drying, 65.57%) and 380 g. of the dried substance was treated as in the foregoing case. Chromatographic separation through alumina yielded a wax-like substance of m.p. 60~81° and crystals of m.p. 180~185° from the mixed solvent (i) and crystals of m.p. 266° from the mixed solvent (ii). The former (0.1 g.) was assumed to be tigogenin, the latter (0.5 g.), gitogenin.

Rhizome of *Yucca recurvifolia* **Salisb.**—The fresh rhizome (2.5 kg.) was treated as in the case of the foregoing rhizome and 50 g. of the precipitate was obtained after hydrolysis. This precipitate yielded 0.2 g. of crystals (III) melting at 135~180°. The mother liquor obtained after the removal of (III) was evaporated and the residue was purified through an alumina column. Elution with the mixed solvent (iii) of EtOH : benzene (2 : 8) yielded 0.15 g. of crystals (Ib) melting at 268°, alone and in admixture with gitogenin.

(III) was purified by chromatography from which a substance of m.p. 68~80° and then 0.05 g. of needle crystals (IIb), m.p. 198~204°, were obtained from the mixed solvent (i). (IIb) showed no depression of the melting point on admixture with (II), obtained from *Yucca gloriosa*.

Yucca aloifolia **L. f.** *tricolor* **Bak.**—a) The leaves were collected from a mature plant (height of the stalk, about 2 m.) in a garden in Nishinomiya, Hyogo Prefecture. After cutting into longitudinal strips, they were dried in air and ground. This dried substance (800 g.) was treated as in the foregoing case and the crude genin was dissolved in the mixed solvent (i) for chromatographic separation. The eluate first yielded a wax-like substance and then needle crystals of m.p. 183° and m.p. 170°. Elution with the mixed solvent (ii) gave crystals of m.p. 206~208°. For further purification, the three products

were combined and again chromatographed from which crystals of m.p. 194°, m.p. 194~205°, and m.p. 205°, were respectively obtained. Recrystallization of the two former crystals from acetone raised the melting point of both to m.p. 205° and the three were thereby seen to be identical substance. No depression of the melting point occurred on admixture of this substance with tigogenin. $[\alpha]_D^{15}$: −61° (in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.46; H, 10.29. Found : C, 77.88; H. 10.58.

Acetate : m.p. 204°. The infrared absorption spectrum of this acetate was identical with that of tigogenin acetate. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.99; H, 10.04. Found : C, 75.51; H, 10.10.

b) Treatment of 70 g. of the half-dried rhizome of the same plant as in the foregoing case yielded needle crystals (V), m.p. 182°, and a small amount of prismatic crystals (VI), m.p. 206°. (V) seemed to be the chief component. $[\alpha]_D^{18}$: −63° (in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.88; H, 10.58. Found : C, 77.41; H, 10.70.

Acetate : m.p. 148°. $[\alpha]_D^{18}$: −56° (in $CHCl_3$). The infrared absorption spectrum of this acetate was identical with that of smilagenin acetate. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.99; H, 10.04. Found: C, 76.45; H, 9.61.

Yucca aloifolia **L.**—The leaves were collected from a medium grown plant in the Kosobe Nursery, Takatsuki, Osaka Prefecture, immediately cut longitudinally, and dried in air for 3 months (loss on drying, 68%). The coarse powder (500 g.) of this half dried product was treated as in the foregoing and the genin (VII) thereby obtained melted at 205°. Yield, 0.5 g. (0.1%). No depression of the melting point occurred on admixture with tigogenin.

The mother liquor obtained after separation of (VII) yielded, after being allowed to stand for some time, crystals of m.p. 181~182°, which showed no depression on admixture with (V).

Yucca filamentosa **L. var.** *flaccida* **Bak.**—The rhizome collected from the plant growing in the Botanical Garden of Saikyo University, Kyoto, was ground coarsely, dried, and reduced to a powder (650 g.) which was treated as in the foregoing. Chromatographic separation of 2 g. (0.31%) of the crude genin, m.p. 180°, thereby obtained yielded crystals of m.p. 178°, 180°, and 183~184°, from the mixed solvent (i), and crystals of m.p. 184° from the mixed solvent (ii). Analytical results indicated that all of these crystals were still impure that they were repeatedly recrystallized from methanol and ethyl acetate by which the melting point was raised to m.p. 194° but the analytical values remained indefinite. The substance was therefore derived to an acetate and chromatographed as a solution in an 1 : 99 mixture of benzene and petroleum ether. The crystals (VIII) of m.p. 100~125° obtained from the effluent were recrystallized from methanol to crystals melting at 141°. The infrared absorption spectrum of this substance was identical with that of sarsasapogenin acetate. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.99; H, 10.04. Found : C, 76.13; H, 10.28.

(VIII) was saponified with 5% alcoholic potash and extracted with ether. The crystals obtained as the ether residue were repeatedly recrystallized from methanol and ethyl acetate to crystals melting at 195° which showed no depression on admixture with sarsasapogenin. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.88; H, 10.58. Found : C, 78.06; H, 10.27.

Yucca Treculeana **Carr.**—The leaves were collected from the mature plant in the Kosobe Nursery, immediately cut longitudinally, and dried in air for 3 months (loss on drying, 67%). The finely ground product (900 g.) was treated as in the foregoing but sapogenin was not obtained. A small amount of crystals melting at around 120°, giving positive reaction for sterols, were obtained.

Summary

Extraction of steroidal sapogenin was carried out on several kinds of Yucca spp. usually cultivated in Japan. The rhizome of *Yucca gloriosa* L. yielded gitogenin (I) and a small amount of tigogenin (II), while its flowers also yielded the same compounds. The flower stalk yielded a small amount of crystals of m.p. 226° and 180~185° which were respectively assumed to be identical with (I) and (II). The rhizome of *Y. recurvifolia* Salisb. yielded (I) and a small amount of crystals, m.p. 198~204°, which showed no depression on admixture with (II). The rhizome of *Y. aloifolia* L. f. *tricolor* Bak. yielded smilagenin (III) and a small amount of (II), while only (II) was isolated from its leaves. The leaves of *Y. aloifolia* L. yielded (II) and a small amount of crystals melting at 180~182°, alone and in admixture with (III). The rhizome of *Y. filamentosa* L. yielded sarsasapogenin. The leaves of *Y. Treculeana* Carr failed to yield any sapogenin but yielded some crystals of m.p. 120°, giving positive sterol reaction. These results were different from those reported by Marker, *et al.*, on Yucca plants of North America.

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